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Oligotrophy biochar stimulates the generation of salicylic acid from soybean roots by increasing nutrient and oxidative stress

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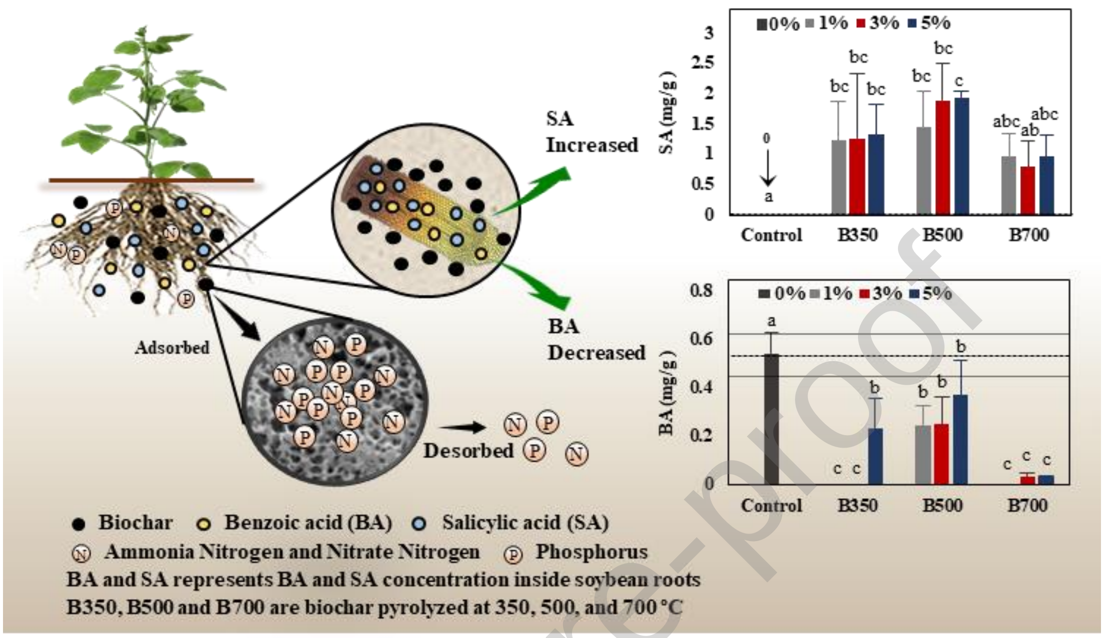
Abstract: The accumulation of allelochemicals in farming land has attracted a great deal of research attention, and biochar has shown positive effects in alleviating allelopathy. This study investigated how oligotrophic biochar application modulated salicylic acid (SA) generation in soybean roots through nutrient and oxidative stress pathways. Biochars were applied to soybean cultivation, with analyses conducted on nutrient adsorption, allelochemical profiles, and plant growth parameters. Results revealed that biochar suppressed benzoic acid (BA) while elevating SA levels, which correlated with the presence of persistent free radicals (PFRs) and nutrient retention. The retention of phosphorus (P) and ammonium (NH_4^+-N) dominated plant height reduction, surpassing oxidative stress effects linked to PFRs. Multivariate linear regression (MLR) identified P retention as the primary driver of SA generation, linked to adaptive phosphorus solubilization via acid secretion. Conversely, malondialdehyde (MDA) accumulation resulted from lipoxygenase-mediated lipid peroxidation under nutrient stress and PFRs-induced oxidative stress. The strong adsorption of P and nitrate (NO_3^--N) by biochar exacerbated soil oligotrophy, triggering SA overproduction as a stress compensation mechanism. The significant correlation between SA and MDA indicated bidirectional stress signaling, wherein allelochemicals exacerbate oxidative damage while activating defense responses. These findings emphasize the dual role of biochar as both a stress inducer and an allelopathy modulator, highlighting the necessity for optimizing pyrolysis and developing soil-specific strategies to balance agricultural benefits with ecological risks.

Graphical abstract



Keywords: allelochemicals, adsorption, retention, persistent free radicals

1. Introduction

The global population is steadily increasing, creating an urgent demand for sustainable and reliable crop yields. While intensive farming practices may alleviate pressures on crop yields, they often lead to the accumulation of allelochemicals — secondary metabolites secreted by plant roots into the soil (Scavo et al., 2019). These compounds contribute to cropping challenges and exacerbate soil degradation (Zhou et al., 2017). Allelochemicals are produced by plants in response to various environmental stresses, including imbalances in microbial communities, nutrient deficiencies, pests, pollutants, and abiotic factors such as heat, cold, and drought (Kirwa et al., 2018). Their accumulation can disrupt soil microbial ecosystems, impair nutrient cycling, reduce seed germination rates, and ultimately diminish crop yields and soil fertility (Weir et al., 2004). Therefore, understanding and regulating the generation of allelochemicals is crucial for overcoming the persistent challenges associated with cropping obstacles.

The generation of allelochemicals is highly related to the environmental conditions in which plants grow (Ma et al., 2022). For instance, under stress from drought, heat, or cold, plants release benzoic acid (BA) to enhance their stress tolerance (Senaratna et al., 2003). BA also exhibits antifungal properties, inhibiting the growth of phytopathogenic fungi upon direct contact (Amin et al., 2021). Similarly, salicylic acid (SA), another allelochemical, can enhance phosphorus (P) availability in soil through acid solubilization (Li et al., 2020). However, the accumulation of BA and SA in soil can lead to allelopathic effects. BA has been shown to reduce the antioxidant capacity of plant cells (Wang et al., 2016), resulting in stunted growth or even seedling mortality (He et al., 2019). Likewise, SA can inhibit the electron transport efficiency of photosystem II (PSII) at the acceptor side (Gao et al., 2018) and reduce seed yield (Abreu and Munne-Bosch, 2009). These findings underscore the need for effective strategies to regulate the generation of allelochemicals and mitigate their advance effects.

