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ORIGINAL PAPER

Biochar application reduces residual napropamide in the rhizosphere and improves soil microbial diversity

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Abstract

The effect and mechanism of the removal of napropamide residues were studied in the rhizosphere after the application of corn straw biochar (CB) combined with low-molecular-weight organic acids (LMWOAs). Adsorption isotherm of napropamide on biochar, tobacco hydroponic, and soil incubation experiments were carried out. After 1 month of incubation, 86.58% of napropamide was dissipated by the combined addition of 2% CB and 10 mg kg^{-1} LMWOAs. During this process, CB strongly adsorbed the napropamide in the soil, rapidly reducing its bioavailability. The relative abundances of microbial species involved in the napropamide biodegradation increased by the combined treatment, enhancing xenobiotic degradation in soil. Moreover, the napropamide desorbed from CB-amended soils by LMWOAs was effectively biodegraded. The combined application of CB and LMWOAs significantly increased the relative abundances of keystone species participating in nutrient cycling and herbicide removal. Taken together, this study can contribute to develop remediation practices of soil contaminated with residual amide herbicides.

Keywords Biochar · Low-molecular-weight organic acid · Napropamide · Removal process · Microbial community composition

Introduction

With the rapid development of intensive agriculture, the amount and frequency of pesticide applications, including herbicides, are increasing. Napropamide (N,N-diethyl-2-(1-naphthalenyloxy)-propionamide) is a polar, nonionizable, and selective chiral amide herbicide used to control annual and perennial grasses and broad-leaved weeds in agricultural soils (Xie et al. 2019). Napropamide has become one of the most popular herbicides worldwide (Cutulle et al. 2019; Musarurwa and Tavengwa 2020). Currently, excessive and improper application of herbicides in agricultural systems has led to many problems, not only polluting tens of millions of hectares of farmland soil but also causing damage

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to sensitive crops (Pan et al. 2018). Gerstl and Yaron (1983) found that napropamide had a relatively long persistence in soil, and the adsorption coefficient was strongly correlated with soil organic matter (OM) content. Napropamide residues in soil can be taken up by tea leaves, soybean, cucumber, and other plants (Biswas et al. 2007; Qi et al. 2015), with the result that agricultural production and even human health could be severely threatened by residual napropamide. Moreover, residual napropamide can inhibit soil activity and decrease soil respiration and microbial biomass (Medo et al. 2021; Sun et al. 2020), perturbing soil functions. Thus, it is of great concern to reduce the environmental hazards posed by residual napropamide in soils.

It is widely accepted that biochar can decrease the availability of herbicides (e.g., acetochlor, atrazine, pyrazosulfuron-ethyl, and terbuthylazine) in soils to crops by chemical or physical adsorption. The large sorption capacity is attributed to the large specific surface area, porous structure, and abundant surface functional groups of biochar (Huang et al. 2020; Li et al. 2018; Sadegh-Zadeh et al. 2017). Acetochlor can be effectively adsorbed by the biochar derived from crofton weed, and the adsorption is enhanced by increasing specific surface area of the biochar (Li et al. 2018). Biodegradation is considered to be the best way to completely remove organic pollutants in soil (Deng et al. 2021a). The influence of biochar on the biodegradation of different organic pollutants in soil is not consistent (Hu et al. 2020). Rice husk biochar pyrolyzed at 300 °C reduced the half-life of imidazolinone from 41 to 26 days by promoting soil microbial activity (Yavari et al. 2019). However, some studies have reported that the strong sorption capacity of biochar reduced the bioavailability of organic pollutants to degrading bacteria, and reduced the bioavailability of soil nutrients, inhibiting the growth of microbial degraders (Ding et al. 2021; Grgić et al. 2019). Additionally, soil physicochemical properties can be improved by biochar amendment, and this can affect soil microbial communities, thereby influencing the biodegradation of organic pollutants in soil (Muhammad et al. 2014; Zhang et al. 2018a). Both the direct and indirect effects of biochar on soil microbial communities are closely related to how biochar affects the biodegradation of organic pollutants in soil (Li et al. 2019). These features of biochar are thought to be effective in reducing the environmental risk of napropamide residues in soil. To date, the mechanism of biochar on napropamide biodegradation and microbial community compositions in soil remains unclear.

