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# Biochar Reduces Nanoplastics Uptake by Lettuce and Alleviates Its Toxicity to the Plant

Anwar A. Aly,*,⋈ Junjie Tang,⋈ Mahmoud Zanaty,⋈ Chenwei Liu, Arjun S. Kulathuvayal, Yanqing Su, Ibukunoluwa Salako, Jennifer Weidhaas, Zeyu Cai, Chuanxin Ma, Joan E. McLean, Arturo Keller, Baoshan Xing, and Yiming Su*

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**ABSTRACT:** The accumulation of micro- and nanoplastics (MPs/NPs) in croplands threatens food quality and human health. This study investigates the effectiveness of pristine biochar (BC) and iron-doped biochar (Fe-BC), produced from pine sawdust via one-step pyrolysis, in reducing NPs uptake by lettuce (*Lactuca sativa L. var. adela*). Fe-BC exhibited greater porosity, higher surface area, and a slightly positive surface charge compared to BC. Using Pd-doped NPs, we confirmed that NPs can penetrate roots and translocate to leaves, accumulating more in older tissues. Soil application of 3 wt % BC or Fe-BC significantly lowered leaf NPs concentrations, with Fe-BC showing a greater reduction, by approximately 60% (from 0.90 to 0.36 mg/kg). While both BC and Fe-BC demonstrated a capacity for alleviating NPs-induced metabolic disturbances and partially restoring soil enzyme activities, Fe-BC presents a more promising amendment for mitigating NPs contamination and protecting crop quality.

**KEYWORDS:** *biochar, Fe-biochar, bioavailability of nanoplastics, nanoplastics immobilization, plant health*



## INTRODUCTION

Since the mid-20th century, vast amounts of plastic, including widely used agricultural films, have been produced, and soil, unfortunately, has been one of the major sinks of plastic waste.[1−3] Microplastics (MPs, with one dimension <5 mm) and nanoplastics (NPs, with one dimension <1 μm) are generally from weathered particles from plastic waste intentionally or unintentionally released to the environment. While MPs are ubiquitous in the environment,[4] the existence/prevalence of NPs in the environment and their impact on soil/plant health remains largely unknown. This uncertainty regarding NPs is due to the lack of reliable extraction and quantification technology, even though the risk of NPs pollution is high.

One of the big threats of NPs contamination in cropland is the potential accumulation of NPs in crops since NPs can be mobile and bioavailable to plants, while most MPs cannot enter crops due to root structure size exclusion. NPs can translocate from water/soil particles to microbes, fungi, algae, invertebrates, and plants.[5,6] Our previous study showed that, while both negatively and positively charged NPs can accumulate in *Arabidopsis thaliana*, positively charged NPs can form hetero aggregates with roots exudates on root surfaces, while negatively charged NPs are frequently found in the apoplast and xylem.[7] This translocation of NPs from soil/water to crops can impact crop health and, potentially, human health. For instance, Jiang et al.[8] demonstrated that NPs can translocate from soil to surfaces of plant roots and then to the grains. A level of 250 mg of NPs per kg of soil can increase empty-shell rice grain numbers by 35.45% and decrease the nutritional value in grains. Wang et al.[9] found that eating crops contaminated with NPs can be one of the reasons NPs accumulate in the human body. Both MPs and NPs have been found in human feces, lungs, placenta, and blood,[10−14] and a recent study by Marfella et al.[15] confirmed that patients with carotid artery plaque containing NPs/MPs had a higher risk of myocardial infarction, stroke, or death than those without NPs/MPs. These studies imply the potential accumulation of MPs/NPs in the human body through the food chain and their subsequent significant health risk. Therefore, it is critical to quantify the potential impact of NPs contamination on soil and crop health and develop a mitigating strategy.

Biochar, produced by pyrolyzing agricultural biomass under limited oxygen, has been widely applied for contaminant immobilization in aqueous systems,[16] but its role in soil, especially regarding NPs mitigation, remains underexplored. In aqueous media, NPs bind to biochar via van der Waals forces, electrostatic interactions, acid−base exchange, or hydrophobic interactions, and reported adsorption capacities vary broadly (e.g., 12 to ∼160 mg/g) depending on biochar type and modification.[17−20] This wide range of removal capacities indicates that biochar needs to be optimized for NPs sequestration. While interactions between biochar and NPs in aqueous media have been investigated, little is known about their interactions in soil and how these interactions affect crop health and nutrition. It is also important to gain a fundamental

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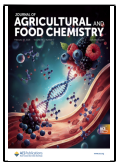

understanding of how biochar competes with soil particles for NPs, how the physical-chemical properties of biochar affect both soil and crop health, and the potential effectiveness of alleviating NPs uptake by crops.

In this study, we test the hypothesis that biochar amendment can reduce NPs uptake by lettuce and mitigate associated negative impacts on soil and plant health. We produced both pristine pine (*genus Pinus*) sawdust biochar (BC) and iron-doped biochar (Fe-BC) in a single pyrolysis step, characterized them, and amended soil at 3% (w/w) in pot experiments. After germination, a single lettuce plant per pot was maintained under NPs exposure via irrigation. At the end of the study, soil biological health, plant growth (e.g., leaf number and biomass), NPs concentrations in roots and leaves, and plant nutritional status were monitored to elucidate the mechanisms by which BC and Fe-BC influence NPs dynamics in soil−plant systems.

## MATERIALS AND METHODS

### Soil Used in the Study

Soil used in this study was collected from an agricultural field near Snowville, Utah (Figure S1). The soil is classified as coarse-silty, mixed, active, mesic *Sodic Calcixerepts*. The soil was air-dried and sieved (2 mm). Soil properties, including pH, EC, cation exchange capacity (CEC), particle size distribution, total carbon, inorganic carbon, and organic carbon, and nutrient availability of nitrate, sulfate, P, and K, and DTPA extractable metals were determined by Atoloye Utah State University Analytical Laboratories (USUAL) following standard methods[21,22] (Tables S1 and S2). The field capacity (FC) of the soil and permanent wilting point (PWP) were determined by the pressure plate method at −100 and −15,000 kPa retention, respectively.[23] The FC was 18%, and the PWP was 8.3%.

### Preparation of Biochar and Iron-Doped Biochar

Pine sawdust was chosen as the biochar feedstock because it is an abundant forestry residue that can be sustainably converted into biochar and has demonstrated promising physicochemical properties for soil remediation and contaminant immobilization.[24,25] The comparison between pristine biochar (BC) and iron-doped biochar (Fe−BC) was designed to distinguish the baseline remediation effects of unmodified biochar from the enhanced sorption and immobilization capacity achieved through iron modification, which is known to increase surface area, functional groups, and affinity for pollutants.[24,25] Together, this design enables a clearer understanding of the mechanisms by which pristine and modified biochars mitigate nanoparticle uptake in leafy vegetables.

BC and Fe-BC were prepared using pine tree (*genus Pinus*) sawdust. For BC, pristine sawdust was washed (removing dust) and dried in an oven (60 °C, until no moisture was observed). For Fe-BC, 150 g $FeCl_3{\cdot}6H_2O$ was dissolved in 400 mL of deionized water and then mixed with 1 kg of sawdust in a large tray. After overnight stabilization, the mixture was then dried at 60 °C for 2 days. The derived (both pristine and Fe-doped) sawdust was then packed into aluminum foil boats and placed in a tube furnace. Pyrolysis was carried out at 600 °C under $N_2$ atmosphere for 2 h. The prepared biochar was ground and sieved (2 mm sieves) and stored for experimental use. The biochar analyses were conducted as described by Subero et al.[26] A 1:5 (w/v) biochar/water extract was used for measuring the pH and EC using a multiparameter pH and EC meter (Table S3). The biochar and Fe-biochar were characterized with SEM-EDS (FEI Quanta 650 F, Thermo Scientific, Hillsboro, OR) for surface morphology, NanoBrook ZetaPALS (Brookhaven Instruments, Holtsville, NY) for surface charge, BET (Monosorb, Quantachrome, FL) for surface area, and X-ray diffraction (XRD, Bruker D2 Phaser, Germany) for crystal structure.

