Supplementary Information

The Role of Nano-Biochar Reduce the Impact of Phenanthrene on Wheat Photosynthesis

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Section 1: The Characteristics of nBCs.

In this study, the biochar was primarily derived from rice and corn straws were selected and pyrolyzed at 350 °C, 500 °C, and 650 °C. Subsequently, the biochar materials underwent nanosizing treatment, followed by ethanol washing and other methods to remove potential polycyclic aromatic hydrocarbons (PAHs) residues from the biochar surface. Finally, the nanosized biochar was ground using a ball mill to obtain nano-scale biochar materials. Therefore, this section mainly focuses on characterizing these six types of nBCs and evaluating their adsorption performance for phenanthrene over time, ultimately selecting the optimal nBC material for further experimental research.

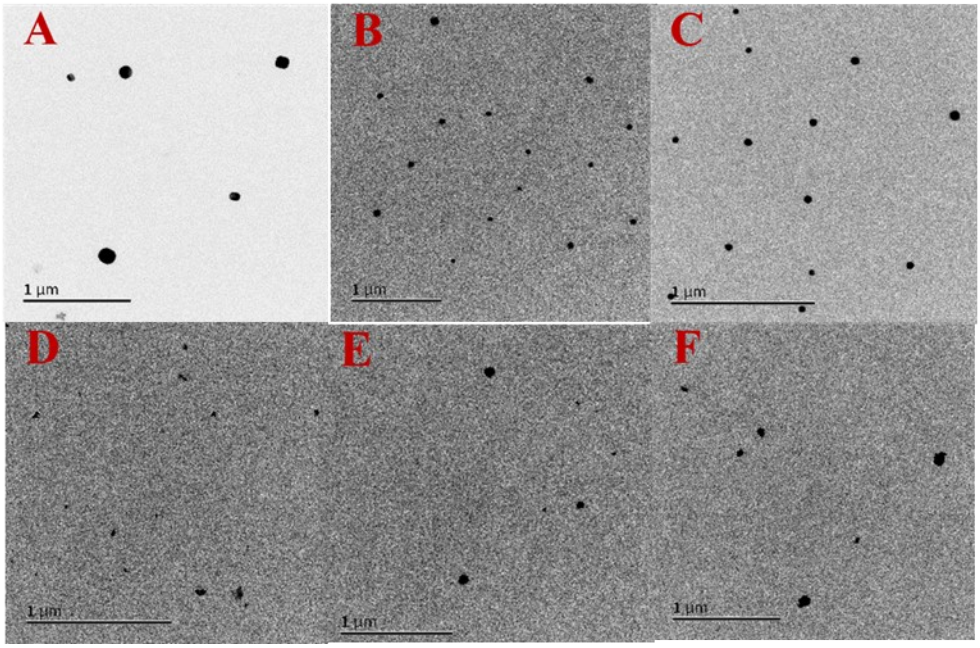

Figure S1. TEM images of six types of nBC.
(Note, the nBC prepared from corn straw at 350°C, 500°C, and 650°C (A - C), the nBC prepared from rice straw at 350°C, 500°C, and 650°C (D, E, F), respectively; nBC, nano-biochar.)

