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Increased removal of ginsenoside Rb1 through the application of capacitance-enhanced biochars in soils

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Abstract

Accumulation of allelochemicals in agricultural soils results in severe obstacles to continuous cropping and poses a notable challenge to global food security. The ginsenosides released into the rhizosphere soil as root secretion or during the degradation of plant residues exhibit autotoxic effects to plants and facilitate the proliferation of root rot bacteria. This study proposes that biochar can be used to alter the electron transfer processes in soil to create a unique “charosphere”, which is beneficial for ginsenoside Rb1 removal in soils planted with *Panax notoginseng*. Biochars were also intentionally modified to enhance their electron transfer properties in charosphere soil. The introduction of biochars activated electro-nutrient microorganisms such as *Lactobacillus*, *Pseudomonas* and *Klebsiella*. However, the abundance of major pathogens of *Panax notoginseng*, including *Fusarium spp.*, *Alternaria*, and *Sarocladium* was reduced by 3.77%–4.79% in Soil-2 and 9.08–17.18% in Soil-3 after applying modified biochars by KHB₄ in soils with different years of planting *P. notoginseng*. Modified biochar also exhibited superior effectiveness in promoting ginsenoside Rb1 chemisorption and biodegradation, and enhancing microbial community diversity, compared to unmodified biochar. This was attributed to the electron-rich (orbital) high-energy alkyne bonds introduced by KHB₄ modification, which improved the capacitance values of the biochar by 14.62–316.2 $\mu\text{F}/\text{cm}^2$. Meanwhile, KBH₄-modification reduces the diffusion impedance layer on biochar surface and the charge transfer resistance inside the biochar matrix. The chemical structures containing π -electron may be the dominant biochar composition to stimulate the growth of electro-nutrient microorganisms and accelerate ginsenoside Rb1 degradation. Our study suggests that alterations in electron transfer processes within the charosphere through the application of biochar regulate the microbiome community structure in the soil. This regulation has the potential to mitigate continuous-cropping disorders in agricultural soils.

Highlights

- A way to mitigate continuous cropping disorders by biochar-regulated microbial communities is proposed.
- Enhancing biochars capacitance by KHB₄ modification increased the removal of ginsenoside Rb1.
- Modified biochar can increase the percentages of bacteria with electron transfer ability in soil.
- Biochars reduced the abundance of major pathogens of *Panax notoginseng* in soil by 3.77–17.18%.

Keywords Agricultural soil, Electron transfer, Continuous cropping obstacle, Root secretion, *Panax notoginseng*

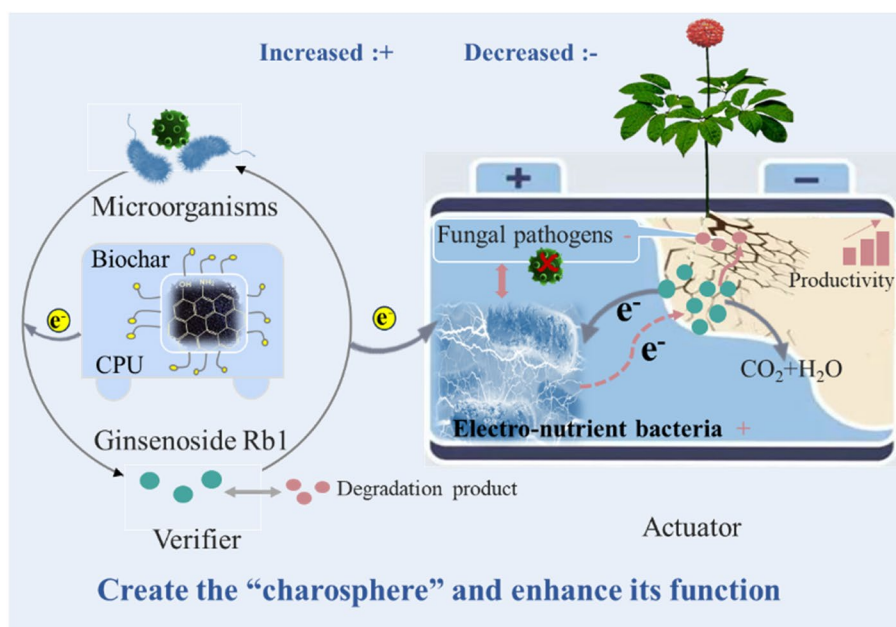
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Graphical Abstract



1 Introduction

Continuous cropping obstacles pose notable threats to global food security. For example, the accumulation of root secretions, most of which are autotoxins, in soil may lead to negative plant-soil feedback and limited agricultural yields worldwide (Li et al. 2023). Various strategies have been utilised to overcome the continuous cropping obstacles, such as crop rotation, soil sterilisation using chemical and physical methods, and exogenous application of plant growth-promoting rhizobacteria (Jia et al. 2023; Liu et al. 2020; Zhao et al. 2023). However, these approaches are often unstable as the interactions between inter-root secretions and soil microorganisms are not considered. Some autotoxins are found in root exudates, such as ginsenoside (Luo et al. 2020) and aliphatic acid (Chen et al. 2011), which worsen root rot by altering the inter-root microbiota, damaging root cells, and boosting the growth and pathogenicity of soil-borne pathogens (Cheng et al. 2020; Deng et al. 2023). Developing strategies to regulate the accumulation of root secrets and foster beneficial rhizosphere microbiota can be a promising approach for addressing continuous cropping challenges.