Many studies have shown that the dissipation of organic pollutants (e.g., atrazine, prometryn, and hexachlorocyclohexane) in the rhizosphere was significantly greater than in bulk soil (Fan et al. 2020; Hand et al. 2020; Rodríguez-Garrido et al. 2020). Rhizodegradation of pollutants may benefit from the key role of low-molecular-weight organic acids (LMWOAs) secreted by plant roots during the dissipation process. In general, LMWOAs will bind with metal cations, breaking the bonds and releasing soil OM, thereby reducing the sorption of organic pollutants in soil (Peña 2022). However, the role played by LMWOAs in altering the sorption behavior of napropamide in biochar-amended soils is still unknown. Organic pollutants can be more susceptible to biodegradation after LMWOAs enhance their bioavailability in soil (Liu et al. 2018; Tong et al. 2021; Vázquez-Cuevas et al. 2020). LMWOAs can act as carbon and energy resources for microbial growth (Thomas and Cébron 2016), or alter the richness of certain microbes (Li et al. 2019), leading to changes in soil microbial diversity. Shifts in soil microbial community composition can reflect changes in microbial functions in soil (Donnison et al. 2000), including the ability of microorganisms to degrade organic pollutants. Tao et al. (2020) found that a mixture of succinate, methylmalonate, oxalate, 3-hydroxybutyrate, citrate, and α-ketoglutarate promoted the biodegradation of atrazine by inducing the expression of the atrazine-degrading genes *trz*N, *atz*B, and *atz*C. It is therefore necessary to thoroughly understand the response of rhizospheric microbial communities, including changes in microbial community composition and function to napropamide stress in biochar-amended soils. An investigation of the microbial responses following biochar application in the presence of LMWOAs can improve our understanding of the microbial strategies in response to biochar to increase napropamide dissipation in the rhizosphere. Such study is crucial to assess the feasibility of using biochar for the in situ remediation of napropamide-contaminated soils.

Therefore, the objectives of this study were to (i) investigate whether the individual/combined effects of biochar and LMWOAs could reduce the residual level of amide herbicides in the soil, (ii) determine the influence of LMWOAs on the desorption and biodegradation of napropamide in biochar-amended soil, and (iii) reveal the relationship between the changes in the composition and activities of microbial communities and napropamide dissipation in soil amended with biochar and LMWOAs. Initially, four kinds of straw biochar were compared to screen out the biochar with the largest adsorption capacity for napropamide for use in the subsequent experiments. Then, tobacco was selected as the model crop because napropamide is one of the most commonly used herbicides applied to tobacco fields. The main LMWOAs secreted by tobacco under napropamide stress were identified, and their impact on the desorption of napropamide on biochar-amended soil was evaluated. Finally, biochar and the LMWOAs were added to the soil separately or in combination and incubated for 1 month. The soil microbial community composition and function were coupled to reveal the microbial response to napropamide-contaminated soil. To the best of our knowledge, this study is the first report to systematically elucidate the effects and

mechanisms of biochar and LMWOAs on the dissipation of napropamide in soil.

Materials and methods

Preparation of the soil and biochar

Soil was sampled from an agricultural field (depth of 0–20 cm) in Linyi, Shandon Province (35°65′93″ N, 118°74′35″ E). The soil was air-dried, ground, and sieved through a 2-mm sieve. The details of the soil properties can be found in the "Supporting information" (SI Table S1).

Corn (*Zea mays* L.), canola (*Brassica campestris* L.), peanut (*Arachis hypogaea* L.), and rice (*Oryza sativa* L.) were selected to produce biochars at 300 °C. These biochar types were referred to as corn straw–derived biochar (CB), canola straw–derived biochar (CAB), peanut straw–derived biochar (PB), and rice straw–derived biochar (RB), respectively. The preparation details and basic characteristics of the biochar are shown in Table S2 and in our previous studies (Ni et al. 2020; Shi et al. 2017).