Biochar, a carbon-rich particle produced under hypoxic conditions, has garnered attention as a cost-effective and efficient soil amendment (Yang et al., 2016). Its application has been shown to enhance soil porosity, degrade organic pollutants, immobilize heavy metals, and improve soil fertility (Mansoor et al., 2021). Additionally, biochar has exhibited potential in alleviating allelopathy and promoting plant growth (Lan et al., 2023; Zhang et al., 2018). However, biochar also has its drawbacks. The presence of persistent free radicals (PFRs) in biochar can induce oxidative stress, adversely affecting plant growth (Liao et al., 2014). Moreover, the adsorption properties of biochar can produce dual effects: while it can function as a slow-release fertilizer, providing a sustained supply of nutrients (Marcińczyk and Oleszczuk, 2022), it may also adsorb limited available nutrients, potentially exacerbating nutrient deficiencies in barren soils (Wang et al., 2023). These complexities raise critical questions: How do the inherent adsorption properties of biochar and its endogenous PFRs influence plant growth? What mechanisms do plants employ to respond to and mitigate the environmental stresses imposed by these factors?

Previous studies have highlighted that the dissolvable components in biochar can provide essential nutrients to plants (Hossain et al., 2020) and serve as carbon sources for soil microorganisms (Palansooriya et al., 2019), thereby alleviating allelopathy (Chen et al., 2022). However, the effect of oligotrophy (removing dissolvable components) biochar on allelopathy remains poorly understood. Research on the generation of allelochemicals in plant roots following the application of oligotrophy biochar is limited. To address this gap, this study constructed oligotrophy biochar systems with varying tenses of PFRs and applied them to soybean cultivation. We hypothesized that oligotrophy biochar might stimulate the generation of allelochemicals in soybean roots, while potentially inhibiting growth due to either limitations in nutrient retention or oxidative stress induced by PFRs.

This study aims to reveal that the application of oligotrophy biochar in soil may not achieve the intended goal of alleviating allelopathy and could instead introduce environmental risks. By exploring how plants response to these risks, this research offers new perspectives for comprehensively evaluating the environmental implications of biochar application and sustainable soil management practices.

2. Materials and methods

2.1 Biochar preparation and characterization

Rice straw was utilized as the feedstock for biochar preparation. The biomass underwent a sequential processing method that included oven-drying, sectioning into fragments smaller than 5 cm, and mechanical pulverization. Pyrolysis temperatures of 350, 500, and 700 ºC were strategically selected to contrast different thermal regimes: 350 ºC prioritized the preservation of oxygenated functional groups (Chen et al., 2022), 500 ºC was aligned with the maximal generation of PFRs identified in prior research (Liao et al., 2014), and 700 ºC targeted the enhancement of specific surface area (SSA). Pyrolysis was conducted in a nitrogen-purged muffle furnace under isothermal conditions for 2 h. After cooling to room temperature, the prepared biochars were washed 6 times with methanol and distilled water, dried at 60 ºC in a drying oven, stored in sealed brown vials, and labeled as B350, B500, and B700, respectively.

The biochars were analyzed using a Fourier transform infrared spectrometer (FTIR, Thermo Fisher Nicolet-Is10). The bulk elemental compositions (C, H, O, N, and S) were determined using an elemental analyzer (MicroCube, Elementar, Germany). The SSA, pore volume, and pore diameter were measured using the Brunauer-Emmett-Teller nitrogen adsorption technique (BET, JW-BK132F). The intensity of PFRs was assessed using an electron paramagnetic resonance (EPR) spectrometer (Bruker, A300- 6/1, USA).

2.2 Soybean planting

Allitic soils were collected from pristine Yunnan uplands (24º 50´ N, 102º 51´ E) at 40 cm depth to exclude surface organic inputs. Plant residues and stones were manually removed from the soil samples. The soils were sieved through a 10-mesh metal sieve, dried at room temperature, and stored for future use. For the concentration range, we adopted 1-5% (w/w) in accordance with established agricultural application standards (Trifunovic et al., 2018; Dong et al., 2019). This approach ensures methodological consistency and facilitates a comprehensive dose-effect analysis. Aliquots of 150 g of soil were mixed with biochar at 1%, 3%, or 5% (w/w) and placed in individual pots. Soybean (*Glycine max [L.] Merr., Zhonghuang-13, China*) seeds were soaked in water for 8 h and then placed in a container for germination. Only seeds with full grains exhibiting approximately 1 cm of germination were selected for sowing, with 4 seeds per pot. Each treatment was replicated 3 times. All samples were cultivated in a climate chamber (QHP-600BE, 2400lux, 12/12h, 25/20 ºC) for 20 d and watered daily with 10 mL of water to keep the soil moist.

2.3 The extraction and determination of BA and SA

After 20 days of planting, all soybean plants were gently removed from the soil. The plant height and root biomass were then measured. The roots were freeze-dried with liquid nitrogen and ground into a powder. A 20 mg root sample was added to an 8 mL hydrochloric acid solution (0.001 mol/L), followed by sonication (300 J/cm^3) for 30 min, and then centrifugation at 10,000 rpm for 20 min. The resulting supernatant was filtered through a 0.45 μm membrane and then extracted three times with 30 mL dichloromethane. The dichloromethane layers were combined and evaporated using a rotary evaporator at 30 ºC until the sample volume was approximately 2 mL. The sample was then transferred to a vial and dried with nitrogen. The derivatization process was accomplished by adding 30 μL pyridine and 150 μL bis(trimethylsilyl)trifluoroacetamide (BSTFA) to the sample vials and placed in an oven set at 60 ºC for 2 h. All the samples were transferred to 250 μL glass conical inserts for analysis.