Previous studies have shown that biochar can remove organic compounds through adsorption and reduction (Geça et al. 2022), and boost soil microbial communities when used as a soil amendment (Zhao et al. 2023). Novel

spatial niches created by biochar formation referred to as the 'charosphere', may extend several millimeters into the adjacent soil (Yu et al. 2021). This unique charosphere often acts as a hotspot for microbial activity, altering microbial composition by promoting the growth of specific bacterial taxa while suppressing other species (Yu et al. 2021). Recent studies have demonstrated that biochar can increase the relative abundance of bacterial groups, such as *Actinobacteria*, *Bacteroidetes*, *Thickettsia*, *Planctomycetes*, and *Gemomonas* at the genus level (Yang et al. 2019). Additionally, biochar has been found to reduce the abundance of pathogens (de Medeiros et al. 2022) and inhibits the growth of certain fungi and bacteria, such as *Kosakonia sacchari*, *Talaromyces helices*, *F. oxysporum*, *F. solan*, *Acidobacteria* and *Chlorobacteria* (Yang et al. 2019), ultimately reducing crop virulence. The potential of biochar to alleviate continuous cropping issues in herbal crops has been emphasised in the literature (Liu et al. 2022). However, the exact mechanism by which biochar influences inter-root microbial communities remains unclear. Interactions among biochar, root secretions and inter-root microbes are often overlooked. Furthermore, biochar directly influences the physicochemical properties of the charosphere (Luo et al. 2023b), not all types of biochar exhibit these positive effects. Studies have suggested that the electron exchange capacity of biochar plays a crucial role in the

transforming of organic compounds (Dorner et al. 2022; Wang et al. 2020). Soil root secretions, which are complex, and contain electron-rich and electron-deficient functional groups (Yan et al. 2023), may serve as carbon resources, and interact with soil electro-nutrient microorganisms (Jiang et al. 2022). Based on these findings, we hypothesised that biochar with a high electron-donating capacity could stimulate the growth of electron-nutrient microorganisms in charosphere, thereby accelerating the degradation of organic compounds.

Here, we used KHB₄ with selective reduction of carbon groups to intentionally increase the electron exchange properties of biochar and investigated the mechanism by which biochar controls autotoxin levels in root secretions and modulates the structure and function of the soil microbiota. Saponins are the most typical autotoxic substances secreted by ginseng root (Deng et al. 2023). Using ginsenoside Rb1 as a model for soot secretion, we specifically explored the application of different biochars that could effectively eliminate autotoxins in this work. The primary focus of our study was to understand how biochar, with its reductive properties, regulates ginsenoside Rb1 and enhances the growth of beneficial microorganisms. The findings of this study will have broad implications for sustainable agriculture, land management, and food production.

2 Materials and method

2.1 Preparation and modification of biochar

Corn stovers were used as feedstocks materials for biochar production. Briefly, the dried and chopped corn stovers were pyrolyzed at 350 °C or 700 °C in flowing N₂ for 24 h. The resulting biochar was milled, sieved through a 100-mesh sieve, washed with water, freeze-dried, stored in brown vials, and labelled as W350 and W700. The electrochemical properties of biochar were enhanced by KHB₄ modification. Briefly, 10 g of the biochar samples were mixed with 500 mL of 5 wt% KHB₄ solution. The solution was gently stirred with a glass rod for homogenisation and then placed on a magnetic stirrer at 25 °C for 24 h. The biochar samples were washed, dried, and denoted as W350-M and W700-M.

2.2 Characterisation of biochar

The biochar suspension was prepared by weighing 5 mg of each biochar in a brown glass bottle and adding deionised water at a solid-to-liquid ratio of 1:800. The samples were shaken in a thermostatic shaker at 25 °C and 150 rpm for 24 h. The surface charge of the biochars were analysed using a zeta potential meter. The pH value was determined using a pH meter (PXSJ-260F). The elemental compositions and functional group structures of the biochar were analysed using an

elemental analyser and infrared spectrometere, respectively (de Medeiros et al. 2022). The specific surface area of the biochar was measured using the Brunauer–Emmett–Teller (BET) method. The apparent electron transfer was measured using a CHI760E electrochemical workstation (Shanghai, China). A glassy carbon disk (Ø = 3 mm), a platinum wire, and a saturated calomel electrode (SCE) were used as the working electrode, counter electrode, and reference electrode, respectively (Li et al. 2021). Phosphate buffered saline (PBS) was used as the electrolyte at pH 7.5. The electronic properties of biochars were studied using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) (Li et al. 2021).

2.3 Removal of ginsenoside Rb1 by biochar

A 250 mL 100 mg/L ginsenoside solution was prepared in a brown glass vial, and supplemented with 312 mg of W350, W700, W350-M, or W700-M (solid–liquid mass ratio of 1:800). The samples were then placed in a constant-temperature shaker at 25 °C and 150 rpm to investigate the removal of ginsenoside Rb1. The initial pH of the mixture was adjusted before shaking. The reaction was conducted for varying durations ranging from 0.08 h to 84 h. Subsequently, the supernatants were filtered through a 0.45 µm aqueous-phase needle-type filter and transferred to a brown vial. The concentration of ginsenoside Rb1 remaining in the system was determined using high performance liquid chromatography (HPLC, Agilent, 1260 infinity).

The HPLC detection parameters included an XDB-C18 column, a mobile phase of acetonitrile and 0.1% phosphoric acid solution (3:7), a UV detector set at 205 nm, a flow rate of 1.0 mL/min, an injection volume of 20 µL, a column temperature of 30 °C, and a retention time of 8 min. The content of ginsenoside Rb1 was quantified by external standard method. All experiments were performed in triplicate to ensure reproducibility. The adsorption of ginsenoside Rb1 by the biochar was calculated using the following equation:

$$q_t = \frac{(C - C_t) \times V}{M} \quad (1)$$

where q_t is the adsorbed ginsenoside Rb1 (mg/g); C and C_t are the initial and equilibrated aqueous concentrations of ginsenoside Rb1 (mg/L), respectively; V is the volume of the solution (L); and M is the mass of biochar (g).

To differentiate between the degradation and adsorption of ginsenoside Rb1, biochar was subjected to methanol extraction after adsorption. Briefly, the supernatant of the biochar sorption experiment was removed, and the particles were extracted using 250 mL methanol under ultrasonication. The process was repeated three times

and the extracts were combined for ginsenoside Rb1 quantification.

The extracted samples were then analysed by HPLC to determine the concentration of ginsenoside Rb1. The difference between the total removal and actual adsorption represents the degradation of ginsenoside Rb1 by the biochar.