### Preparation of Pd-Doped Polystyrene NPs

To track the translocation of NPs, palladium-doped polystyrene nanoplastics (Pd-NPs) were employed. The Pd-NPs were synthesized according to our previous study,[27] and as described, both the number and mass concentration of Pd-NPs can be calculated through a triple quadrupole inductively coupled plasma mass spectrometer (ICP-MS, Agilent 8900 ICPQQQ, Santa Clara). Briefly, copolymerization occurred during the synthesis. Acrylonitrile (monomer), potassium persulfate (initiator), sodium dodecyl sulfate, and potassium poly-(ethylene glycol) 4-nonylphenyl 3-sulfopropylether (surfactants) were used. Potassium chloropalladite ($K_2PdCl_4$) was introduced as the metal precursor for chemical entrapment of the Pd-NPs. At the later phase of synthesis, $H_2O$, styrene, and divinylbenzene (DVB) were added to the reactor, followed by washing three times with deionized water (DI water). Pd-NPs were characterized using SEM, ICPQQQ, and ZetaPals.[27]

### Experimental Setup and Design

Lettuce (*Lactuca sativa* L. var. Adela), a globally consumed leafy vegetable, was selected as the test crop due to its fast growth, high surface area-to-biomass ratio, and known propensity to accumulate environmental contaminants such as nanoparticles, posing potential food safety risks.[28,29] The experiment was conducted in a constant-temperature room (16 °C) at Utah Water Research Laboratory, Utah State University. The biochar was thoroughly mixed with the air-dry soil on a weight basis of 3% (*w/w*), and all treatments were replicated four times. After germination, one selected plant was kept in each pot, and during the experiment, the pots were randomized to avoid positional bias. Plants were cultivated under 100% light intensity (Viparspectra, Model BQE96C-2400-40-PV) with a 16 h light/8 h dark photoperiod.

The experimental plants were irrigated to reach 100% field capacity on a weight-loss basis every day with tap water and Hoagland solution. From the 30th day after germination to the 66th day, lettuce was exposed to a Pd-NPs suspension (100 mg/L, total 1 mg of Pd-NPs per dosage per pot) in irrigation water every other day. A total of 18 mg of Pd-NPs was added to each pot (0.9 kg soil) under investigation. NPs exposure was initiated on the 30th day after germination to ensure uniform seedling establishment and to simulate an acute contamination event.[30,31]

### Pd, Nutrients, and Other Inorganic Elements Analyses

The lettuce plants were harvested, weighed, and divided into leaves and roots prior to analysis. The leaves were counted and divided into three parts for each plant as follows: part A for old (outer) leaves, part B for middle-aged leaves, and part C for recent (inner) leaves (Table S4). The plant parts were washed sequentially with DI water, 0.01 M HCl, and nanopure water to remove surface residues and loosely bound nanoparticles. Samples were then dried for 2 days at 65 °C and ground to pass through a 60-mesh screen. Leaves and roots were digested using $HNO_3-H_2O_2$ for Pd and other elements analyses as described by Pequerul et al.[32] The elements Pd, Ca, Mg, Na, K, P, Cu, Fe, Mn, Zn, V, Si, Be, and Al were determined using ICP-MS (Agilent 8900 ICPQQQ). The detection limits for each element were: Pd, 0.002 μg/L; Ca, 0.007 mg/L; Mg, 0.002 mg/L; Na, 0.006 mg/L; K, 0.007 mg/L; P, 0.037 mg/L; Cu, 0.066 μg/L; Fe, 0.083 μg/L; Mn, 0.028 μg/L; Zn, 0.611 μg/L; V, 0.037 μg/L; Si, 0.004 mg/L; Be, 0.035 μg/L; and Al, 0.425 μg/L.

### Soil Enzyme Analysis

Enzyme activity in mixed soil and biochar samples was determined using previously published spectrophotometric and colorimetric methods. β-glucosidase and β-N-acetylglucosaminidase (NAGase) activities were determined using the method of Eivazi and Tabatabai.[33] The soil samples were incubated with p-nitrophenyl-β-D-glucopyranoside and p-nitrophenyl-N-acetyl-β-D-glucosamine as substrates, respectively, and the released p-nitrophenol (PNP) was measured spectrophotometrically at 410 nm.[33,34] Protease activity was quantified by incubating soil samples with sodium caseinate at 50 °C for 2 h, followed by termination with 10% trichloroacetic acid (TCA). The resulting supernatant was reacted with sodium carbonate ($Na_2CO_3$) and Folin-Ciocalteu reagent, and absorbance was measured at 650 nm.[35,36] Cellulase activity was determined using



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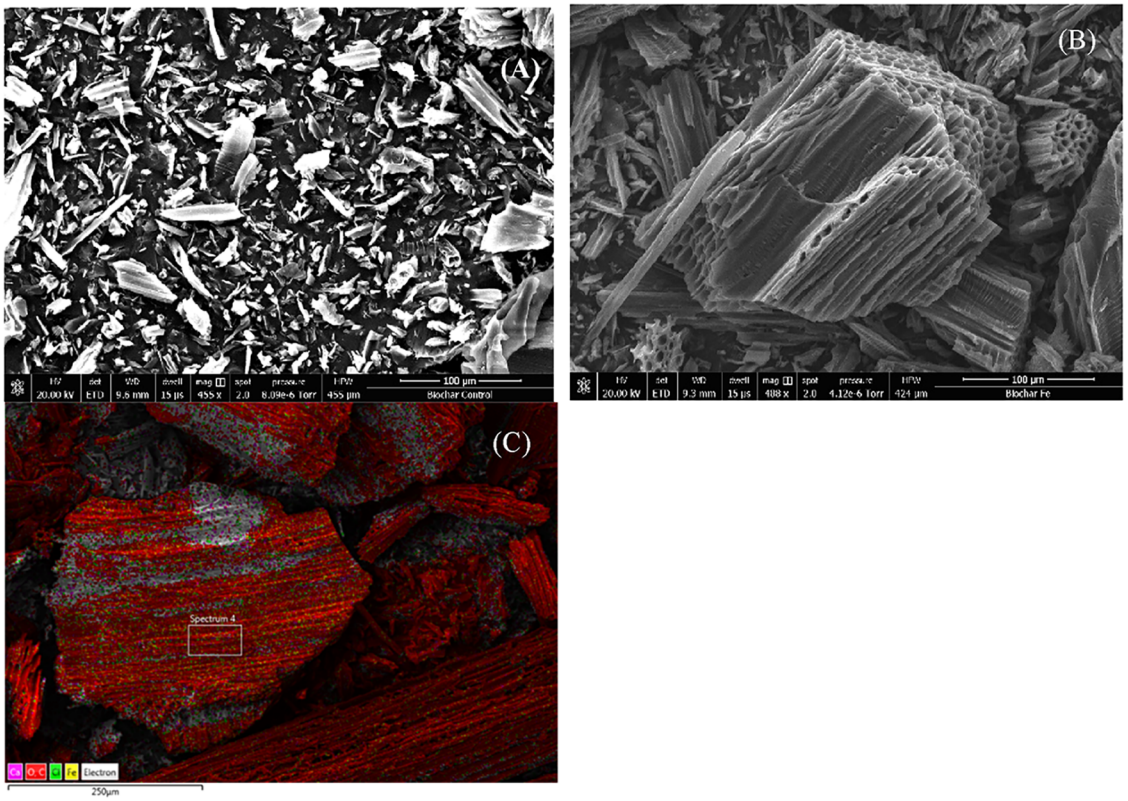


**Figure 1.** SEM images of biochar (A) and Fe-biochar (B). Notably, while biochar does not have clear Fe atom signals (Figure S3), the overall Fe content in (C) is about 2.3% (Figure S4A), and that in selected area 4 (white rectangle) is about 6.0% (Figure S4B) (a clear EDS mapping of Fe element of (C) is provided in Figure S4C).

carboxymethyl cellulose as a substrate, with reducing sugars quantified using Amplex Red glucose assay reagent at 540 nm[37,38]

Amidase activity was determined by incubating the soil samples with L-leucine $\beta$-naphthylamide hydrochloride at 37 °C for 1 h. The supernatant was then treated with acidified ethanol and p-dimethylaminocinnamaldehyde reagent, and absorbance was measured at 540 nm.[39−41] Alkaline phosphatase activity was quantified by adding p-nitrophenyl phosphate to samples and incubating for 1 h to form p-nitrophenol with the PNP concentration. The PNP formed by alkaline phosphatase was then measured by absorbance at 410 nm.[34] Dehydrogenase activity (DHA) was determined based on the reduction of 2,3,5-triphenyl tetrazolium chloride to triphenyl formazan (TPF). The TPF was then extracted with methanol, and its concentration was determined by absorbance at 485 nm.[42,43] Phenol oxidase activity was assessed by incubating soil samples with ABTS ((2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt)) at 30 °C for 5 min, and absorbance was measured at 420 nm.[44] Arginine deaminase activity was determined by incubating soil with L-arginine substrate at 37 °C for 3 h, then the supernatant was mixed with sodium phenolate, sodium hypochlorite, and sodium nitroprusside to extract the color at 630 nm.[45,46]

All enzyme activities were expressed on a dry soil weight basis, and assays were conducted in duplicate.