The concentrations of BA and SA in soybean roots were analyzed using gas chromatography-mass spectrometry (GC-MS, Agilent 5975C 7980A) equipped with a DB-5 capillary column (30m×0.25mm×0.25μm). A solvent delay time of 10 min was used with a column flow of 1mL/min. The initial temperature was set at 50 °C for 2 min and then increased to 250 °C at a rate of 6 °C/min. The peaks of BA and SA were observed at 16.1 min and 23.2 min, respectively. Malondialdehyde (MDA), a lipid peroxidation biomarker, was quantified within the root using MDA detection kits

(GeRuiSi-G0110F) and ultraviolet spectrophotometer (Shimadzu, UV-2600). Thiobarbituric acid (TBA) was utilized to facilitate chromogenic reaction with MDA in the roots under acidic conditions, and then the absorbance at 532 nm and 600 nm was used to calculate the MDA concentrations.

2.4 Adsorption and extraction of P, NO_3^--N, and NH_4^+-N on biochars

Stock solutions of P, nitrate nitrogen (NO_3^--N), and ammonia nitrogen (NH_4^+-N) were respectively prepared using NaH_2PO_4, $NaNO_3$, and NH_4Cl. The adsorption of nutrients on biochars was examined at room temperature. Batch adsorption experiments were conducted with nutrient concentrations of 0, 5, 10, 15, 20, 25, 30, and 40 mg/L. To prevent bacterial interference, all solutions were supplemented with 200 mg/L of NaN_3, and the pH was adjusted to 7 with 0.1 mol/L HCl and NaOH. Each treatment consisted of 16 mg of biochar in an 8 mL background solution, with 3 replications for each. The samples were mixed on a shaker at 60 rpm for 24 h, filtered through a 0.45 μm membrane, and stored in 8 mL vials.

After the sorption experiment, the biochar samples were also extracted to examine the retention of nutrients. Each treatment was replicated 3 times. P was extracted using 0.5 mol/L $NaHCO_3$ for 30 min (HJ 704-2014), NH_4^+-N with 2 mol/L KCl for 60 min (HJ 634-2012), and NO_3^--N with 2 mol/L KCl for 10 min (HJ 634-2012). All samples were placed into 8 mL vials. P was quantified at 700 nm using a microplate reader (WD-2102B) through the ammonium molybdate spectrophotometric method (GB 11893-89). NH_4^+-N was determined at 420 nm using a microplate reader with Nath's reagent spectrophotometric method (HJ 535-2009).

NO_3^--N was measured at 220 nm and 275 nm using an ultraviolet spectrophotometer in the ultraviolet spectrophotometric method (HJ/T 346-2007).

2.5 Analytical methods

The retention ratios of P, NO_3^--N, and NH_4^+-N were calculated by Equation 1 as follows.

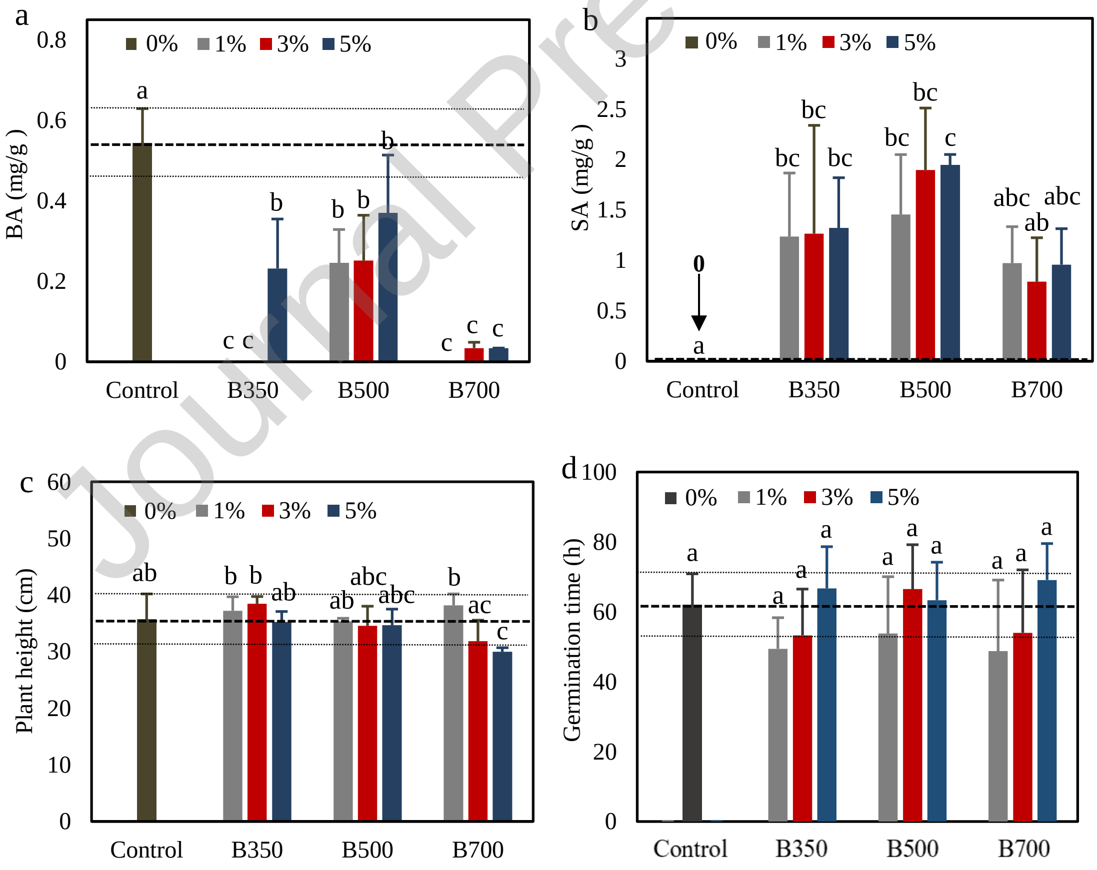
$$\text{Retention ratio (\%)} = (C_0 - C) / C_0 \times 100 \quad (1)$$

The adsorption and extraction concentration of P, NO_3^--N, and NH_4^+-N (mg/g) were denoted as C_0 and C, respectively. Pearson correlation analysis and multiple linear regression (MLR) analysis were conducted using the software SPSS 26.0.