2.4 Analysis of microbial communities in soil with biochar application

According to the standard soil sampling method, soil samples were collected from 23.5° north latitude, Pingba town, Wenshan under various conditions, including soil without crops and soils planted with *Panax notoginseng* (*P. notoginseng*) for two and three years. The soil type is ferralsol. These samples were labelled as Soil-0, Soil-2, and Soil-3, respectively. After removing plant debris and roots, the sample was stored in refrigerator at 4 °C and used for biochar amendment experiment within 7 d. The soil samples were then mixed with 5% (0.5 g) biochar and incubated for 7 d at 25 °C. Sterile water was added daily to maintain soil moisture at 35%, as *P. notoginseng* is a shade-loving plant that typically requires a soil moisture content of 22% to 40% (Xia et al. 2016; Zang et al. 2023). The bottle was covered with cling film with several holes to allow gas exchange and slowed down water evaporation. To ensure the reliability of the data, three replications were included in each measurement. To analyse *Alpha* and *Beta* diversity, bacterial 16SrDNA from V3 and V4 regions was amplified using primers F: ACTCCTACGGGGAGGCAGCA and R: GGACTACHVGGGTWCTCTAAT. The fungal ITS1 region was amplified using primers F: CTTGGTCATTTAGAGGAAGTAA and R: GCTGCGTTCTTCATCGATGC. Trimmomatic v0.33 software was used to filter the sequenced raw reads to analysis data. Then cutadapt 1.9.1 software was used to identify and remove primer sequences, and clean reads without primer sequences were obtained. The dada2 method in QIIME2 2020.6 was used for denoising, the double-ended sequences were spliced, and the chimeric sequences were removed to obtain the final effective data (Non-chimeric Reads).

Soil bacteria are divided into strong electron transfer bacteria and weak electron transfer bacteria based on their electron transfer ability (Qin et al. 2024). Typical iron-reducing bacteria, such as *Geobacter*, are categorized as “strong exoelectrogens” (Doyle and Marsili 2018). Weak exoelectrogens do not have the ability to transfer electrons directly to extracellular solid electron acceptors, but they likely display potential to transfer electrons to soluble electron shuttles (Doyle and Marsili 2018). Based on this rule and classification results reported in

literature, bacteria are divided into two categories: strong electron transfer and weak electron transfer.

2.5 Modified biochar-mediated soil microbial degradation of ginsenoside Rb1

Ten g soil sample was evenly mixed with 5% (0.5 g) biochar, and cultured at 25 °C for 7 d. Sterile water is added daily to ensure 35% water retention. Soil samples without biochar treatment were used as blanks. Microorganisms were extracted from soil samples. A microbial solution (10 mL) was prepared and mixed with ginsenoside Rb1 (250 mL, 100 mg/L). The mixture was then agitated at 25 °C and 150 rpm in the dark. Subsequently, 1 mL sample were collected at specific time points (0.08 h, 0.17 h, 0.5 h, 1 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 36 h, 48 h, 60 h, 72 h, and 84 h) to explore the degradation kinetics and stored in 1.5 mL EP tubes. The samples were centrifuged at 8000 rpm for 5 min, and the supernatant was filtered using a 0.45 µm microporous membrane. Ginsenoside Rb1 content in the supernatant was then analysed using HPLC.

2.6 Statistics and reproducibility

All data are presented as mean ± standard deviation (SD). Statistical analyses and graphs presentations were prepared using OriginLab 2023 and Graph-Pad Prism 8.0.1. Significant differences were determined by Duncan's multiple range test at $p < 0.05$. One-way analysis of variance (ANOVA) was used to compare the data difference (means ± SD, $n = 3$).

3 Results and discussion

3.1 Ginsenoside Rb1 degradation by biochar-stimulated microbes

Microbes extracted from the original three soils showed different potentials for the degradation of ginsenoside Rb1. Specifically, ginsenoside Rb1 was hardly degraded in Soil-0, however, was degraded in Soil-2 and Soil-3 (Fig. 1). This difference may be attributed to the enrichment of the soil microbial community structure by the root secretions of *P. notoginseng*, which is discussed extensively in the following section. We must emphasise that this experiment focused on the ginsenoside Rb1-degradation ability of the microbes extracted from the soils. The presence of root secrets may induce the domestication of ginsenoside Rb1-degrading microbes. However, this observation does not necessarily mean that the root secrets were completely degraded. The accumulated ginsenoside Rb1 will result in obvious obstacles to continuous cropping.

After incubation with biochar for 7 d, microbes extracted from all the soil systems showed increased activity in degrading ginsenoside Rb1. The extent of

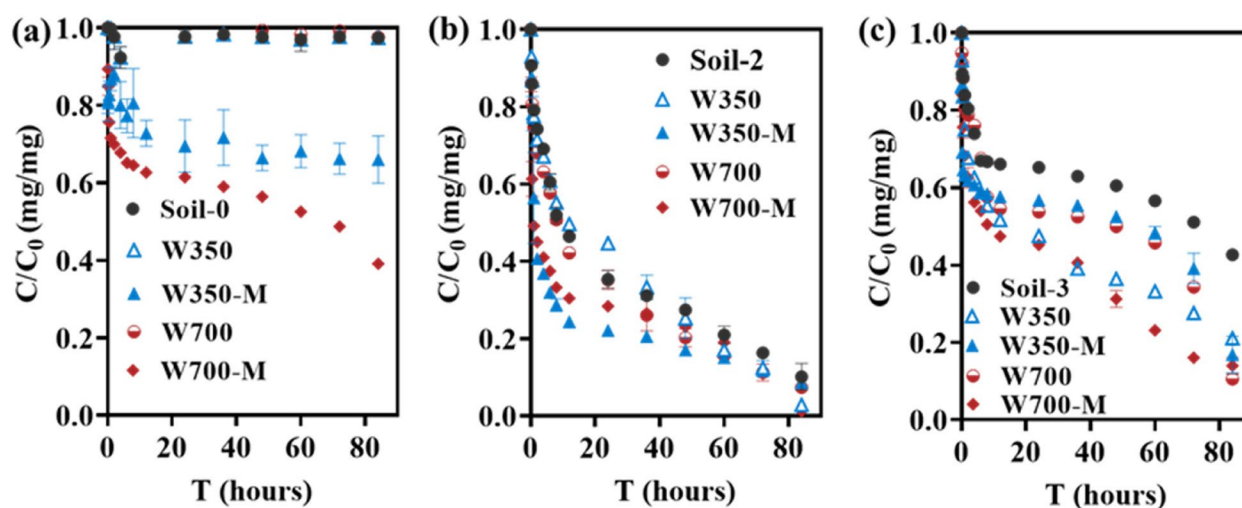


Fig. 1 Degradation of ginsenoside Rb1 by biochar-mediated microbes in soil (a) Soil-0, (b) Soil-2 and (c) Soil-3

degradation was the highest for microbes extracted from soil amended with W700-M, followed by W350-M, W700 and W350. For example, the degradation ratio of ginsenoside Rb1 by microbes in the W700-M/Soil-2 system reached 90% at 84 h. According to pseudo-one order kinetics model fitting results, the rate constants (k) for Rb1 degradation in Soil-2 and Soil-3 were significantly higher than that in Soil-0 (Table S1). This increase may be primarily attributed to the regulation of the soil microbial community structure by biochar application, resulting in enhanced abundance of soil microorganisms. Notably, the efficiency and rate of ginsenoside Rb1 degradation were higher in microorganisms from KBH_4 -modified biochar systems than in those from unmodified biochar. This may be related to the electrochemical properties of biochar such as capacitance, transfer resistance and electron exchange capacity. The microbial structures were then analysed to explore the reason for the altered ginsenoside Rb1 degradation.