Adsorption isotherm of napropamide on biochar

A batch equilibrium experiment was conducted to compare the sorption capacities of the straw biochar for napropamide. Napropamide solution in acetonitrile was added to 20 mL of 10 mmol L^{-1} $CaCl_2$ solution in a 50-mL glass tube, resulting in initial concentrations of napropamide ranging from 0.5 to 5 μg mL^{-1}. Then, 0.5 g of the biochar was added to the tubes, which were immediately closed with Teflon-lined screw caps and then rotated on an overhead shaker at 200 rpm for 72 h at 25 °C. The solutions were centrifuged at 3644 g for 10 min, and then the supernatant was filtered through a 0.22-μm microporous membrane for subsequent determination via an ultra-high-performance liquid chromatography (UHPLC) 1290 infinity system coupled with an AB-4500 Qtrap mass spectrometer (AB-SCIEX, Framingham, MA, USA) and equipped with a Poroshell 120EC-C18 column (2.7 μm, 2.1 × 75 mm internal diameter, Agilent, Santa Clara, CA, USA). The details of the various parameters of the UHPLC instruments used for measuring napropamide are shown in SI, Text S1.

Soil incubation experiment

Twenty grams of sampled soil was weighed, placed into a beaker, and mixed with 1 mL of 1000 mg L^{-1} napropamide until the acetonitrile was completely volatilized. Then, the 20 g polluted soil sample was mixed with 180 g of clean soil sample in the same beaker to have 200 g of soil with an initial napropamide concentration of 5 mg kg^{-1}. This was repeated for a total of 12 beakers, which were incubated in a growth chamber at 25 °C, and at 25% moisture content for 2 weeks. Then, 2% of the biochar with the largest adsorption capacity according to the results of the batch equilibrium experiment, and a mixture of 10 mg kg^{-1} LMWOAs (acetic acid:glycolic acid:maleic acid:succinic acid = 1:8:6:2, Table S3), based on the data from the hydroponics experiment (SI Text S2), were added to the soil to have the following treatments: control (no biochar and LMWOAs), 2% CB, 10 mg kg^{-1} LMWOAs, and 2% CB + 10 mg kg^{-1} LMWOAs. Each treatment was replicated three times. All the beakers were placed in the growth chamber, regularly watered, and sampled at days 0, 2, 7, 14, and 30. The soil samples were then used to determine the residual level of napropamide.

The contents of soil OM, dissolved organic C (DOC), total N (TN), nitrate-nitrogen (NO_3^--N), ammonium-nitrogen (NH_4^+-N), total P (TP), available P (AP), total K (TK), and available K (AK) were measured before and after the cultivation (Carter and Gregorich 2007; Pansu and Gautheyrou 2007). At the last time point, a high-throughput sequencing analysis of soil microbes was also conducted.

Residual napropamide data were fitted to the following first-order elimination kinetic equation:

$$C_t = C_0 e^{-kt}$$

where C_t is the napropamide concentration in the soil (mg kg^{-1}) at time t, C_0 is the initial napropamide concentration in the soil (mg kg^{-1}), and k is the digestion rate constant. The half-life ($T_{1/2}$) of napropamide was determined via the algorithm $T_{1/2} = \ln 2/k$.

Napropamide desorption experiment

Desorption of napropamide from the control and 2% CB-amended soils by LMWOAs was conducted after the soil incubation. Briefly, 0.5 g of soil was amended with 20 mL of 1, 10, and 20 mg L^{-1} LMWOA solutions. Mercuric chloride at a concentration of 100 mg L^{-1} was added to prevent microbial degradation. Napropamide in the soil samples was extracted after shaking for 24 h and high-speed centrifugation (at 2879 g) and determined by UHPLC. The DOC content in the supernatant was determined by a multi N/C 3100 analyzer (Analytik Jena, Germany).

Extraction and determination of napropamide

Briefly, 5 g of soil was extracted with 10 mL of deionized water and 10 mL of acetonitrile in a glass centrifuge tube by shaking for 30 min followed by centrifugation for 5 min. The method used for determining the napropamide concentration of the acetonitrile layer was the same as that used for

the samples in the batch equilibrium experiment described in SI, Text S1.

High-throughput sequencing and functional gene prediction

The genomic DNA of the soil samples sampled during the incubation was extracted using a PowerSoil DNA isolation kit (MoBio Laboratories, Inc., Carlsbad, CA, USA). The primers 806R and 338F were used to amplify the bacterial V3–V4 region, and the primers ITS1-F and ITS2 were used to amplify the fungal internal transcribed spacer (ITS) regions (Hou et al. 2020a, b; Zhang et al. 2018b), followed by a polymerase chain reaction (PCR) (SI Text S3). Total numbers of 16S and ITS reading were 25,099 and 29,787, respectively. The bacterial and fungal DNA sequences were submitted to the Sequence Read Archive of the National Center for Biotechnology Information (NCBI) database under accession number PRJNA808253. Then, the operational taxonomic units (OTUs) of the 16S rRNA sequences were normalized via the Phylogenetic Investigations of Communities by Reconstruction of Unobserved States (PICRUSt) software package, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) ortholog (KO) functional profiles were then calculated with the PICRUSt algorithm based on the KO output (http://www.kegg.jp/). The functional groups of fungal OTUs associated with specific ecosystem functions were inferred via FUNGuild 1.0 (Nguyen et al. 2016).