3 Results and discussion

3.1 Effects of biochars on allelochemicals generation and soybean growth

BA and SA, as key functional constituents of phenolic acid allelochemicals, are widely recognized for their potent allelopathic activity, with extensive studies confirming their superior allelopathic potential compared to other allelochemicals (Senaratna et al., 2003). Especially, during the soybean seedling development, BA and its derivatives exhibit significantly stronger allelopathic potential and higher rhizospheric detection concentrations than other allelochemicals, such as citric acid, thereby justifying their prioritization in this experimental framework (Wu et al., 2009). As illustrated in Fig. 1, biochar-amendment markedly suppressed the generation of BA from soybean roots, reducing its levels below the instrumental detection limit while concurrently stimulating SA accumulation. This divergence likely reflects a biochar-induced shift in plant stress signaling. While BA is generally generated to enhance plant tolerance to temperature, humidity, and light stressors (Widhalm and Dudareva, 2015), SA demonstrates broader adaptive efficacy by mitigating drought, thermal extremes, and nutrient deficiency, thereby promoting physiological homeostasis under multifactorial stress conditions (Li et al., 2020; Vlot et al., 2009). Notably, B500 exhibited a unique dual response characterized by residual BA levels alongside sharply elevated SA concentrations, indicating intensified composite stress effects. This finding underscores the ecological implications of biochar characteristics, particularly the necessity to balance the benefits of soil amendment against potential risks of stress induction.



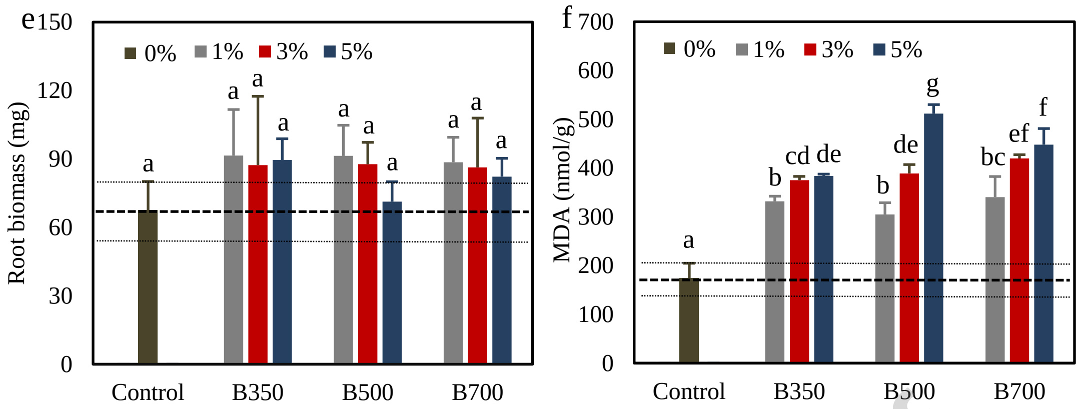


Fig. 1 BA (**a**) and SA (**b**) generation, plant height (**c**), soybean seed germination time (**d**), root biomass (**e**), and MDA (**f**) affected by biochar addition. B350, B500, and B700 are biochars produced at 350, 500, and 700 ºC from rice straws. The ratios of biochar application in percent are denoted by 1%, 3%, and 5%. Error bars indicate the standard error. Different letters represent significant differences at $P < 0.05$

Plant height, germination time, and root biomass are important growth indicators for evaluating plant performance, reflecting both developmental progression and stress adaptation capacity (Jabborova et al., 2021; Morales and Munné-Bosch, 2019). The morphological analysis of soybean (Fig. 1c) revealed that biochar produced at 700 °C with a 5% application ratio negatively impacted plant height. However, no statistically significant differences were observed in germination time or root biomass in the biochar-amended systems. This outcome may be attributed to the fact that seed germination primarily relies on endogenous nutrient reserves, with limited reliance on external soil nutrient during this autonomous phase (Trelease, 1942). Similarly, the stability of root biomass under biochar treatment may be attributed to adaptive rhizosphere modulation: under mild environmental stress, plants regulate allelochemicals to improve the rhizosphere environment, optimize nutrient utilization efficiency, and alleviate stress, thus maintaining the stability of root biomass at minimal energetic cost (Craine, 2006; Scavo et al., 2019). Fig. 2 illustrated a significant negative correlation (p<0.05) between BA and root biomass, indicating that higher BA concentrations correspond to reduced root biomass, supporting previous findings that BA inhibited root growth by disrupting aquaporin-mediated water channels dynamics (Kaur et al., 2005). Although plant height and germination time were not directly correlated with BA and SA levels (Fig. 2), these parameters remain valuable for a comprehensive growth assessment, particularly in identifying stress thresholds.