### Metabolites Content and Nutritional Values

NPs accumulation in lettuce could potentially affect the nutritional composition of the produce. To comprehensively assess the potential effects of NPs on the nutritional quality of crops, key nutritional parameters were measured in lettuce plants. Metabolites content was measured using the modified method described by Li and Keller.[47] Briefly, 100 mg of plant tissue from each homogenized sample was combined with 1 mL of 80% methanol in water containing 2% formic acid in a 1.5 mL centrifuge tube. The mixture was vortexed at 3000 rpm for 20 min, followed by sonication in a water bath at room temperature for 20 min. After extraction, the sample was centrifuged at 20,000g for 20 min. The resulting 1 mL of supernatant was divided into four vials, each containing 200 $\mu$L, and reconstituted in the appropriate solvent for analysis using liquid chromatography-tandem mass spectrometry (LC−MS/MS).

### Statistical Analysis

Lettuce yield and plant vigor data were analyzed statistically using analysis of variance (ANOVA) in SAS (SAS Campus Drive, Cary, NC 27513-2414), and the mean differences were tested for significance with the LSD test.

The partial least squares-discriminant analysis (PLS-DA) was conducted by R 4.1.3. The orthogonal partial least squares-discriminant analysis (OPLS-DA) for different metabolite (DM) identification was conducted by SIMCA 14.1 (Umetrics, Sweden). The DMs in the compared groups were identified based on a fold change <0.78 or fold change >1.3 and variable importance in projection (VIP) > 1 in the OPLS-DA model. The heat map was conducted by R-package heatmap. The correlation analysis of DMs was conducted by R-package psych. The significantly correlated differential metabolites were used to construct correlation networks. The correlation network was conducted by Cytoscape 3.10.1.

## RESULTS

### Biochar Characterization and NPs Removal Capacity

BC and Fe-BC have distinctly different microstructures. It is seen from Figure 1A,B that Fe doping successfully preserved the majority of the porous structure of the raw material and resulted in an even distribution of Fe on biochar (Figure 1C). This aligns well with the XRD analysis result (Figure S2), which confirms the presence of amorphous carbon but not crystal iron oxide on both BC and Fe-BC.[48] However, the abundant porous structure from SEM analysis explains the higher surface areas of Fe-BC than that of BC. The specific


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**Table 1. Enzyme Activities under Different Treatments**[a]

| enzyme activities[b] | ContFW | ContNPs | B1 | B2 | B1NPs | B2NPs |
|---|---|---|---|---|---|---|
| $\beta$-glucosidase ($\mu$g PNP/g/h) | 55.1 ± 0.01 a | 19.8 ± 0.05 b | 25.9 ± 0.2 c | 25.1 ± 0.4 d | 18.6 ± 0.3 e | 24.7 ± 0.3 d |
| $\beta$-N-acetylglucosaminidase (NAGase) ($\mu$g PNP/g/h) | 7.4 ± 0.3 a | 3.3 ± 0.07 b | 11.4 ± 0.07 c | 8.0 ± 0.5 a | 3.7 ± 0.1 a,b | 2.7 ± 0.3 b |
| protease (mmol TYR/g/h) | 93.5 ± 10.8 a | 32.7 ± 35.9 b,c | 15.7 ± 1.9 c | 9.3 ± 6.5 c | 8.9 ± 10.1 c | 49.0 ± 6.1 b,c |
| cellulase ($\mu$mol glucose/g/h) | 82.4 ± 48.3 a | 52.3 ± 4.7 a | 69.2 ± 23.9 a | 46.4 ± 23.0 a | 57.2 ± 12.0 a | 35.5 ± 17.0 a |
| amidase ($\mu$g $\beta$-naphthylamine/g/h) | 8.4 ± 0.3 a | 7.3 ± 0.03 b | 7.2 ± 0.04 b | 5.6 ± 0.04 c | 5.9 ± 0.04 c | 5.7 ± 0.01 c |
| alkaline phosphatase ($\mu$g PNP/g/h) | 28.4 ± 0.12 a | 29.3 ± 0.08 b | 58.0 ± 0.07 c | 40.3 ± 0.12 d | 39.8 ± 0.2 e | 45.4 ± 0.05 f |
| dehydrogenase (DHA) ($\mu$g TPF/g/h) | 16.1 ± 0.33 a | 36.6 ± 0.11 b | 13.0 ± 0.46 c | 12.5 ± 0.3 c | 22.0 ± 0.25 d | 17.2 ± 0.01 e |
| phenol oxidase (mmol/g/min DM) | 31.4 ± 0.2 a | 32.4 ± 0.1 b | 30.7 ± 0.03 a | 29.3 ± 0.3 c | 33.4 ± 0.03 d | 34.5 ± 0.3 e |
| arginine deaminase ($\mu$g $NH_3$–N/g/h) | 0.7 ± 0.01 a | 1.9 ± 0.05 b | 1.5 ± 0.03 c | 0.7 ± 0.04 a | 1.3 ± 0.07 d | 1.1 ± 0.01 e |

[a]Average and standard deviation of $n = 2$ samples for each treatment are shown. Mean enzyme activities with a different letter were significantly different by Tukey HSD at an $\alpha = 0.05$. [b]PNP: p-nitrophenol, TPF: triphenyl formazan, TYR: tyrosine.

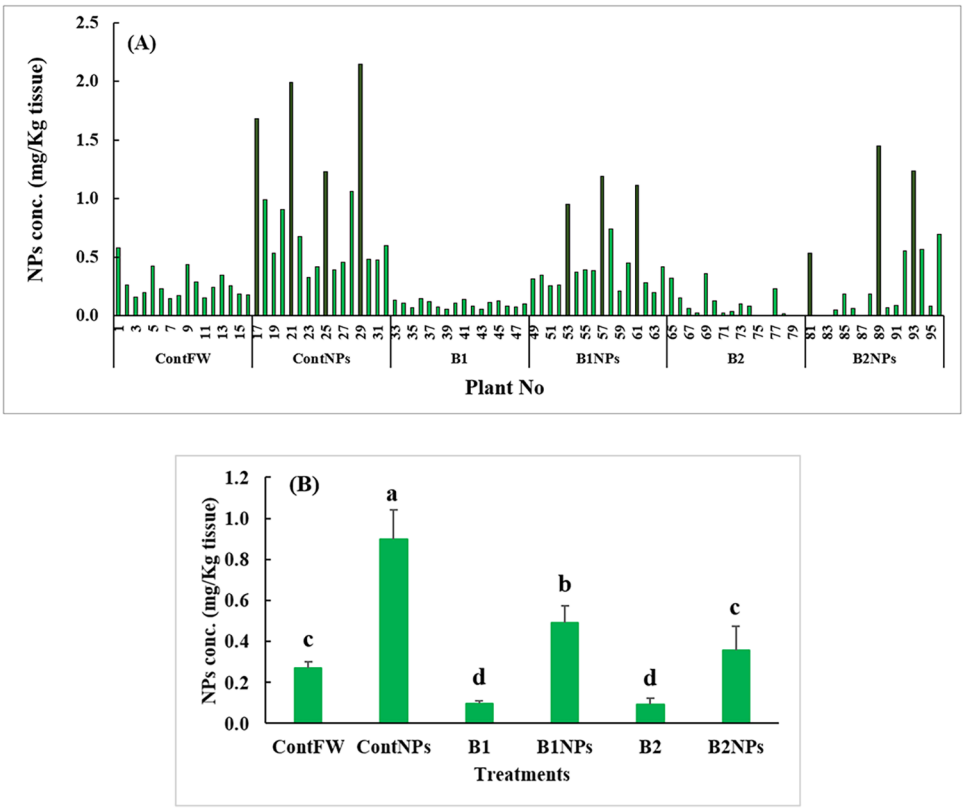


**Figure 2.** Translocation of NPs into lettuce leaves (black coloration indicating older leaves) (A) and the average of NPs content for each treatment ($n = 4$) (B). Treatment means with the same letters are not significant by LSD at the 0.05 level.