3.2 Biochar-stimulated structural change of microbe community in charosphere beneficial to ginsenoside Rb1 removal

P. notoginseng plantation significantly altered the structure of soil microbial communities. The first observation was that the pathogenic fungi, such as *Alternaria*, *Meyerozyma*, *Sarocladium*, and *Kazachstania*, increased in Soil-2 and Soil-3 (Figs. 2a and S1). *Fusarium*, the primary fungus responsible for *P. notoginseng* root rot was more prevalent in Soil-3 than in Soil-2, indicating an increase in harmful strains over *P. notoginseng* growing time. However, the application of biochars decreased the abundance of pathogenic fungi in Soil-2 and Soil-3. Generally, this fungal inhibition effect was more significant in

biochars generated at higher pyrolysis temperatures, and further increased after biochar modification. Specifically, the use of W700-M led to a reduction in pathogenic fungi in Soil-3 by up to 17.18% compared to the soil without biochar (Fig. 2c).

P. notoginseng plantations increased the abundance of bacteria (Figs. S3-S5). This could partly explain the higher degradation of ginsenoside Rb1 by microbes extracted from Soil-2 and Soil-3 than by those extracted from Soil-0. After the application of biochar, the abundance of some microorganisms increased in charosphere, whereas that of others decreased (Fig. 3a-b). Most bacteria in soils can be separated based on their electron transfer abilities (Qin et al. 2024). The percentages of bacteria with strong electron transfer ability increased to 37.75% after the application of W700-M in Soil-2 (Fig. 3d). The application of W700-M to Soil-3 elevated the level of strong electroactive bacteria by 7.22% (Fig. 3e). Meanwhile, the levels of bacteria with weak electron transfer abilities were significantly decreased in Soil-2 and Soil-3 compared to the original soil (Figs. 3g-h). Based on these observations, we conclude that after applying biochars in soil, the percentages of bacteria with strong electron transfer ability increased, however, the abundance of bacteria with weak electron transfer ability decreased (Figs. 3 and S6). Therefore, Ginsenoside Rb1 degradation in soil is related to the abundance and species of electroactive bacteria and the highly electroactive bacteria such as *Klebsiella*, may dominate its transformation (Fig. 3c-e).

3.3 Enhanced removal of ginsenoside Rb1 by biochars

The removal of ginsenoside Rb1 by biochars was also enhanced by KBH_4 -modification (Fig. 4a). W700 exhibited a removal of 29.1 mg/g for ginsenoside Rb1 at 84 h,

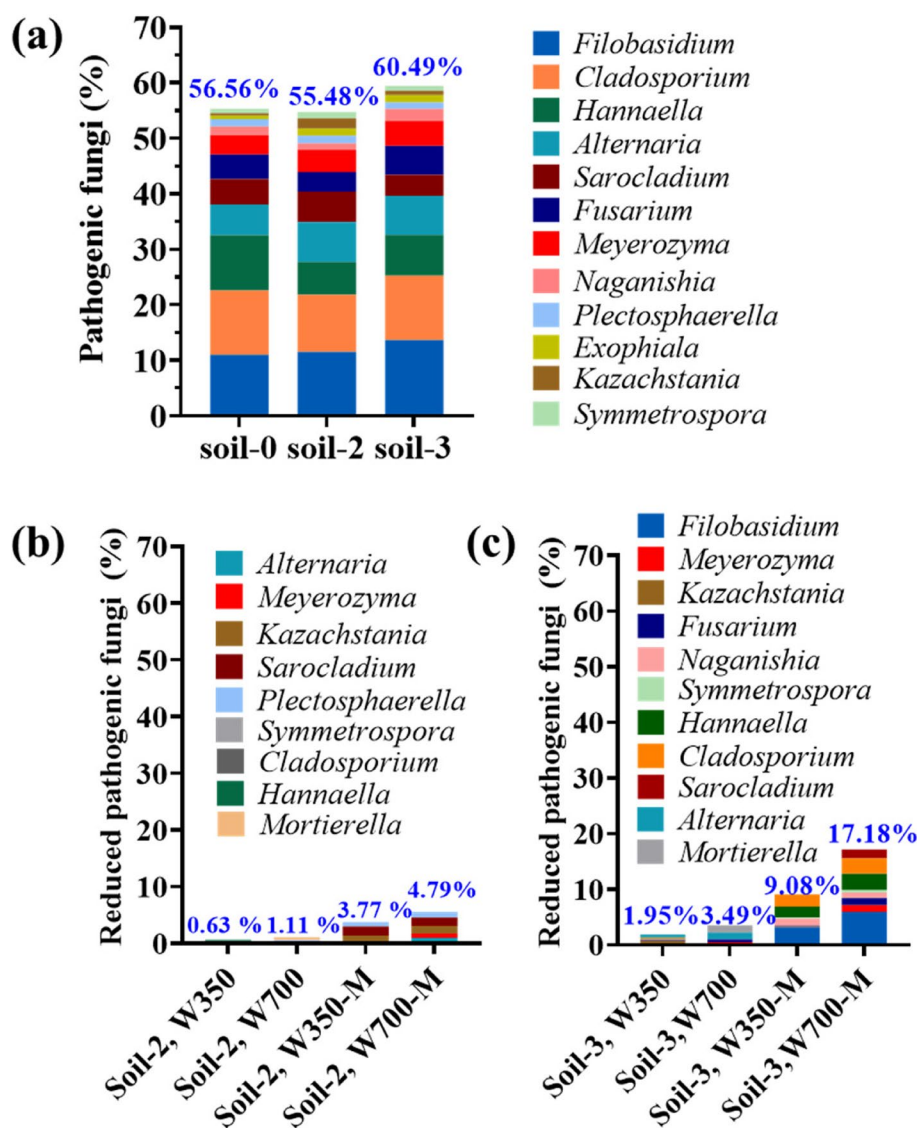


Fig. 2 Biochar-mediated rhizosphere fungal community structural change in 3 soils (Soil-0 represents original soil without crops, Soil-2 is the soil planted with *P. notoginseng* for 2 yr, and Soil-3 is the soil with *P. notoginseng* for 3 yr). The percentages of pathogenic fungi in the overall fungi increased after *P. notoginseng* plantation (a), but reduced after applying biochars in Soil-2 (b) and Soil-3 (c)