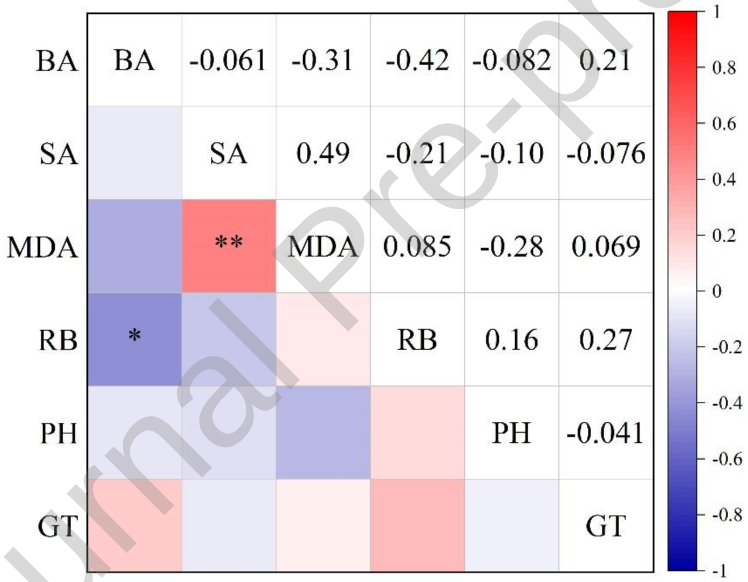


Fig. 2 Correlation matrix between allelochemicals concentrations (BA and SA) and soybean growth (MDA, root biomass (RB), plant height (PH), germination time (GT)) in different biochar applications. The biochar pyrolyzed temperatures are denoted by 350, 500, and 700 ºC. The ratios of biochar application in percent are denoted by 1%, 3%, and 5%
* Significant correlation at 95% confidence level (*p*<0.05).
** Significant correlation at 99% confidence level (*p*<0.01)

MDA is an important product of lipid peroxidation in plants when exposed to environmental stressors, serving as a key indicator of oxidative damage to membrane lipids (Morales and Munné-Bosch, 2019). As demonstrated in Fig. 1f, biochar application significantly elevated MDA concentrations in a dose-dependent manner, indicating that the experimental conditions induced systemic stress, prompting compensatory responses in plants. Notably, MDA exhibited a strong positive correlation ($p<0.01$) with SA in Fig. 2, implying that biochar-derived stressors stimulated SA biosynthesis in soybean roots as part of the adaptive response. Given that the dissolvable compositions were leached from the biochars, we proposed two plausible drivers for the observed MDA accumulation: (1) PFRs formed during biomass pyrolysis could trigger oxidative stress in plant cell membranes by stimulating the overproduction of reactive oxygen species (ROS) (Liao et al., 2014), and (2) nutrient limitation may upregulate lipoxygenase (LOX)-mediated lipid peroxidation, as observed in nutrient-stressed plants (Dokwal et al., 2021). These hypotheses were systematically investigated in subsequent experiments.

PFR intensity varied markedly with pyrolysis temperatures, with the strongest signal observed in B500 (Fig. 3), a finding consistent with previous literature (Liao et al., 2014). Notably, BA generation was also the highest in the B500 system (Fig. 1a), following a similar pattern to PFRs, potentially indicating the significant role of BA in alleviating oxidative stress induced by free radicals. It has been demonstrated that BA mitigated radicals-mediated damage by enhancing both enzymatic (e.g., catalase, peroxidase) and non-enzymatic (e.g., phenolic compounds) antioxidant defenses (Nehela et al., 2021). While SA — a hydroxylated BA derivative — showed a significant positive correlation with MDA (Fig. 2), consisting of the fact that SA could be generated to alleviate the chemical stress on plants. However, SA generation in B500 systems did not significantly differ from those in B350 and B700 systems (Fig.

1b). This observation indicated that SA synthesis is regulated not solely by PFRs and may involve nutrient-dependent pathways, given the specialized role of SA in alleviating nutrient stress. Therefore, further mechanistic studies should integrate analyses of the availability of essential nutrients.

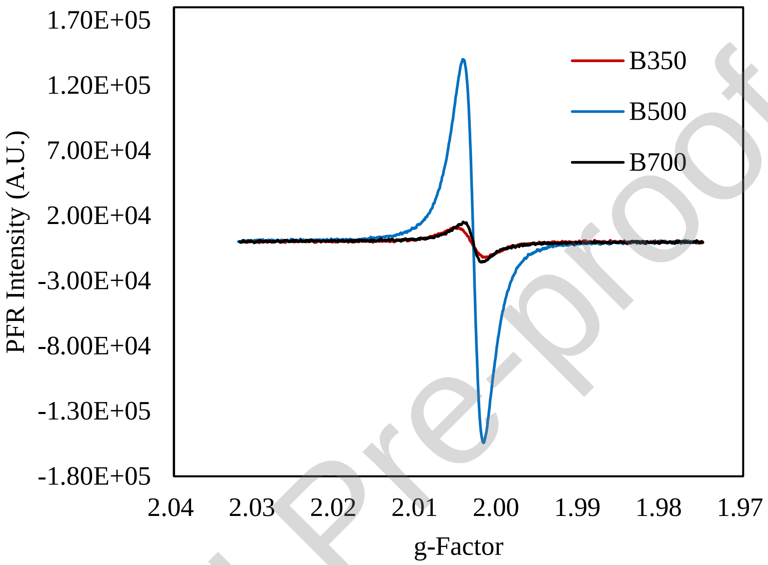


Fig. 3 The intensity of PFRs on biochars. B350, B500, and B700 are biochars produced at 350, 500, and 700 ºC from rice straws, respectively