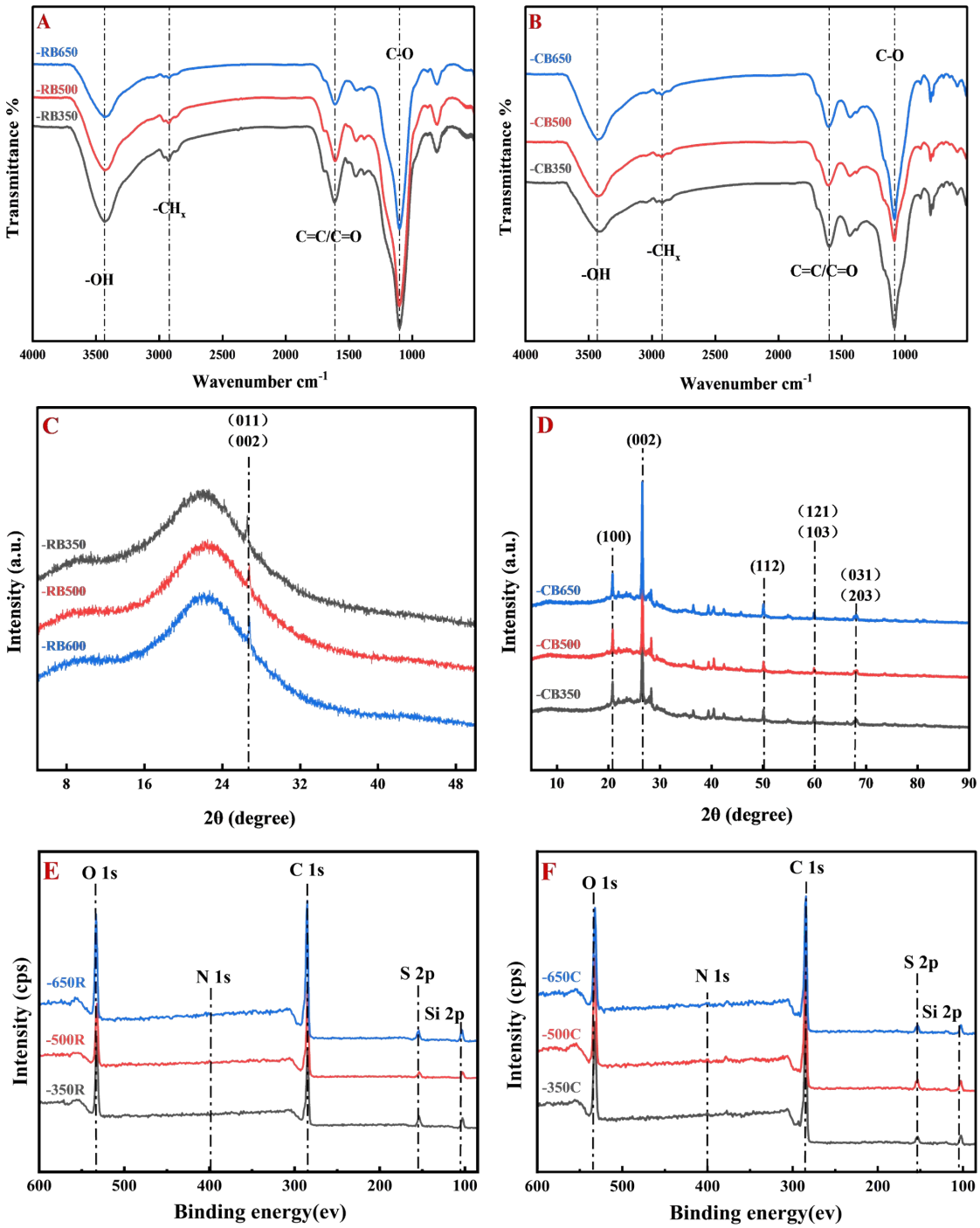

Figure S2. (A, B) FTIR, (C, D) XRD spectra, and (E, F) XPS whole pattern of nBC. (Note, RB650, RB500, RB350 represent the nBC prepared from rice straw at 650 °C, 500 °C and 350 °C, and CB650, CB500, CB350 represent nBC prepared from corn stover at 650 °C, 500 °C and 350 °C, respectively; nBC, nano-biochar.)

Table S1. Biochar nanoparticles colloidal stability

Label	Description	Hydrated Particle Size (nm)	Polydispersity Index (PdI)	Zeta Potential (mV)
Nano rice biochar - 350°C treated	RSB 350	853.2 ± 12.4	0.38 ± 0.04	-43.8 ± 4.3
Nano rice biochar - 500°C treated	RSB 500	740.0 ± 12.2	0.34 ± 0.03	-47.5 ± 2.5
Nano rice biochar - 650°C treated	RSB 650	715.0 ± 14.2	0.31 ± 0.03	-52.5 ± 5.4
Nano corn biochar - 350°C treated	CSB 350	959.7 ± 9.2	0.41 ± 0.05	-38.5 ± 4.2
Nano corn biochar - 500°C treated	CSB 500	850.0 ± 13.2	0.36 ± 0.04	-42.5 ± 3.5
Nano corn biochar - 650°C treated	CSB 650	825.0 ± 15.8	0.33 ± 0.03	-47.5 ± 5.5

Section 2: Adsorption Kinetics of PHE on nBCs.

Investigation of the time-dependent adsorption performance of nBCs for PHE. Six nano-biochar materials, prepared as described above, were individually added at a concentration of 1.0 mg L^{-1} to a solution containing 1.0 mg L^{-1} phenanthrene. Adsorption experiments were conducted at 25 °C and 200 rpm. During the adsorption process, samples were taken at 0.25, 1, 2, 4, 8, 12, 24, 48, and 96 h, centrifuged at 4000 rpm for 10 minutes, and the supernatant was collected to determine the residual PHE concentration. By comparing the adsorption rates of phenanthrene at checkpoints, the nBCs with the optimal adsorption performance for phenanthrene was selected. Each treatment was performed in triplicate times.