BET surface area of BC and Fe-BC is 12.49 $m^2$/g and 19.03 $m^2$/g, respectively, with removal capacities of 0.12 mg/g for BC and 0.13 mg/g for Fe-BC. Moreover, Fe doping increased the point of zero charge (PZC) from pH = 3.36 to pH = 5.43, which could be caused by the high PZC of iron oxides (with ZPC ≥ 7.0) on biochar (with ZPC ∼ 4.0).[49,50] It implies that NPs removal can be facilitated by Fe doping since electrostatic repulsion between NPs and BC surfaces can possibly be reduced.

## Effect of NPs and Biochar on Soil Enzymes

NPs had varying effects on soil enzyme activities (Table 1). When NP were added to the irrigation water, enzymes involved with carbon and nitrogen cycling in soils, such as $\beta$-glucosidase, $\beta$-N-acetylglucosaminidase, protease, and amidase, decreased by 64, 55, 65, and 13%, respectively, compared to the control soils. In contrast, dehydrogenase and arginine deaminase activities increased by 127.2 and 186%, respectively, while a slight increase was observed for alkaline phosphatase and phenol oxidase compared to the control soil. The addition of BC and Fe-BC mitigated some of these adverse effects. For example, alkaline phosphatase increased from 28.4 ± 0.12 in the control to 58.0 ± 0.09 $\mu$g PNP/g/h (B1) and 40.3 ± 0.12 $\mu$g PNP/g/h (B2), and NAGase rose from 7.4 ± 0.3 to 11.4 ± 0.07 and 8.0 ± 0.5 $\mu$g PNP/g/h, respectively. When biochar and NPs were combined, enzyme activities were generally lower than those in biochar-only treatments but higher than those in NPs-only soils, indicating partial mitigation of NP-induced inhibition. For instance, $\beta$-glucosidase recovered to 24.7 ± 0.3 $\mu$g PNP/g/h in B2 + NPs, while alkaline phosphatase increased to 39.8 ± 0.2 and 45.4 ± 0.05 $\mu$g PNP/g/h in B1 + NPs and B2 + NPs, respectively. Dehydrogenase activity showed an opposite pattern, rising


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from 16.1 ± 0.33 μg TPF/g/h (ContFW) to 36.6 ± 0.11 μg TPF/g/h with NPs (ContNPs), but decreasing with biochar dosage (17.2−22.0 μg TPF/g/h, in B1+NPs/B2+NPs). These findings demonstrate the potential of biochar to alleviate the negative impact of NPs on soil enzyme activities and maintain soil functionality.

## BC and Fe-BC Alleviate the Accumulation of NPs in Lettuce

After NPs exposure through irrigation water, the NPs content in lettuce in all growth media increased significantly, compared to those counter-experiments without NPs dosage (due to the high sensitivity of ICPQQQ, trace level of Pd was also detected in the Control group, possibly due to the existence of Pd in soil[51]). However, it is noted that lettuce from soil without BC or Fe-BC dosage recorded higher NPs content in plant leaves (ContNPs compared to ContFW), while BC and Fe-BC amendment significantly decreased leaf NPs content (Figure 2b), which aligns well with the finding of NPs' entrance into plants reported by Huang et al.[52] and Li et al.[53] Moreover, it was found that in general, NPs content in old leaves (e.g., No. 17, 21, 25 and 29:1.68, 2.0, 1.23, and 2.15 mg/kg, respectively) were much higher than those of young leaves (e.g., No. 11, 47, 51, 55, 79 and 95:0.15, 0.07, 0.25, 0.39, 0.00, and 0.08 mg/kg, respectively), and similarly, BC and Fe-BC amendment brought down the (average) NPs content in both old and young leaves (Figure 2A). Overall, BC and Fe-BC amendment decreased NPs accumulation in leaf from 0.90 ± 0.59 mg/kg in the ContNPs group to 0.52 ± 0.36 mg/kg for B1NPs and 0.36 ± 0.45 mg/kg for B2NPs (Figure 2B), indicating both BC and Fe-BC can potentially be employed for alleviating NP uptake by crops. However, we noticed that, after exposure, the root NPs content from lettuce grown in soil with BC dosage (B1NPs) was significantly lower than those from groups of ContNP and B2NP treatments (Figure 3). The NPs content in lettuce roots from the B2NPs group was even closer to that from the ContNP group. We found that during harvesting, there was still a noticeable amount of Fe-BC retained on roots surface even after careful washing, while little to unnoticeable BC was retained on roots surfaces in B1NPs treatments. Therefore, (modified) biochar is effective for immobilizing NPs from being uptaken by plants, yet it is possible that the retained Fe-BC on root surfaces may cause the high root NPs content in lettuce seen from the B2NPs group since Fe-BC was loaded with NPs from irrigation water.

## Impact of NPs Exposure and Biochar Dosage on Plant Vigor

The accumulation of NPs in lettuce has a negative impact on crop health and yield, which is in line with previous finding.[54] NPs significantly decreased the total fresh weight and water content of both leaves and roots, regardless of whether BC or Fe-BC was applied (except for the fresh weight of roots for the controls) (Table 2). By dosing BC into soil, the fresh weight of both shoots and roots increased by 17.1% and 38.5%, respectively, compared to those from ContFW, leading to a significant increase in the total dry mass of leaves. Coincidentally, BC treatment also led to a significant increase in the leaf number (Table 2). Amending soil with Fe-BC increased root fresh weight and water content, but no impact was recorded on the fresh weight of leaves. In addition, Fe-BC application reduced leaf water content. Overall, Fe-BC amendment slightly increased the total dry mass of leaves compared to that of the ContFW group (data not shown here).

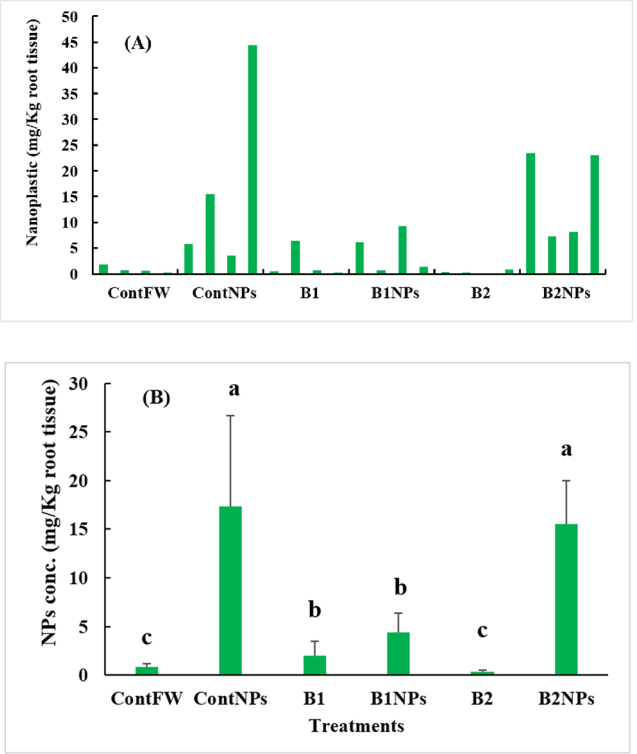


**Figure 3.** Translocation of NPs into lettuce roots: (A) four plants replicate ($n$ = 4); (B) the average of four plants. Treatment means with the same letters are not significant by LSD at the 0.05 level. (Note: values in ContFW do not imply the presence of NPs, but the possible background of Pd in soil).

**Table 2. Recorded Plant Vigor of Lettuces from Different Groups[a]**

| | shoot | | | root | | |
|---|---|---|---|---|---|---|
| treatments | total FW (g) | water Content (%) | no. of leaves | total FW (g) | water content (%) | main secondary roots |
| ContFW | 44.8b | 93.2a | 13.3b | 5.2c | 85.7c | |
| ContNPs | 43.2d | 90.6b | 12.3c | 5.2c | 79.4d | 2.0 |
| B1 | 52.5a | 87.0d | 14.3a | 7.2a | 89.0a | |
| B1NPs | 40.9d | 86.6e | 12.5c | 6.5b | 86.2b | 2.0 |
| B2 | 44.3b | 89.6c | 12.0c | 6.9a | 88.9a | |
| B2NPs | 41.2d | 88.6d | 11.3d | 5.0c | 86.9b | 2.0 |

[a]Note: in each column, values labeled with the same letter are not significantly different from each other by LSD at the 0.05 level, meaning there is no statistically significant variation between those groups.

This different impact of BC and Fe-BC on plant vigor could be caused by the high salinity in soil pore water induced by Fe-BC dosage[55,56] since Fe-BC brought up salinity from 0.40 (with BC) to 1.02 dS/m. However, notably, neither BC nor Fe-BC dosage alleviated the decline of the fresh weight of leaves caused by NPs contamination from irrigation water.