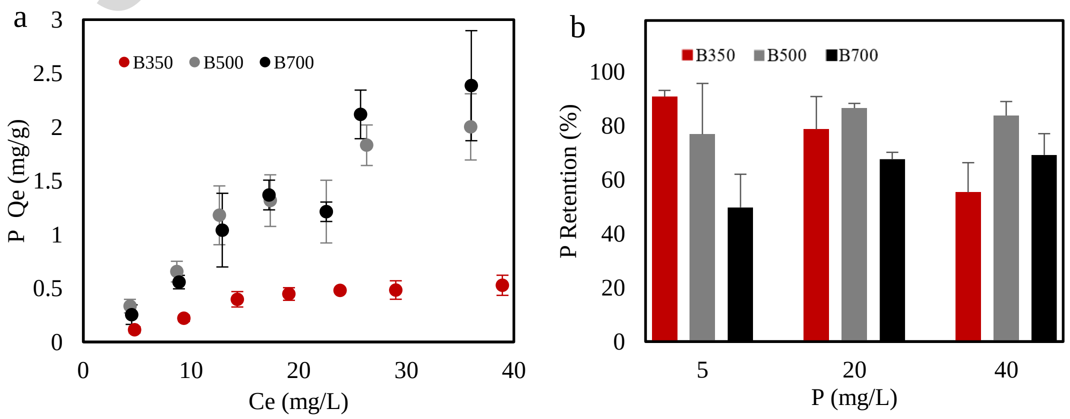
3.2 Adsorption and retention of essential nutrients on biochars

Our previous research demonstrated that the addition of biochar to allite significantly promoted soybean growth (Lan et al., 2023). In this experiment, the biochars were thoroughly washed to remove dissolvable components, thereby becoming oligotrophic and exposing more adsorption sites (Boakye et al., 2019; Sun et al., 2021). The adsorption of P (as phosphate) on biochar increased with the increased preparation temperature (Fig. 4a). Specifically, B500 and B700 exhibited 4-5 times higher P adsorption compared to B350. Moreover, the extraction process revealed that the P adsorbed on the biochar was not extracted completely, with retention rates of the adsorbed P ranging from 50-91% (Fig. 4b). The SSA of the biochar increased with the preparation temperature (Table 1), aligning with the trend of P adsorption. High-temperature biochar exhibited smaller pore size and larger pore volume through the fracture and shrinkage of carbon framework and the generation of fused rings to form micropores, showing advantages in P adsorption (Zhang et al., 2021). It is worth noting that the soil used in this study contains very low available P (9.73 mg/kg, as presented in Table S1). Therefore, the strong sorption and retention of P on these biochars may result in poor nutrition stress.

Table. 1 Characterization of biochar and soil

Biochar		B350	B500	B700	Soil
Elemental mass composition (%)	N	1.14±0.05	1.21±0.01	0.69±0.15	0.04±0.01
	C	53.49±1.15	58.55±0.10	60.41±1.69	0.53±0.30
	H	3.78±0.11	2.57±0.06	1.37±0.06	1.43±0.14
	O	20.90±0.20	11.22±0.43	10.01±0.44	13.08±0.18
Atomic ratio	H/C	0.85±0.01	0.53±0.01	0.27±0.01	32.26±5.45
	O/C	0.29±0.01	0.14±0.01	0.12±0.01	18.45±0.46
	(N+O)/C	0.31±0.01	0.16±0.01	0.13±0.01	0.13±0.01
BET	SSA*(m^2/g)	10.72±0.99	25.75±8.07	214.71±18.54	72.49±1.86
	PV*(cm^3/g)	0.04±0.02	0.04±0.01	0.16±0.01	0.20±0.01
	PD*(nm)	15.31±4.85	5.172±0.62	2.94±0.03	10.83±0.29
pH value		6.96±0.01	10.13±0.01	10.36±0.02	4.91±0.04

*SSA: specific surface area; PV: pore volume; PD: pore diameter; BET: Brunauer-Emmett-Teller nitrogen adsorption technique. B350, B500, and B700 are biochars pyrolyzed at 350, 500, and 700 °C by rice straws