which is approximately two times higher than that of W350 (Fig. 4b), attributing to the favorable porous structure of biochar formed at relatively high pyrolysis temperature (Ji et al. 2021). The removal of ginsenoside Rb1 by W700-M reached 64.1 mg/g at 84 h, with an apparent adsorption of 49.9 mg/g, which was two times higher than unmodified W700 (Fig. 4a-b). In comparison to the fitting parameters of adsorption kinetics, the R^2 value of the quasi-second-order kinetic equation is higher than that of the quasi-first-order kinetic equation (Table S2). Additionally, the equilibrium adsorption capacity predicted by the quasi-second-order kinetic equation is

closer to the measured adsorption capacity. Therefore, the adsorption of ginsenoside Rb1 by modified biochar follows pseudo-second order kinetics model (Table S2). This indicates that the adsorption process between ginsenoside Rb1 and biochar involves diffusion, surface adsorption, and intraparticle diffusion, along with the formation of chemical bonds. Significance analysis between the groups showed significant variations in the adsorption and degradation of ginsenoside Rb1 by biochar before and after the KBH_4 modification (Fig. 4c-d). All the investigated biochars showed rapid removal of ginsenoside Rb1 during the initial reaction. The rapid

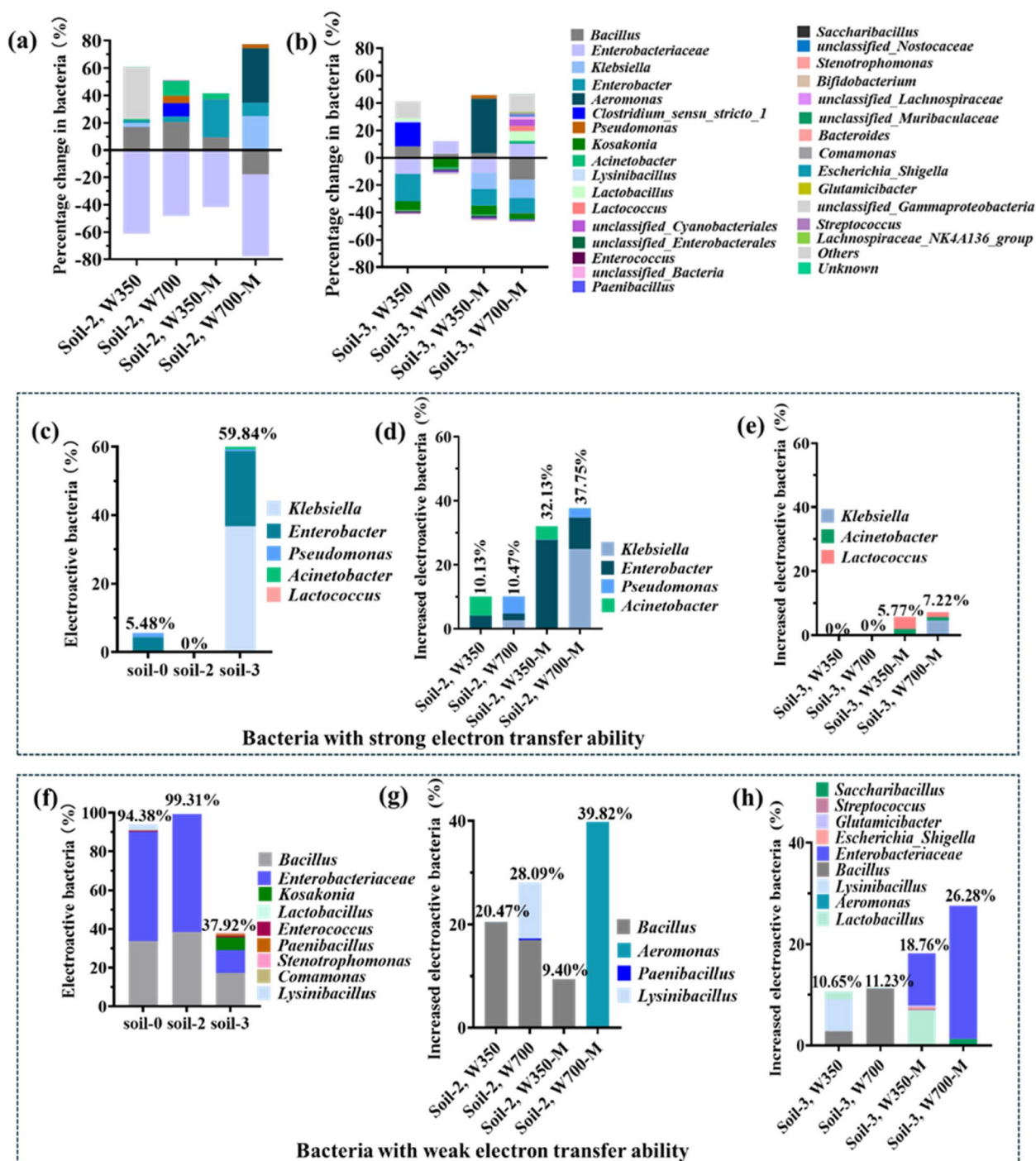


Fig. 3 Biochar-mediated rhizosphere bacterial community structural change in 3 soils (Soil-0 represents original soil without crops, Soil-2 is the soil planted with *P. notoginseng* for 2 yr, and Soil-3 is the soil with *P. notoginseng* for 3 yr). The electroactive bacteria community structures changed substantially after *P. notoginseng* plantation (c and f), and after applying biochar in Soil-2 (a) and Soil-3 (b). Bacteria with strong electron transfer ability in Soil-0, Soil-2 and Soil-3 (c). The percentages of bacteria with strong electron transfer ability increased after applying biochars in Soil-2 (d) and Soil-3 (e). Bacteria with weak electron transfer ability in Soil-0, Soil-2 and Soil-3 (f), but bacteria with weak electron transfer ability reduced after applying biochars in Soil-2 (g) and Soil-3 (h). The above data are the average values of the three test samples