Regarding the impact of NPs on roots, similar to previous findings,[57,58] NPs exposure changed the root structure, i.e., irregular shape, shallow distribution, and lower density of its root hair (Figure S5A). The most significant change noticed is that two horizontal main lateral roots were developed after NPs exposure in all treated groups (Figure S5A). Again,


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neither BC nor Fe-BC dosage can clearly relieve NPs' effect on roots.

## Impact of NPs and Biochar on Plants Nutrition

To reveal the impact of both NPs and biochar on crop nutrition, we quantified inorganic elements/nutrient content in mature lettuce leaves from different groups. The average values of inorganic elements/nutrients content in lettuce leaves from various treatment groups were normalized with data from the control group (Control Soil) and presented in a heatmap (Figure 4) (Text S2 for detailed method information). In the

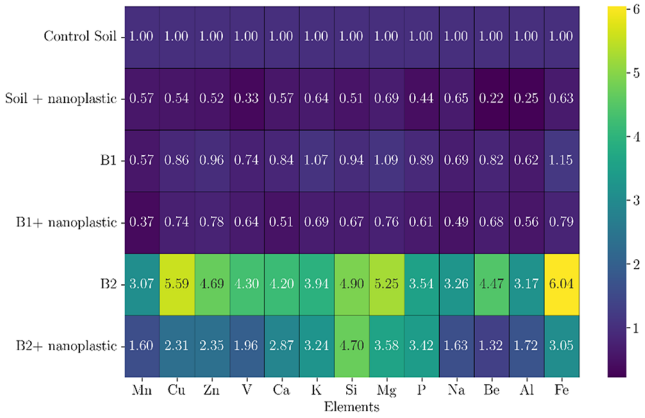


**Figure 4.** Impact of NPs and biochar on inorganic element/nutrient content in lettuce leaf.

heatmap, a blue gradient represents a decrease in nutrient content, while a yellow gradient indicates an increase in nutrient content in leaves. As demonstrated in Figure 4, the long-term exposure to irrigation water containing NPs caused a significant decline (approximately 50%) of inorganic nutrients (e.g., Zn, Ca, K, P, Na, and Fe) in lettuce leaves. While BC alone did not significantly improve the nutrient content (but it did greatly increase the total fresh weight of lettuce, Table 2), Fe-BC significantly increased all the inorganic nutrient content by at least 3 times. Even though NPs exposure caused nutrient content to decline, the levels of nutrients in leaves from lettuces with Fe-BC amendment were much higher than those from the ContFW group. Notably, the calcareous soil we used is nutrient-deficient (such as Zn and maybe Fe, Table S2), and NPs are negatively charged. Therefore, it is possible that interactions between NPs and cationic metals in soil caused the significant decline of inorganic elements/nutrients in lettuce leaves. In addition, the significant root morphology change caused by NPs stress (Figure S5) may contribute to the decline in nutrient uptake efficiency of the roots, which deserves further investigation. In sum, regarding the capability of improving the nutritional value of lettuce, Fe-BC has better performance than BC in both senarios, with or without the presence of NPs.

## Impact of NPs on the Metabolites Content of Lettuce Leaves

Since lettuce is one of the major nutritious vegetables, it is critical to gain comprehensive insight into the impact of NPs on plant metabolism in the presence/absence of biochar. A total of 68 metabolites were identified in lettuce leaves, including 22 amino acids, 3 antioxidants, 6 fatty acids, 12 nucleobase/side/tide, 10 organic acid/phenolics, 8 vitamins, and 8 sugar/sugar alcohol (Figure 5, Table S5). The score plot of the PLS-DA model indicated that BC exhibited the greatest impact on the metabolism of lettuce growth in normal soil, while Fe-BC had a more significant effect on the metabolism of lettuce in soil contaminated with NPs compared to BC (Figure 6). Compared to those lettuces grown in healthy soil, 25

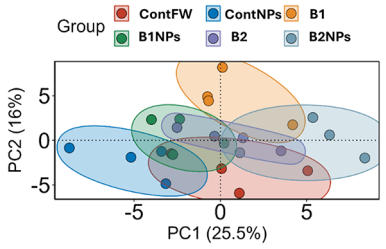


**Figure 6.** PLS-DA score plot of metabolites in lettuce leaves.

metabolites in leaves of the lettuce treated with NPs were significantly decreased, accounting for 36.7% of the total identified metabolites (Figure 7). Specifically, 13 out of 25 downregulated metabolites belonged to the class of amino acids, indicating that NPs inhibit amino acid accumulation in lettuce leaves. Importantly, the addition of Fe-BC and BC to NPs-treated soils reduced the number of metabolites down-regulated by NPs, and increased the levels of some metabolites above those in the untreated control. In comparison to the lettuce exposed to NPs, Fe-BC and BC increased the levels of 19 and 15 metabolites in the lettuce leaves, respectively, with

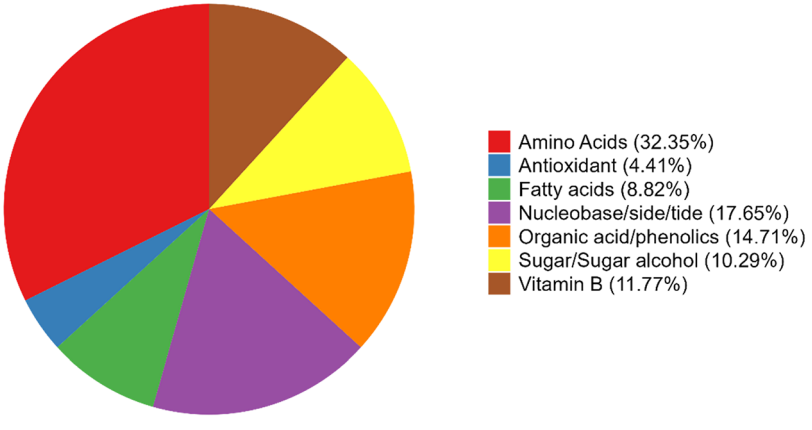


**Figure 5.** Classification of identified metabolites in lettuce from the control group.



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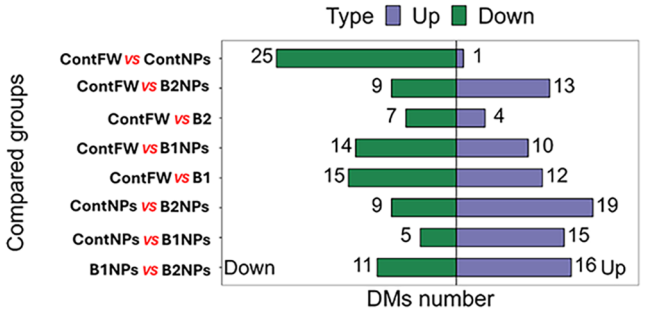


**Figure 7.** Number of differential metabolites in compared groups in X vs Y; up (down) indicates upregulated (downregulated) DMs in group Y.

the majority being amino acids (Figure 7). A total of 60 differential metabolites were extracted from all of the comparison groups and subjected to correlation network analysis. The results showed that ribitol/xylitol, a-tocopherol, glycine, AMP, and leucine had the top five betweenness in network, indicating that BC and NPs mainly changed these five metabolites and caused changes in amino acids and vitamins amino acids, sugar, and vitamin B (Figure 8).

## DISCUSSION

In this study, Pd was introduced into the soil via Pd-NPs, which were synthesized using the same method developed in previous literature.[59] Also, in our recent work,[27] we have conducted a thorough characterization of Pd-NPs, particularly their stability under various conditions (e.g., acidic solutions, soil leachate), and confirmed their great stability as reported.[59] The observed effects were therefore not caused by the leaching of Pd from Pd-NPs. In addition, according to our calculation, each 22.1 g of NPs material contained 150 mg of Pd (i.e., 6.79 mg of Pd/g of NPs). The concentrations of Pd (total Pd inside Pd-NPs) introduced through Pd-NPs were estimated and compared to known phytotoxicity thresholds reported in the literature. Previous studies have shown that ionic Pd ($Pd^{2+}$) can cause growth inhibition and metabolic alterations in plants at concentrations above 1−10 mg $L^{-1}$ in hydroponic systems and 10−50 mg $kg^{-1}$ in soils.[60−62] However, the total Pd levels applied in this study were far lower, 0.0068−0.6787 mg $L^{-1}$, in irrigation water and approximately 0.1358 mg $kg^{-1}$ in soil, representing less than 1% of the lowest reported toxic range. Moreover, Pd accumulation in lettuce tissues in the present study (0.0013−0.0153 mg $kg^{-1}$) is orders of magnitude below tissue/soil Pd levels associated with phytotoxicity reported in the literature, where biomass or growth reductions were generally observed only at much higher exposures (tens to hundreds mg Pd $kg^{-1}$ in growth substrate or elevated mg $L^{-1}$ in solution).[61,63] Besides, as Pd was immobilized within the polystyrene matrix, its bioavailability would be greatly limited compared to soluble ionic forms.[63] Therefore, these data collectively indicate that the effects observed in this study can be confidently attributed to exposure of NPs itself rather than to Pd.