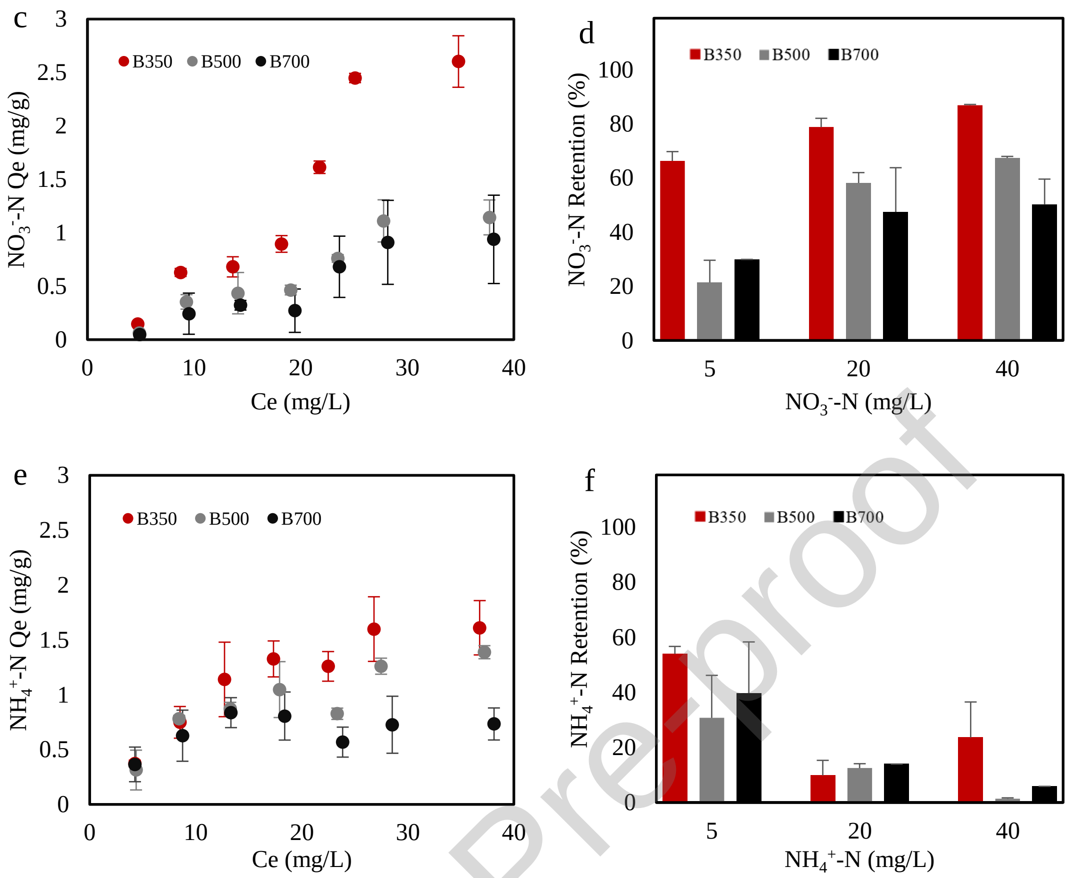


Fig. 4 Adsorption (**a**) and retention (**b**) of P on biochar. Adsorption (**c**) and retention (**d**) of NO$_3^-$-N on biochar. Adsorption (**e**) and retention (**f**) of NH$_4^+$-N on biochar. B350, B500, and B700 are biochars produced at 350, 500, and 700 ºC from rice straws. Error bars indicate the standard

Organic acids can dissolve insoluble phosphorus compounds through acid solubilization (Lazo et al., 2017) or compete with P for adsorption sites on biochar, thereby alleviating the stress induced by P deficiency (Yi et al., 2022). With a lower pKa value compared to BA, SA exhibits stronger acid solubilization properties. Consequently, SA is more effective than BA in alleviating phosphorus stress induced by biochar application. Furthermore, BA serves as an intermediate in SA biosynthesis, and the carbon initially utilized for BA production may be redirected to produce SA (Vlot et al., 2009). As a result, BA decreased and SA increased in soybean roots following biochar application.

The adsorption of both NO$_3^-$-N and NH$_4^-$-N on biochar decreased with increasing preparation temperature, with B350 exhibiting the highest adsorption (Fig. 4c). The NO_3^--N adsorbed on the biochar was not extracted completely, such as B350, with retention rates ranging between 66-87% (Fig. 4d). In contrast, most of the NH_4^+-N adsorbed on biochar could be extracted, with lower retention compared to P and NO_3^--N (Fig. 4f). It was previously reported that NO_3^--N could be adsorbed through regular or charge-assisted hydrogen bonds (Acelas et al., 2017). The O-containing functional groups on biochar, such as -OH and -COOH, as instructed by peaks at 3438 cm^{-1} and 1720 cm^{-1} in Fig. 5, promoted NO_3^--N adsorption through hydrogen bond formation (Zhang et al., 2023). This strong bond made NO_3^--N desorption difficult and could be retained on the biochar. Conversely, NH_4^--N adsorption primarily relies on weaker electrostatic attraction with biochar surfaces (Wang et al., 2015), a physisorption process that allows for easy desorption.

Despite the low retention rate of NH_4^--N, its transient adsorption may still impose nutrient stress by temporarily limiting nitrogen bioavailability. Under such oligotrophic conditions induced by biochar-amended soils, legumes may activate adaptive strategies: root-secreted phenolic acids, traditionally viewed as allelochemicals, can function as symbiotic signals to recruit rhizobia (Dakora et al., 2002). This microbial collaboration enhances biological nitrogen fixation (BNF), reducing atmospheric N_2 to ammonia in root nodules — a compensatory mechanism that simultaneously elevates SA production as part of systemic stress signaling (Dobbelaere et al., 2002). Collectively, these findings suggest that biochar-induced nitrogen limitation acts as a dual stressor, promoting both nutrient conservation through adsorption and adaptive biochemical responses via SA-mediated pathways.

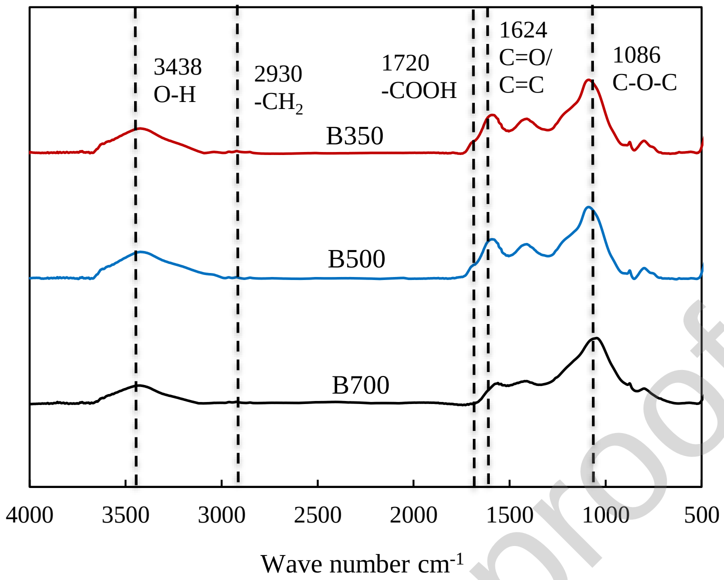


Fig. 5 FTIR spectra of biochar. B350, B500, and B700 are biochars pyrolyzed at 350, 500, and 700 °C by rice straws

3.3 Biochar-mediated nutrient and oxidative stress modulate soybean growth and allelochemicals

The MLR analysis revealed the simultaneous roles of biochar-mediated stressors in modulating soybean responses (Table 2). It was observed that the independent variables of P retention, NH_4^+-N retention, and PFR intensity significantly predicted the dependent variables of MDA, BA, SA, and plant height after excluding the effect of NO_3^--N retention. The absolute values of the standardized coefficient showed that the retention of P and NH_4^+-N on biochar had the highest effect on plant height, while the effect of PFRs was relatively low, indicating that nutrient stress exerted a stronger effect than oxidative stress on plant height. Notably, BA generation exhibited comparable contributions from both nutrient and oxidative stressors. In contrast, SA generation correlated more strongly with P and NH_4^+-N retention than with PFR intensity, particularly identifying P retention as the primary driver. Intriguingly, NO_3^--

N retention had no measurable influence on allelochemical profiles or growth parameters.