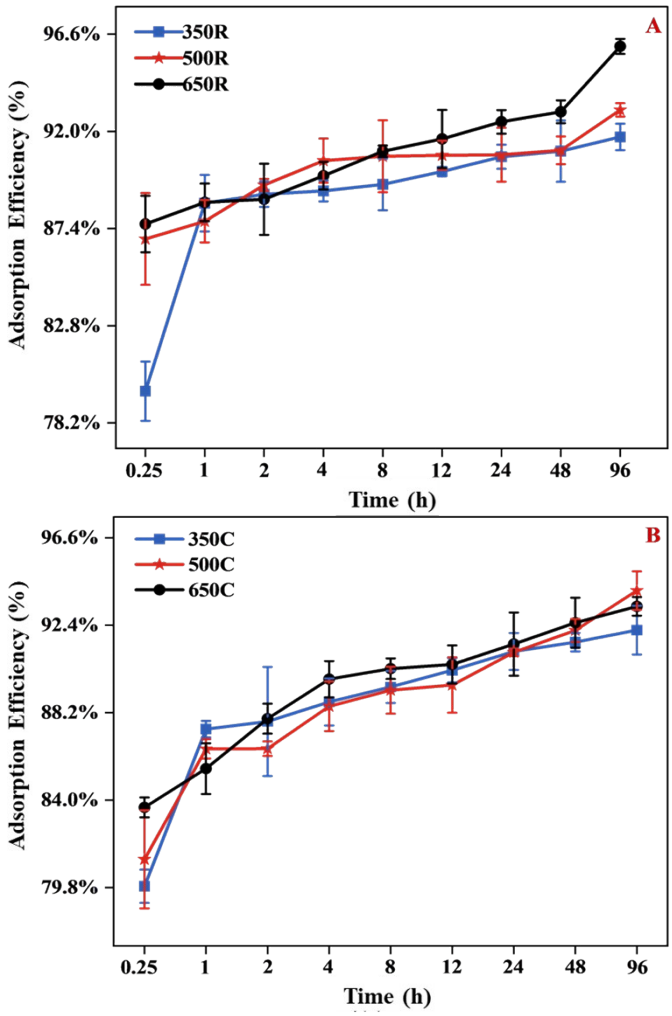

Figure S3. Adsorption kinetic of PHE by six types of nBC.

(Note, 350R, 500R, and 650R represent nBC made from rice straw fired at 350 °C, 500 °C, and 650 °C, and 350C, 500C, and 650C represent nBC made from corn stover fired at 350 °C, 500 °C, and 650 °C, respectively; nBC, nano-biochar; PHE, phenanthrene.)

Section 3: The Growth and SPAD of the Wheat Seedings under PHE Treatments.

The biomass data corroborated our visual observations and provided quantitative evidence of the effects of PHE stress and nBC treatment on wheat seedling growth after 30 days (Table S2).

Table S2. Fresh and dry weights of per wheat seedlings under different treatments at day 9.

Treatment	Shoot Fresh Weight	Root Fresh Weight	Shoot Dry Weight	Root Dry Weight
Control	3.52 ± 0.15 a	1.28 ± 0.08 a	0.42 ± 0.03 a	0.15 ± 0.01 a
nBC	3.61 ± 0.18 a	1.33 ± 0.09 a	0.44 ± 0.03 a	0.16 ± 0.01 a
PHE	2.48 ± 0.22 c	0.89 ± 0.07 c	0.29 ± 0.02 c	0.10 ± 0.01 c
PHE + 0.5 nBC	2.89 ± 0.19 b	1.05 ± 0.08 b	0.34 ± 0.02 b	0.12 ± 0.01 b
PHE + 1.0 nBC	3.18 ± 0.17 ab	1.16 ± 0.09 ab	0.38 ± 0.03 ab	0.14 ± 0.01 ab

(Note, Data are means ± SD (n = 3); Different letters indicate significant differences among treatments ($p < 0.05$))

A Chlorosis Severity Index (CSI) was calculated based on the affected area percentage and reduction in green intensity. The CSI values confirmed the visual observations.

Chlorosis Severity Index (CSI) Calculation is followed the equation below,

$$CSI = (Ac/At) \times (1 - GIc/GIh) \times 100$$

where,

Ac, Area showing chlorosis (pixels); At, Total leaf area (pixels); GIc, Green intensity of chlorotic area; and GIh, Green intensity of healthy tissue.