Finally, a NPs concentration of 100 mg $L^{-1}$, equivalent to a total application of 20 mg $kg^{-1}$ soil, was selected in this study to represent a high yet experimentally relevant exposure level. This dose allows us to simulate worst-case contamination scenarios, to better elucidate potential toxic mechanisms under stress conditions, and to better explore the effectiveness of the biochar remediation strategy. Although environmental concentrations are typically lower, several recent studies have employed comparable or even higher NPs doses to investigate dose−response thresholds and mechanistic effects on plants and soil systems. For instance, Giorgetti et al.[64] exposed *Allium cepa* roots to 100−1000 mg $L^{-1}$ of polystyrene NPs, reporting significant cytogenetic and oxidative stress responses. Similarly, Maity et al.[65] observed DNA damage, reactive oxygen species accumulation, and cell death in onion roots exposed to 25−100 mg $L^{-1}$ nanopolystyrene. In soil systems, Beggio et al.[66] applied 400 mg $kg^{-1}$ polystyrene NPs to evaluate their phytoavailability and translocation in tomato plants, while the review by Kögel et al.[67] summarized laboratory experiments employing NPs concentrations up to several hundred mg $kg^{-1}$. Furthermore, Tourinho et al.[68] investigated soil exposures using NPs concentrations ranging from 0.01 to 300,000 mg $kg^{-1}$, which were applied to assess dose−response relationships and potential environmental hazards. Accordingly, the

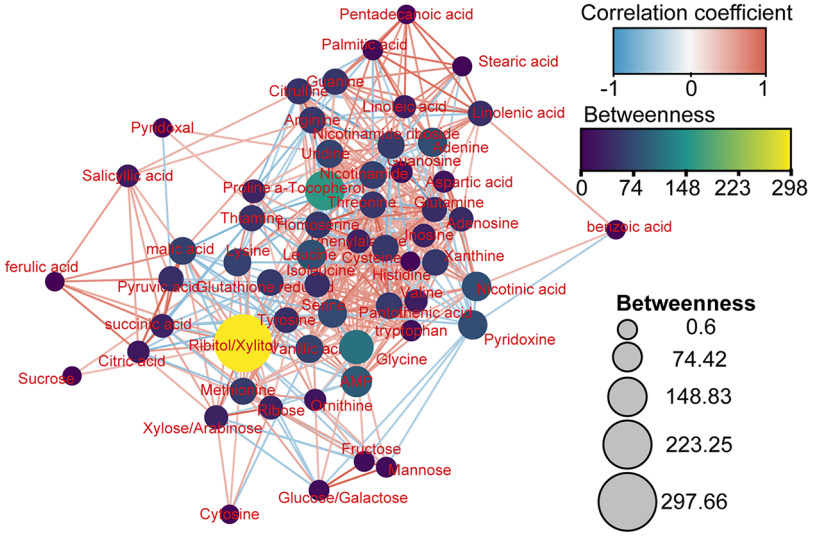


**Figure 8.** Correlation network of all differential metabolites among all compared groups. The color palette and size of nodes represent the difference values of metabolites. The color palette of the edge represents the correlation coefficient between metabolites.



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concentration selected in this study falls within the experimental ranges commonly used in the literature and is suitable for assessing NPs-induced physiological and biochemical responses under controlled conditions. By positioning our applied dose between environmentally observed baselines and the extreme concentrations used in stress assays, we aimed to strike a balance—high enough to ensure measurable uptake and biological response, yet within the realistic spiking levels reported in previous studies. Nevertheless, it is acknowledged that actual soil burdens are generally much lower; thus, this work should be interpreted as a mechanistic stress test of biochar's capacity to mitigate NPs' impacts rather than a direct mimic of field-level exposures.

## Impact of NPs and Biochar on Soil Enzyme Activities

It has been reported that soil enzyme activities can be affected by NPs and biochar, but questions on the impact of their coexistence remain to be answered. In this study, we found that BC increased the NAGase activity by 7.35%. As a key nitrogen-cycling enzyme, NAGase plays a critical role in influencing nitrogen availability in soil.[81] However, NAGase was reduced from 7.4 ± 0.3 μg PNP/g/h in ContFW to 3.3 ± 0.07 μg PNP/g/h in ContNPs, similar to findings by Wang et al.[69] on NP-induced inhibition of nitrogen mineralization. Biochar amendments slightly restored NAGase activity (B1:11.4 ± 0.07 μg PNP/g/h; B2:8.0 ± 0.5 μg PNP/g/h), but B1NPs (3.7 ± 0.1 μg PNP/g/h) and B2NPs (2.7 ± 0.3 μg PNP/g/h) remained low. Arginine Deaminase, which is involved in ammonification, remained stable across treatments but slightly increased with the biochar presence. Protease activity remained suppressed, indicating potential disruptions in nitrogen cycling.

β-Glucosidase, a key enzyme for cellulose breakdown, dropped from 55.1 ± 0.01 μg of PNP/g/h for ContFW to 19.8 ± 0.05 μg of PNP/g/h for ContNPs, confirming the negative effect of NPs on this carbon cycle-associated gene. This aligns with Qian et al.,[70] who reported a 24.9−29.9% reduction of β-glucosidase activity in soils containing degraded plastic films. While pristine BC did not demonstrate any noticeable alleviation on this key enzyme, modified biochar (Fe-BC) addition successfully maintained soil enzyme activity, with B2 (25.07 ± 0.4 μg PNP/g/h) and B2NPs (24.7 ± 0.3 μg PNP/g/h), although both remained below ContFW levels (55.1 ± 0.01 μg PNP/g/h). These reductions in β-glucosidase activity can be associated with impaired cellulose decomposition and a lower level of soil organic matter accumulation.

In terms of cellulase activity, it declined from 82.4 μmol glucose/g/h for ContFW to 52.3 μmol glucose/g/h for ContNPs, while Su et al.[71] reported that the presence of microplastics caused a 35.6% reduction in cellulase activity; in this study, the dosage of NPs did not lead to a significant change in cellulase activity. Dehydrogenase activity (DHA), a microbial respiration indicator, was higher in NP-contaminated soils (36.6 μg TPF/g/h in ContNPs vs 16.1 μg TPF/g/h in ContFW), reflecting microbial activity enhancement, in contrast to the result from Wang et al.,[72] who observed a 14.9−59% decrease in DHA when degraded plastic film is present in soils.

Alkaline phosphatase, essential for phosphorus mineralization, was significantly higher in B1 (58.0 ± 0.07 μg PNP/g/h) and B2 (40.3 ± 0.12 μg PNP/g/h) compared to ContFW (28.4 ± 0.12 μg PNP/g/h). This aligns with the study by Palansooriya et al.,[73] which showed an increase in alkaline phosphatase activity after the addition of biochar. Despite nanoplastic exposure, B1NPs (39.8 ± 0.2 μg PNP/g/h) and B2NPs (45.4 ± 0.05 μg PNP/g/h) still maintained relatively high activity compared to ContNPs (29.3 ± 0.08 μg PNP/g/h).[74] This suggests that biochar helped maintain phosphorus availability in nanoplastic-contaminated soil.[73] However, in Su et al.[71] and Qian et al.,[70] phosphatase activity was slightly inhibited in NP-polluted soils. Phenol oxidase activity was largely unaffected, suggesting that its role in degrading complex organic compounds may be less sensitive to nanoplastic contamination than hydrolytic enzymes.

In summary, NPs exert stress on soil microorganisms, broadly affecting nutrient cycling processes (C, N, P) mediated by microbial communities. The addition of BC and Fe-BC demonstrates promising potential to alleviate some of these stresses, aiding the sustainment of soil functionality.