The MLR results identified a contribution for SA generation: P retention > NH_4^+-N retention > PFR intensity. The retention of P on biochar exacerbated P deficiency in the soil, which triggered adaptive SA generation in soybean roots, facilitating acid-mediated solubilization of insoluble phosphorus compounds — a well-documented phosphorus-scavenging strategy in legumes (Lazo et al., 2017). Concurrently, NH_4^+-N retention exacerbates nitrogen limitation, further amplifying SA synthesis as part of systemic nutrient stress signaling.

The results identified that MDA generation arose from two synergistic pathways. (1) Nutrient stress: LOX-mediated lipid peroxidation triggered by P/NH_4^+-N deficits (Dokwal et al., 2021), and (2) biochar-derived PFRs induced oxidative stress (Liao et al., 2014). The significant positive correlation between SA and MDA (Fig. 2) suggested that SA accumulation may intensify oxidative damage in the plant, which further promoted the generation of MDA (Nehela et al., 2021). The increased of MDA is usually indicated when plants were under stress, and this stress would also activate the defense mechanism of plant to stimulate the generation of allelochemical (SA) in soybean roots (Chen et al., 2022). The retention of P and NH_4^+-N had a much stronger effect on plant height than oxidative stress, this is because the deficiency of essential nutrients directly affected plant growth stages, especially cell division and tissue growth (Singh et al., 2024). Therefore, the accumulation of SA and MDA in the soybean root system and the alteration of soybean growth after oligotrophic biochar application resulted from a combination of oxidative and nutrient stresses.

The interaction between biochar and soil nutrients is a complex process, characterized by varying retention rates for different nutrients in biochar and differing nutrient backgrounds across various soil types. Based on the soil nutrient background, biochar and specific nutrients can be applied to regulate the generation of allelochemicals.

Table. 2 Multiple linear regressions (MLR) of allelochemicals concentrations (BA and SA) and soybean growth (MDA, root biomass (RB), plant height (PH), germination time (GT)) as functions of biochar (P retention, NO_3^--N retention, NH_4^+-N retention, and PFR intensity) amendments

Variable	Multiple regression	R^2	*p*	Standardization coefficient (*Beta*)			
				P retention	NO_3^--N retention	NH_4^+-N retention	PFR intensity
MDA	256.866 + (1.726 × P) + (295.729 × NH_4^+-N) + (7.599E-5 × EPR)	0.787 ***	< 0.001	0.028	-	0.754	0.188
BA	0.201 + (0.046 × P) + (-0.613 × NH_4^+-N) + (4.772E-7 × EPR)	0.274 *	0.043	0.343	-	-0.697	0.549
SA	0.791 + (0.616 × P) + (-2.329 × NH_4^+-N) + (3.709E-7 × EPR)	0.400 **	0.005	1.172	-	-0.712	0.101
PH	37.170 + (2.798 × P) + (-24.015 × NH_4^+-N) + (-2.691E-7 × EPR)	0.440 **	0.002	1.186	-	-1.613	-0.018
RB	—						
GT	—						

* Significant correlation at 95% confidence level ($p<0.05$)

** Significant correlation at 99% confidence level ($p<0.01$)

*** Significant correlation at 99.9% confidence level ($p<0.001$)

4. Conclusion

The application of oligotrophic biochar induces dual stress pathways in soybean systems, linking nutrient immobilization and oxidative damage. Biochar’s strong adsorption affinity for P and NO_3^--N exacerbates soil nutrient deficiencies, triggering compensatory SA biosynthesis in roots to enhance phosphorus solubilization through acid secretion — an essential adaptive response in nutrient-deficiency environments. Concurrently, PFRs in biochar, particularly in B500, amplify oxidative stress via ROS overproduction, as evidenced by elevated MDA levels. MLR analysis identified nutrient stress (P and NH_4^+-N retention) as the primary growth inhibitor, overshadowing oxidative effects. A bidirectional feedback loop emerged: SA accumulation intensified oxidative damage, while MDA elevation activated SA synthesis as a defense mechanism, underscoring the interdependence of stress adaptation pathways.

The dualistic nature of biochar — enhancing soil structure and carbon sequestration while posing risks of nutrient retention. Key priorities include tailoring biochar properties (e.g., pyrolysis temperature, functional groups) to align with soil nutrient profiles, optimizing formulations to minimize PFRs generation, and integrating biochar with slow-release fertilizers or phosphorus-solubilizing microbes to mitigate adsorption-induced deficiencies. Such approaches balance biochar’s agronomic benefits with ecological safety, transitioning it from a contested amendment to a precision tool in sustainable agriculture.

CRediT authorship contribution statement

Pinyao Lan: Investigation, writing-original draft. **Quan Chen:** Investigation. **Min Wu:** Investigation, writing and editing, funding acquisition. **Patryk Oleszczuk:** writing and editing. **Bo Pan:** Investigation, writing and editing, funding acquisition. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

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

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Highlights

- Biochar application decreases BA and increases SA content in soybean roots.
- Persistent free radicals in biochars can stimulate the generation of both BA and SA.
- Oligotrophy biochar application can exacerbate nutritional stress in soil.
- Biochar can increase phosphorus and nitrate nitrogen deficiencies.