And the result was shown below,

Table S3. Chlorosis Severity Index (CSI) of wheat seedlings under different treatments

Treatment	Day 7	Day 9
Control	2.1±0.3 a	2.3±0.4 a
nBC	2.3±0.4 a	2.4±0.3 a
PHE	45.6±3.2 d	68.7±4.1 d
PHE+0.5nBC	31.2±2.8 c	42.3±3.5 c
PHE+1.0nBC	18.4±2.1 b	25.6±2.8 b

Note, values represent mean ± SD (n=3). Different superscript letters indicate significant differences between treatments ($p < 0.05$).

Relative chlorophyll content (SPAD) is one of the most important biochemical parameters affecting crop growth, and it is also an indicator of the photosynthetic capacity of plants as well as an important indicator for evaluating crop growth. The portable handheld multifunctional plant photosynthesis meter Photosynq MultiseQ v2.0 was utilized to determine the SPAD values of wheat seedling leaves.

The formulas developed by Wood et al. (1993) to calculate chlorophyll content from SPAD values as below,

$$(1)\ C_{Chl\,a} = 0.05 \times SPAD - 0.27 \qquad (1)$$

$$(2)\ C_{Chl\,b} = 0.013 \times SPAD + 0.04 \qquad (2)$$

$$(3)\ C_{total\ Chl} = 0.062 \times SPAD - 0.24 \qquad (3)$$

Among them:

C_{Chl} is the chlorophyll content (in mg g^{-1}); and SPAD is the measured SPAD value.

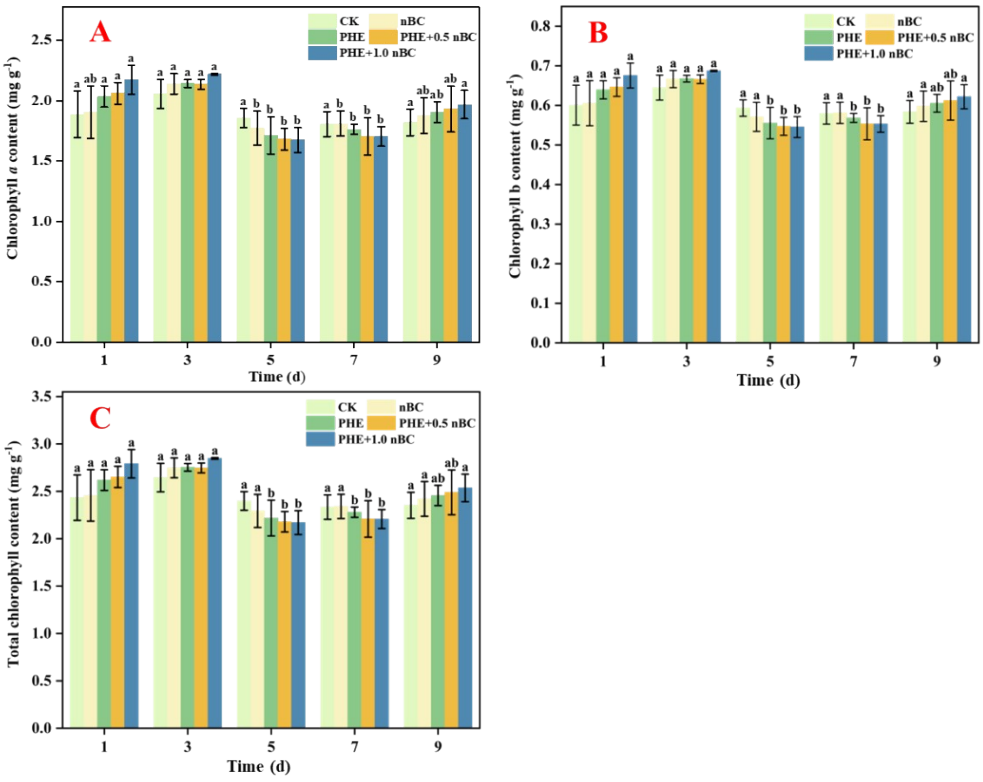

Figure S4. Changes of SPAD calculated chlorophyll *a* (A), chlorophyll *b* (B), and total chlorophyll (C) in wheat leaves.

(Note, CK, control; nBC, 1.0 mg L^{-1} nBC; PHE, 1.0 mg L^{-1} PHE; PHE+0.5 nBC, 1.0 mg L^{-1} PHE + 0.5 mg L^{-1} nBC; PHE+1.0 nBC, 1.0 mg L^{-1} PHE + 1.0 mg L^{-1} nBC; different letters

indicate significant differences ($p < 0.05$).)