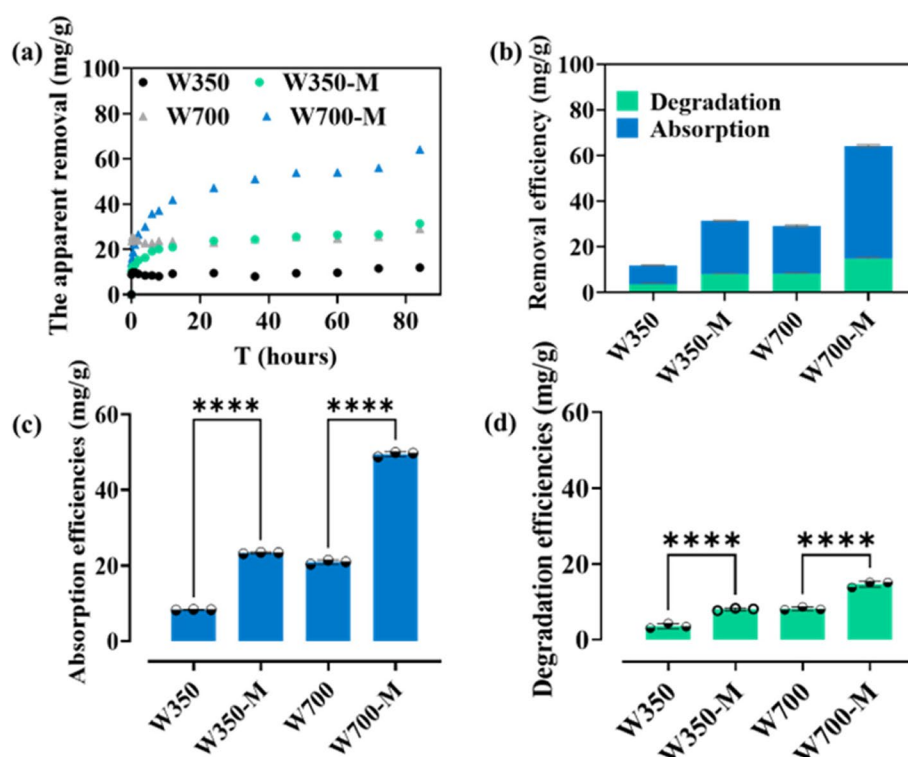


Fig. 4 (a) Removal capacity of ginsenoside Rb₁ by biochar prepared in different ways; (b) Contribution of adsorption and degradation in the removal of ginsenoside Rb₁ by biochar; (c) and (d) Differential analysis

equilibrium for the unmodified biochar may suggest that the removal was mostly associated with the outer surface (Feng et al. 2020). KBH₄-modification not only enhances the electrochemical properties (as will be discussed in the following section), but also exposes more inner pores, which is why the apparent equilibrium time was extended.

Biochar was previously reported to may mediate the chemical degradation of organic chemicals after adsorbed on biochar surface (Lan et al. 2023; Zeng et al. 2023). Similar degradation was expected in this current study. We conducted a solid-phase extraction experiment to quantify the apparent sorption and calculated the degradation based on the mass balance. Obvious degradation was observed, accounting for 1/4 of the overall ginsenoside Rb₁ removal. Higher sorption was generally associated with greater degradation, which agrees with our previous statement that organic chemicals are first adsorbed and then degraded after direct contact with biochar particles (Chen et al. 2020).

3.4 Beneficial properties of biochars after KHB₄ modification

The results of biochar mediated biodegradation demonstrated the notable role of KBH₄-modified biochars

in ginsenoside Rb₁ (Fig. 1). The surface areas of the biochars increased substantially after modification (Table S3), which was beneficial for ginsenoside Rb₁ removal through adsorption. However, no general correlation was observed between the removal of ginsenoside Rb₁ and the biochar properties, including pH, surface area, zeta potential, and ash contents (Fig. S7). We further analysed the electrochemical properties of biochar. The integrated areas in the cyclic voltammetry curves indicative of capacitance were larger for KBH₄-modified biochars compared to unmodified biochar (Fig. S8) and exhibited a strong positive correlation with the pore and specific surface area of biochar (Figs. S7 and S9). The capacitance value of W350-M was measured to be 89.5 $\mu\text{F}/\text{cm}^2$, representing an improvement of 14.6 $\mu\text{F}/\text{cm}^2$ to the unmodified biochar W350 (Fig. 5a). The capacitance value of W700-M (388 $\mu\text{F}/\text{cm}^2$) was also notably higher, in comparison to the unmodified biochar (Fig. 5a).

Besides capacitance, the electron transfer is also determined by the resistance R , which can be reliably analyzed using EIS under an open circuit voltage (Panigrahi et al. 2024). Nyquist plot is a widely applied method for analysing EIS data, and the result showed that the R of the KBH₄-modified biochar was significantly smaller than unmodified biochar in the middle-high frequency