## NPs Uptake by Lettuce in the Absence/Presence of Biochar

Our results confirmed that NPs can penetrate through root surfaces, enter plants' vascular system, and translocate to leaves. This is in good agreement with our previous findings. After 1-month NPs exposure, approximately 0.0061%, 0.0034%, and 0.0025% of total NPs dosed along with irrigation water ended up in lettuce leaves grown from soil alone, soil with BC, and soil with Fe-BC, respectively. While the percentage remains low, given the small size of the NPs, the plastic particle number concentration in these different groups of lettuces can be as high as $2.94 \times 10^{10}$, $1.65 \times 10^{10}$, and $1.10 \times 10^{10}$ per gram of dried leaf, respectively. Notably, old leaves accumulated more NPs than young leaves (Figure 9), which

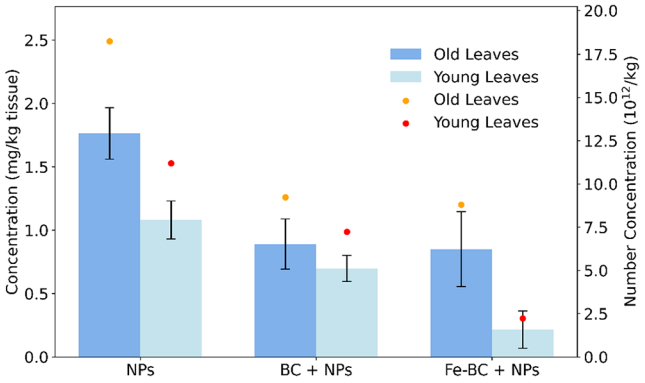


**Figure 9.** Accumulation of NPs in old and young (the rest leaves except the old ones) leaves.

may be due to a longer exposure time and faster transpiration induced by the larger surface area. It is also possible that plants can move the toxin, NPs in this study, from young tissue to old tissue (less productive tissue that will fall off) through the phloem.[74] BC and Fe-BC amendment in soil can alleviate NPs accumulation in leaves, possibly through immobilizing them onto biochar's inner surfaces of the porous structures. However, different types (compositions) of biochar can interact differently with plant and soil microbial communities due to the nutrients that they carry. Overall, seeing from these results, it is suggested that eating young leaves can reduce our possible exposure to NPs in polluted lettuce (Figure 9).[75,76]

In fact, BC and Fe-BC can intercept NPs, such as polystyrene NPs, in the rhizosphere through a combination of adsorption, aggregation, and microenvironmental modifica-



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tions. BC provides a porous structure and hydrophobic surface that facilitate the adsorption of hydrophobic NPs via van der Waals forces and $\pi-\pi$ interactions.[77] Fe-biochar further enhances this capacity by introducing iron oxides or hydroxides onto the surface, creating positively charged sites that attract negatively charged or neutral NPs through electrostatic interactions.[52] Fe-BC outperformed BC due to its higher surface area and porosity, as well as the presence of Fe oxides that enhance electrostatic and magnetic interactions with nanoplastics, promoting their aggregation and immobilization and thereby reducing plant uptake.[78] In calcareous soils, such as those used in this study, although high pH and ionic strength may reduce some electrostatic interactions, Fe-biochar can still promote NP aggregation and immobilization, especially through heteroaggregation with biochar colloids.[79] Additionally, biochar alters the rhizosphere microenvironment by modifying pH, increasing nutrient availability, and enhancing microbial activity, which in turn influences root exudation profiles.[80] These changes can reduce the release of certain exudates that otherwise stabilize NPs,[81] thereby indirectly reducing NP migration toward roots. Enhanced microbial biofilms promoted by biochar may also physically trap NPs or produce extracellular polymeric substances that further bind NPs within the rhizosphere.[82] Overall, biochar and Fe-biochar act both directly—via sorption and aggregation, and indirectly, by modifying root exudates and microbial interactions, to intercept and reduce the mobility of NPs in the rhizosphere.

Despite the clear mitigation effects observed, this study has several limitations that should be acknowledged. First, the experiment was conducted as a short-term pot study under controlled conditions, which may not fully capture the complexity of the field environments. Soil heterogeneity, climatic variability, microbial dynamics, and long-term aging of biochar can substantially influence biochar performance and contaminant behavior in real agricultural systems. Therefore, results from pot experiments may not be directly extrapolated to field-scale or long-term conditions, highlighting the need for extended field trials.[80,83] Second, although Fe-modified biochar exhibited enhanced effectiveness compared to pristine biochar, the potential leaching or release of Fe from Fe-BC was not directly assessed in this study. Previous studies have shown that metal-modified biochars may release metals under certain soil conditions, particularly at low pH, which could affect the soil chemistry and plant metal uptake. Long-term assessments are therefore necessary to evaluate the stability, mobility, and ecological safety of Fe-BC in soil−plant systems.[84,85] Overall, while the present findings demonstrate the potential of Fe-BC to reduce nanoplastic uptake, long-term and field-based investigations are required to confirm its effectiveness and environmental safety under realistic agricultural conditions.

## Impact of NPs on Water and Nutrient Uptake by Lettuce

Accumulated NPs can cause toxicity and harmful impact to plants through inhibiting water and nutrients uptake[86−88] and damaging cell membranes, thus reducing crop yield.[89] As shown in Table 1, NPs significantly reduced the water content in both roots and leaves, and the BC/Fe-BC dosage did not resolve this issue. We hypothesized that this change is due to the change of water availability in soil and the blockage of root surfaces by NPs. According to a previous study, negatively charged NPs along with positively charged minerals in soil can block the pores in soil,[90] preventing the deep permeation of water and leading to a significant change of water flow path in soil. This can be supported by the appearance of horizontal lateral roots after NPs exposure, which is for water uptake.[91,92] Once NPs are adsorbed onto roots surfaces, they can block the surface pores, which can further deteriorate water (and nutrient) uptake.[93,94] Since after NPs exposure we also observed a clear decline of root hair density, it is very likely that NPs can block the roots surface, especially the surface of root hairs, for water uptake. Under these stresses caused by NPs' appearance in soil, instead of using dense root hairs to adsorb water, lettuce developed shallow lateral roots to overcome the negative impact. Moreover, the high content of NPs in irrigation water/soil can also be toxic to roots, leading to genotoxicity, oxidative damage, and biomass reduction.[86,95] This could further affect water and nutrient uptake by the roots. In addition, NPs can interact with coexisting pollutants, such as heavy metals, arsenic, and emerging contaminants, imposing a high risk on food safety.[96] For instance, Dong et al.[89] reported that MPs increase the toxicity of arsenic to rice. Therefore, how NPs interact with coexisting pollutants in soil-crop systems and how biochar can be optimized for effectively immobilizing a wide range of pollutants deserve further investigation.

## Impact of NPs on Lettuce Metabolism

Similar to previous findings,[89,97−99] it is found that the NPs negatively affect the plant's metabolic system and cause critical interruption. For instance, in spinach, pathways related to glycine, serine, and threonine metabolism were also disrupted under NPs treatment, and aged NPs induced a more extensive downregulation of glycine metabolism.[100] However, it is very likely that BC and Fe-BC have the capacity to alleviate harmful impacts (Figure 10). According to Zhang et al.,[88] and Wu et al.,[101] it is clear that NPs exposure can significantly change gene expression patterns (such as metal ion transport gene expression),[102] the metabolism pathways of carbon, mitogen activated protein kinase signaling pathways (responding to abiotic stress), biosynthesis of amino acids, fatty acids, sugars, plant hormone signal transduction in crops (such as wheat, cucumber, and corn). Lian et al.[102] observed a similar phenomenon, reporting that NPs significantly disrupted the biosynthesis of amino acids and the metabolism of glycine,

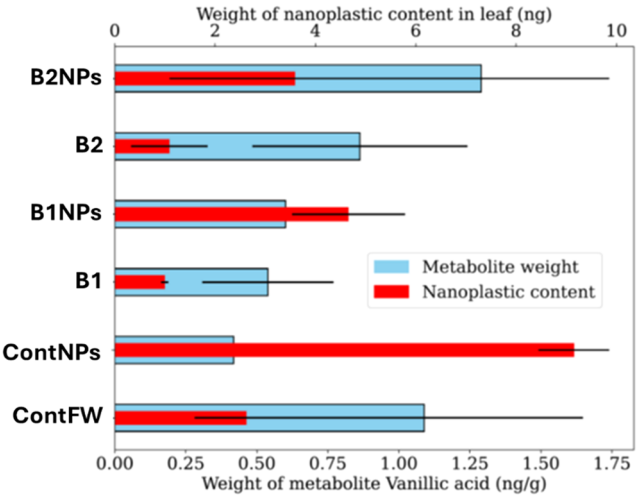


**Figure 10.** Antioxidants, vanillic acid, content in lettuce leaves among different treatments.



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serine, and threonine in wheat leaves. Gene ontology analysis revealed downregulation of terms related to amino acid metabolism and biosynthesis, indicating an inhibitory effect of NPs on the synthesis of various amino acid groups, including serine family amino acids, glutamine family amino acids, aspartate family amino acids, sulfur-containing amino acids, cysteine, lysine, and histidine. Additionally, Wu et al.[99] found that MPs exposure disrupted the saturated and unsaturated fatty acid contents in rice grains, which was attributed to the significantly downregulated expression of genes encoding enzymes crucial for fatty acid metabolism. Furthermore, studies have identified a reduction in uridine and thymine, key catabolic products of nucleobases, in plant leaves, suggesting disturbances in pyrimidine metabolism, one of the primary metabolic pathways.[103] These findings collectively highlight the profound metabolic disruptions caused by MPs and NPs in plant systems.