Table S4. The Paired Sample T-test of measured chlorophyll content and chlorophyll content obtained from SPAD values.

Paired Sample T-test	Day	T-statistic	P-value	Conclusion
Chlorophyll *a*	Day 1vs Day1.1	0.244466	0.810416	Reject the null hypothesis (No statistically significant difference between each two sets of measurements)
	Day 3 vs Day3.1	0.079482	0.937774	
	Day 5 vs Day5.1	-0.096010	0.924873	
	Day 7 vs Day7.1	-0.074877	0.941371	
	Day 9 vs Day9.1	-0.026344	0.979354	
Chlorophyll *b*	Day 1vs Day1.1	-0.372192	0.715322	Reject the null hypothesis (No statistically significant difference between each two sets of measurements)
	Day 3 vs Day3.1	2.364263	0.005631	
	Day 5 vs Day5.1	-1.611507	0.129377	
	Day 7 vs Day7.1	-0.713350	0.487351	
	Day 9 vs Day9.1	-1.028433	0.321187	
Total chlorophyll	Day 1vs Day1.1	0.054193	0.957546	Reject the null hypothesis (No statistically significant difference between each two sets of measurements)
	Day 3 vs Day3.1	-1.912142	0.076541	
	Day 5 vs Day5.1	0.339714	0.739114	
	Day 7 vs Day7.1	-0.0759054	0.946041	
	Day 9 vs Day9.1	-1.177454	0.067044	

Section S4. Data Analysis

(1) Data Normality, ANOVA, and Independence Check.

Table S5 Data Tests.

Data	Test	Statistic	p-value	Conclusion
Chlorophyll a	Shapiro-Wilk (Normality)	0.968436	0.057911	Reject null hypothesis (Data is normally distributed)
	Welch's ANOVA	4.315984	0.006481	Reject null hypothesis (Data is normally distributed)
	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)
Chlorophyll b	Shapiro-Wilk (Normality)	0.988821	0.756031	Reject null hypothesis (Significant difference between groups)
	Welch's ANOVA	8.581324	0.000065	Reject null hypothesis (Data is normally distributed)
	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)
Total chlorophyll	Shapiro-Wilk (Normality)	0.982779	0.401402	Reject null hypothesis (Significant difference between groups)
	Welch's ANOVA	6.865857	0.000363	Reject null hypothesis (Data is normally distributed)
	Chi-square test of independence	10.0	1.0	Reject the null hypothesis (Two variables are independent)
PHE concentration of root	Shapiro-Wilk (Normality)	0.943325	0.135477	Reject null hypothesis (Data is normally distributed)
	Welch's ANOVA	5.895114	0.000024	Reject null hypothesis (Significant difference between groups)
	Chi-square test of independence	8.0	1.0	Reject the null hypothesis (Two variables are independent)
PHE concentration of shoot	Shapiro-Wilk Test (Normality)	0.877011	0.000196	Reject null hypothesis (Data is normally distributed)
	Levene's Test (Variance Homogeneity)	2.10211	0.158206	Reject null hypothesis (Levene's Test hypothesis is valid)
	Chi-square test of independence	8.0	1.0	Reject the null hypothesis (Two variables are independent)
Fv/Fm	Shapiro-Wilk Test (Normality)	0.976021	0.165389	Reject null hypothesis (Data is normally distributed)
	Levene's Test (Variance Homogeneity)	0.006925	0.934269	Reject null hypothesis (Levene's Test hypothesis is valid)

	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)
Φ_{PSII}	Shapiro-Wilk Test (Normality)	0.972161	0.097076	Reject null hypothesis (Data is normally distributed)
	Levene's Test (Variance Homogeneity)	0.139275	0.711814	Reject null hypothesis (Levene's Test hypothesis is valid)
	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)
qP	Shapiro-Wilk Test (Normality)	0.947907	0.058341	Reject null hypothesis (Data is normally distributed)
	Levene's Test (Variance Homogeneity)	0.090141	0.766216	Reject null hypothesis (Levene's Test hypothesis is valid)
	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)
NPQ	Shapiro-Wilk Test (Normality)	0.939119	0.532072	Reject null hypothesis (Data is normally distributed)
	Levene's Test (Variance Homogeneity)	1.247622	0.273501	Reject null hypothesis (Levene's Test hypothesis is valid)
	Chi-square test of independence	16.0	1.0	Reject the null hypothesis (Two variables are independent)