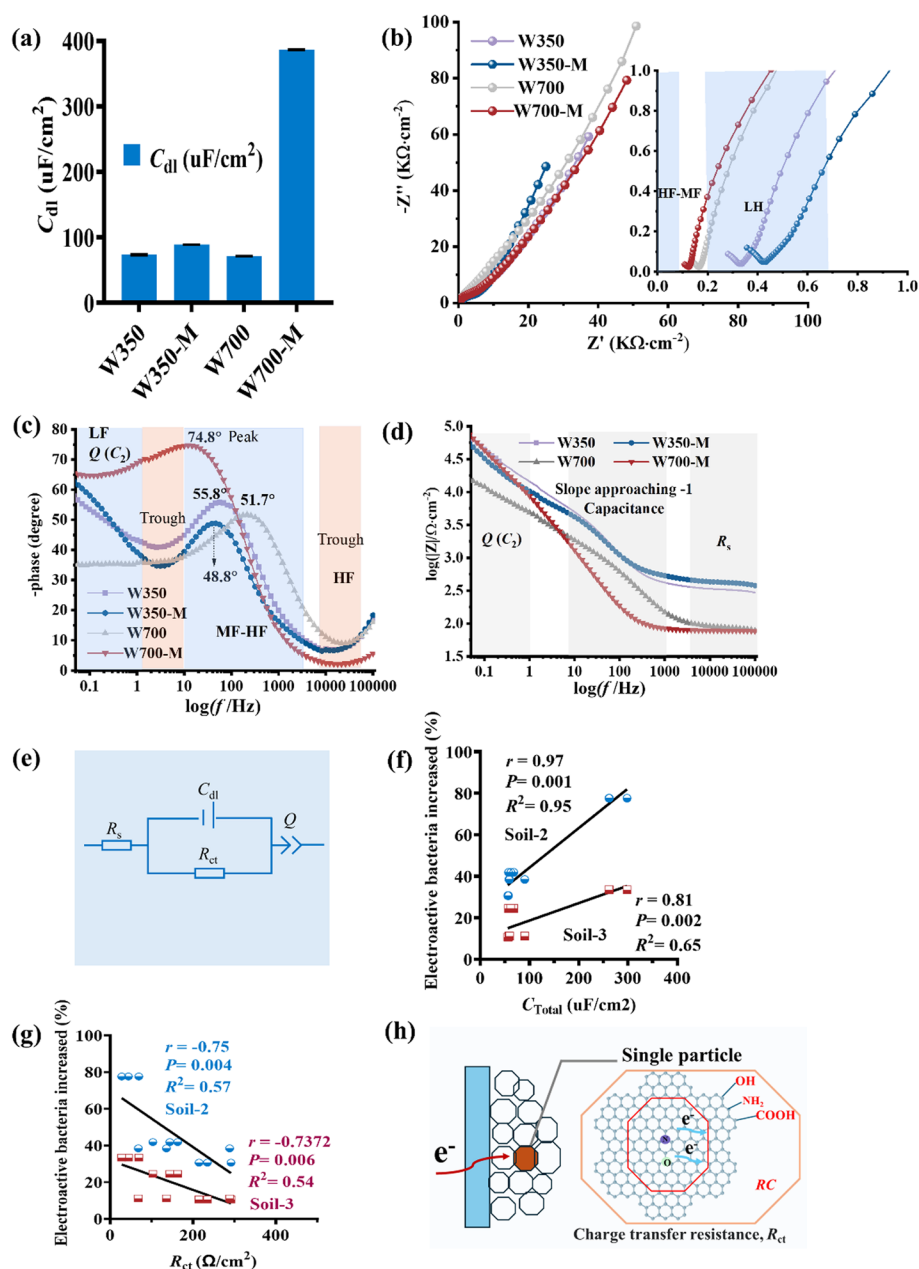


Fig. 5 Electrochemical properties of biochar. Capacitances of biochar prepared at different temperatures (a); Nyquist of biochar (b); Bode plots of biochar including Bode phase degree plot (c) and Bode modulus plot (d); Equivalent electric circuit (e); The relationship between the increased percentage of electroactive microorganisms in soils after biochar application and the capacitance value (f) or transfer resistance (g); Diagram of electron transfer model at biochar interface (h)

(MF-HF) range (Fig. 5b). Biochar structure is highly heterogeneous, and thus biochar may be involved in multiple electrochemical processes, with different time constants. However, the Nyquist plot is in linear axes and only sensitive to one electrochemical process (Huang et al. 2016). Furthermore, the limitation of Nyquist plot is caused by the distortion of the semicircles at high frequencies due

to inductive effect and at low frequencies due to diffusive effect (Huang et al. 2016). Bode plots are an essential complement to Nyquist plots.

Bode plots comprise by phase angle plot and modulus plot, providing more details about resistors and capacitors because of the direct illustration of frequency. The phase angle Bode plot can be employed to count the

number of interfacial electrochemical processes involved in EIS. A peak in the MF-HF region and two troughs were observed (Fig. 5c), indicating the presence of two-time constants, corresponding to different electrochemical processes. The maximum phase angle of W350-M (48.8°) is lower than that of W350 (55.8°) in the MF-HF region (Fig. 5c), indicating that KBH_4 -modification enhanced the capacitance of biochar. The phase angle lines of W350 and W350-M were not parallel to the x axis, suggesting that faradaic type electron transfer or redox reaction was the dominant mechanism for charge storage (Saha et al. 2022). The phase angle lines of W700 and W700-M were parallel to the x axis in the low-frequency region, implying excellent capacitive behaviour (Fig. 5c). In addition, previous studies have confirmed that the initiate point at x axis in MF region could be attributed to solution resistance (R_s), the slope of line approached -1 for a capacitor, and approaches zero for a resistor in Bode modulus plot (El-Kady et al. 2012). Our observations illustrated that the slopes of Bode modulus plots for all the investigated biochars were close to -1 MF-HF region, indicating a typical capacitive behavior (Fig. 5d). Collectively, we observed a general capacitance-dominant charge storage process (Saha et al. 2022), and that the modified biochar exhibited higher capacitance ability than the unmodified biochar.

By combining the Bode phase angle and modulus plots, the multiple electrochemical processes of biochar can be analysed using the equivalent electric circuit model $R(CR)Q$ (Tables S3–S4). The number of peaks or troughs in the phase angle Bode plot equals the number of (CR) or (QR) elements in the equivalent circuit, and a (CR) or (QR) unit corresponding to an interfacial electrochemical process (2021). The components in equivalent circuit may include solution resistance (R_s) (the intercept of Nyquist at x axis at high frequencies (HF)), charge transfer resistance (R_{ct}), electrical double-layer capacitor (C_{dl}), and the constant phase element (Q) (Ter Heijne et al. 2011; Wang et al. 2021a, b). The double-layer capacitance of biochar is assumed to be heterogeneous, and the exponent n ($0 < n < 1$) represents the deviation between Q and C, implying the degree of biochar electrode inhomogeneity. When n is close to 1, Q is replaced with an ideal capacitor.