Li et al.[104] reported that the NPs have an adverse impact on sugar metabolism due to the impact of the derived benzene ring formation from the polystyrene degradation in the leaves. Zhou et al.[105] revealed that exposure to NPs can cause overproduction of reactive oxygen species such as hydrogen peroxide, hydroxyl radical, superoxide anion, and singlet oxygen, which leads to irreversible damage of plant cells. It is observed that the vanillic acid content on lettuce leaves increased (Figure 10). When plants are exposed to abiotic stress, various phytohormones can be impacted, which further regulate the activity of antioxidant enzymes.[106−108]

The degree of linear association between metabolites was assessed in this study using the simple correlation coefficient ($r$) (Table S5). Correlation analysis evaluates the strength of the relationship between selected variables; when the correlation coefficient approaches +1 or −1, the linear relationship between the two variables becomes nearly perfect.[109] In lettuce leaves, highly significant correlations were observed between several metabolite pairs, including arginine and citrulline, homoserine and cysteine, threonine and cysteine, homoserine and threonine, inosine and adenosine, glucose/galactose and fructose, mannose and fructose, and glucose/galactose and mannose. The r values for these pairs were close to +1 (greater than 0.996; Table S5), indicating that these metabolites exhibited very similar responses under varying environmental conditions. These findings are consistent with those of Gao et al.[110] and Kusano et al.,[111] who reported strong positive correlations among sugars and amino acids in lettuce leaves under environmental stress conditions.

## CONCLUSIONS

The study confirmed that NPs accumulate in lettuce, especially in older leaves, and negatively impact plant health by disrupting metabolic processes, such as decreasing amino acid levels and reducing the uptake of essential inorganic nutrients (e.g., Zn, Ca, K, P, Na, and Fe). Introducing both pristine biochar (BC) and iron-doped biochar (Fe-BC) to the soil was shown to be an effective mitigation strategy, with Fe-BC demonstrating superior performance by achieving a 60% reduction in NPs accumulation in lettuce leaves compared to the NPs-only control group. This reduction is attributed to biochars' ability to immobilize NPs through sorption, aggregation, and modification of the rhizosphere environment. Furthermore, the biochar amendments partially alleviated NPs-induced metabolic disturbances, helped to restore essential nutrient levels (significantly so with Fe-BC), and maintained soil functionality by mitigating the adverse effects on key soil enzyme activities (e.g., $\beta$-glucosidase and alkaline phosphatase). Overall, the iron-doped biochar presents a promising and effective approach for safeguarding crop quality and human health in agricultural systems facing NPs contamination.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jafc.5c14785.

Soil sampling location, XRD spectra, EDS, and SEM−EDS analyses of biochar and iron-modified biochar, and visual effects of nanoplastics exposure on lettuce roots (Figures S1−S5); physicochemical properties of the soil, soil macro- and micronutrient concentrations, chemical characteristics of biochar materials, and detailed treatment and plant sampling identifier (Tables S1−S4); statistical analysis using Fisher's least significant difference test for metabolite comparison (Text S1) and normalization approach used to evaluate nanoplastics effects on plant nutrient uptake (Text S2) (PDF)

Correlation coefficient matrix of metabolites in lettuce leaves (Table S5) (XLSX)

## AUTHOR INFORMATION


### Corresponding Authors

**Anwar A. Aly** − *Soil and Water Science Department, Faculty of Agriculture, Alexandria University, Alexandria 21545, Egypt*; orcid.org/0000-0003-2427-1115; Email: aaaly@alexu.edu.eg

**Yiming Su** − *Utah Water Research Laboratory, Utah State University, Logan, Utah 84322, United States; Department of Biological Engineering, Utah State University, Logan, Utah 84322, United States*; orcid.org/0000-0001-6035-7384; Email: yiming.su@usu.edu

### Authors

**Junjie Tang** − *Utah Water Research Laboratory, Utah State University, Logan, Utah 84322, United States; Department of Civil and Environmental Engineering, Utah State University, Logan, Utah 84322, United States*; orcid.org/0000-0001-6587-1761

**Mahmoud Zanaty** − *Material Science and Nanotechnology Department, Faculty of Postgraduate Studies for Advanced Sciences (PSAS), Beni-Suef University, Beni-Suef 62511, Egypt*

**Chenwei Liu** − *Utah Water Research Laboratory, Utah State University, Logan, Utah 84322, United States; Department of Civil and Environmental Engineering, Utah State University, Logan, Utah 84322, United States*

**Arjun S. Kulathuvayal** − *School of Aerospace and Mechanical Engineering, University of Oklahoma, Norman, Oklahoma 73019, United States*

**Yanqing Su** − *School of Aerospace and Mechanical Engineering, University of Oklahoma, Norman, Oklahoma 73019, United States*; orcid.org/0000-0003-0790-5905

**Ibukunoluwa Salako** − *Civil and Environmental Engineering, University of Utah, Salt Lake City, Utah 84112, United States*

**Jennifer Weidhaas** − *Civil and Environmental Engineering, University of Utah, Salt Lake City, Utah 84112, United States*

**Zeyu Cai** − *Guangdong Basic Research Center of Excellence for Ecological Security and Green Development, Key Laboratory for City Cluster Environmental Safety and Green Development of the Ministry of Education, School of Ecology, Environment and Resources, Guangdong University of Technology, Guangzhou 510006, China*

**Chuanxin Ma** − *Guangdong Basic Research Center of Excellence for Ecological Security and Green Development, Key Laboratory for City Cluster Environmental Safety and Green Development of the Ministry of Education, School of Ecology, Environment and Resources, Guangdong University of Technology, Guangzhou 510006, China*

**Joan E. McLean** − *Utah Water Research Laboratory, Utah State University, Logan, Utah 84322, United States; Department of Civil and Environmental Engineering, Utah State University, Logan, Utah 84322, United States*

**Arturo Keller** − *Bren School of Environmental Science and Management, University of California, Santa Barbara, California 93106, United States;* orcid.org/0000-0002-7638-662X

**Baoshan Xing** − *Stockbridge School of Agriculture, University of Massachusetts, Amherst, Massachusetts 01003, United States;* orcid.org/0000-0003-2028-1295

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jafc.5c14785

## Author Contributions

⋈A.A.A., J.T. and M.Z. contributed equally to this work. A.A.A. and J.T.: Methodology, investigation, data analysis, writing—original draft; A.A.A., M.Z. and C.L.: methodology, investigation, data analysis; A.S.K. and Y.S.: data analysis, writing—original draft; I.S. and J.W.: methodology, data analysis, writing—original draft; Z.C. and C.M.: data analysis, writing—original draft; A.K.: conceptualization, methodology; B.X. and J.M.: writing—review and editing, supervision, methodology, conceptualization; Y.S. and A.A.A.: conceptualization, writing—original draft review and editing, supervision, project administration; Y.S.: funding acquisition.

## Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

NPs:nanoplastics; Pd-NPs:palladium-doped polystyrene nanoplastics; ContFW:soil irrigated with fresh water; ContNPs:soil irrigated with water containing 100 μg/mL NPs; B1:soil with 3% sawdust biochar and irrigated with fresh water; B2:soil with 3% iron-doped sawdust biochar and irrigated with fresh water; B1NPs:soil with 3% sawdust biochar and irrigated with water containing 100 μg/mL NPs; B2NPs:soil with 3% iron-doped sawdust biochar and irrigated with water containing 100 μg/mL NPs; FC:field capacity

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