To compare the capacitance ability between different biochars, Q was converted to C using the equation $C = Q(\omega_{max})^{n-1} = Q(2\pi f)^{n-1}$ (Li et al. 2019). The double layer capacitance C_{dl} of W350, W350-M, W700 and W700-M were $57.38 \mu\text{F}/\text{cm}^2$, $60.78 \mu\text{F}/\text{cm}^2$, $93.22 \mu\text{F}/\text{cm}^2$ and $564.6 \mu\text{F}/\text{cm}^2$, respectively (Table S4). This result is consistent with the results of CV results, but with more detailed and accurate information after excluding the external factors (such as solution resistance). Interestingly, a non-ideal capacitor is connected in series that is

not affected by resistance, which implies a rapid electron exchange. The overall capacitances of W350, W350-M, W700 and W700-M in the equivalent circuit were $56.88 \mu\text{F}/\text{cm}^2$, $57.02 \mu\text{F}/\text{cm}^2$, $90.10 \mu\text{F}/\text{cm}^2$ and $297.95 \mu\text{F}/\text{cm}^2$, respectively (Table S4). The KBH_4 -modified biochar (W350-M and W700-M) exhibited a higher capacitance and lower resistance than the unmodified biochar. Correlation analysis showed that the increase in electroactive bacteria was related positively related to biochar capacitance (Fig. 5f), however, negatively related to biochar resistance (Fig. 5h). Therefore, we concluded that the electrochemical properties of biochar are essential for soil charosphere ecosystems and further investigations are warranted.

Using FTIR, we aimed to further illustrate the structural basis of biochar electrochemical properties. The FTIR analysis reveals prominent peaks at 3424 cm^{-1} , 2071 cm^{-1} , 1626 cm^{-1} , 1505 cm^{-1} , 1391 cm^{-1} , 1086 cm^{-1} and 800 cm^{-1} in both unmodified and modified biochars at 350°C and 700°C , corresponding to $-\text{NH}_2/-\text{OH}$, $\text{C}\equiv\text{C}$, $\text{C}=\text{C}$ of aromatic ring/ $\text{C}=\text{O}/\text{C}=\text{N}$, pyridinic-N, $\text{C}=\text{C}$, C-O, saturated aromatic hydrocarbons and aromatic C-H groups, respectively (Gao et al. 2021; Luo et al. 2023a; Shao et al. 2018; Wu et al. 2017). After modification, the intensity of $\text{C}\equiv\text{C}$ absorption peak increased, while that of C-O decreased, suggesting an elevated electron-rich (orbital) high-energy alkyne bond in KBH_4 -modified biochar (Fig. 6). The abundant π -electron system associated with aromatic structure and $\text{C}\equiv\text{C}$ provide more electron donating capacities, and thus promote the removal of ginsenoside Rb1. The N- and O-containing functional groups could increase the hydrophilicity of the electrochemical electrodes which reduces their mass transfer resistance (Chen et al. 2024). The aromatic properties of biochar were characterized by the molar ratios of H to C, while the atomic ratio of O/ and (O+N)/C can be used as indices to evaluate the degree of aromatization and polarity of biochar. The results showed that the content of N in KBH_4 -modification biochar decreased, whereas the H/C, O/C, and (O+N)/C values increased (Table S5). These findings imply that N and O content can increase biochar polarity, which consequently decreases biochar capacitance but enhances the electron exchange ability between of biochar and microorganisms. The increased electron exchange capacity may increase the activity of enzymes such as sucrase, urease and peroxidase, thus accelerating the degradation of ginsenoside Rb1.

4 Conclusions

Our study provides direct evidence that biochar can alter the electron transfer to promote the growth of electro-trophic microorganisms in charosphere, which is crucial process for the microbial degradation of ginsenoside Rb1.

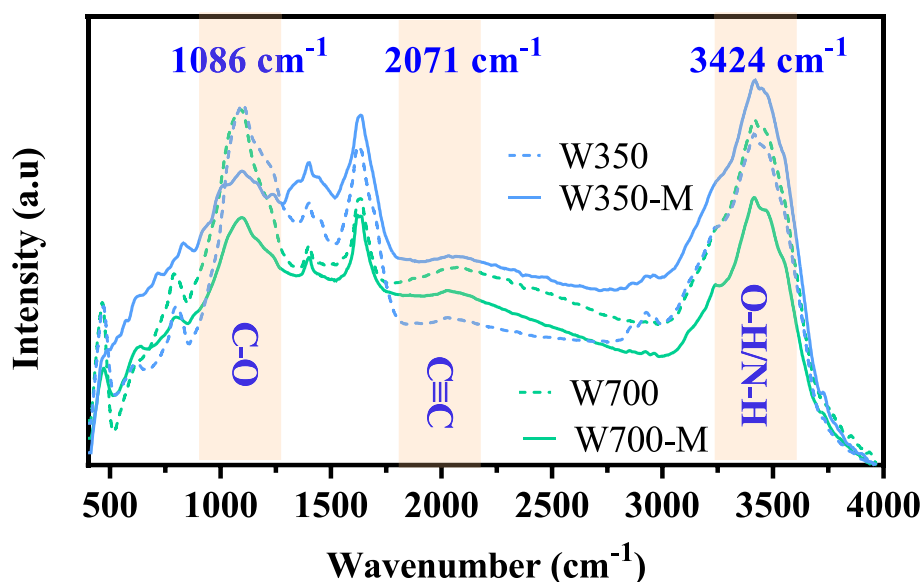


Fig. 6 Changes in oxidizing and reducing functional groups on the surface of biochar by KBH_4 -modified

This process may also be applicable for other carbonaceous materials rich in $\text{C}\equiv\text{C}$ groups with π -electron. Biochar enhances the population of electroactive bacteria, such as *Bacillus*, *Paenibacillus* and *Klebsiella* in *P. pseudoginseng* plantation soil, while suppressing fungal pathogens such as *Fusarium* and *Alternaria*. In particular, biochar with high levels of electron-rich groups improves the overall microbial community structure and facilitates the degradation of organic compounds. This study highlights the importance of biochar capacitive properties for electron exchange capacity and contributes to the ongoing efforts to efficiently manage ginsenoside Rb1 levels for controlling continuous cropping challenges through the strategic use of carbonaceous materials. In addition to ginsenoside Rb1, electron transfer may occur between root exudates (such as phenolic acids and enzymes), carbon materials containing electron-rich groups, and electrophilic microorganisms, which should all be considered when overcoming continuous cropping obstacles.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s44246-025-00199-4>.

Supplementary Material 1.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Shunling Li and Xuemei Wang. The first draft of the manuscript was written by Shunling Li and Bo Pan. The manuscript was reviewed and edited by Weiheng Qin, Min Wu, Bo Pan, Christian Stainberg. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability

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

Declarations

Competing interests

Bo Pan is an editor of Carbon Research and was not involved in the editorial review, or the decision to publish, this article. All authors declare that there are no competing interests.

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