Statistical analysis

Two different napropamide concentrations (5 mg kg^{-1} and 500 µg kg^{-1}) were added to the original soil samples to estimate the recovery of the herbicide in the test samples. The extraction and determination procedures used were the same as those described above. The details of the quality assurance and quality controls are shown in SI, Text S4. The average recovery of napropamide in the soil was 102 ± 8%.

The Chao1 and Shannon indices were calculated via MOTHUR 1.30.2, and a canonical correlation analysis was used to analyze the soil microbial communities via R Studio 4.1.3. The significantly different bacteria/fungal responses to the various treatments were screened on the basis of a linear discriminant effect size of 3.5.

Results and discussion

Sorption of napropamide on the biochars

The sorption isotherms of napropamide on the four types of straw biochar, i.e., CB, CAB, RB, and PB, are shown in Fig. 1. All adsorption isotherms fitted the Freundlich and Langmuir equations well ($R^2 = 0.907–0.997$). The values of 1/n were less than 1 (Table S4), indicating a nonlinear sorption caused by surface adsorption (Al-Ghouti and Da'ana 2020). The Q_m values indicated that the napropamide adsorption on the biochars followed the order of CB (44.61 mg g^{-1}) > CAB (39.58 mg g^{-1}) > RB (36.44 mg g^{-1}) > PB (27.92 mg g^{-1}). The surface functional groups on CB were similar to those on the other three kinds of biochar (Fig. S1). As shown from the surface morphology of the biochars, CB and CAB had abundant pore structures (Fig. S2). Additionally, the *Q*m values of the biochars were positively correlated with their surface areas ($r = 0.98$, $p < 0.05$). In this study, the high adsorption capacity of the CB was mainly dependent on its large surface area and developed pore structure, which provided active adsorption sites for napropamide (Tan et al. 2021). As shown in Table S4, the *E* values of RB, PB, and CB varied from 0 to 8 kJ mol^{-1} (1.64–3.24 kJ mol^{-1}), indicating that the sorption process of napropamide on these biochars was dominated by physical adsorption, and the high *E* values of CAB (8.39 kJ mol^{-1}) indicated that chemical processes, including the formation of

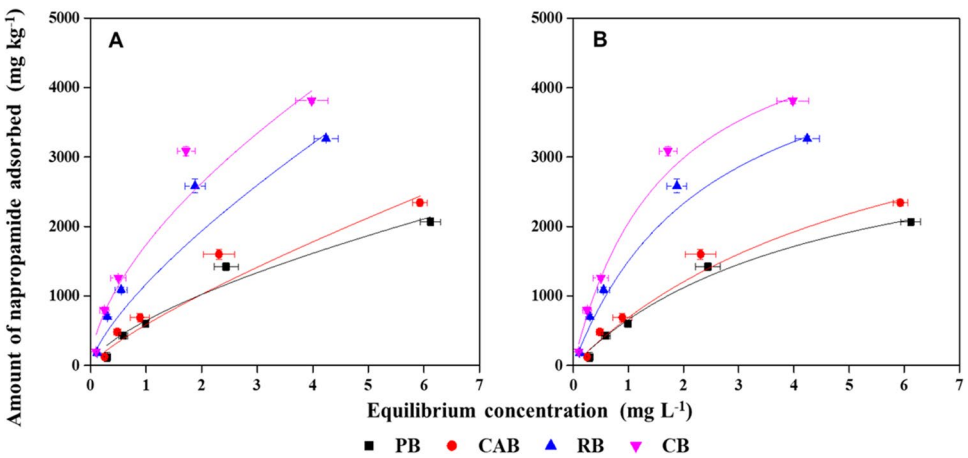

Fig. 1 Sorption isotherms of napropamide on the four types of straw biochars, i.e., corn (CB)-, canola (CAB)-, peanut (PB)-, and rice (RB) straw–derived biochars (curves of **A** were fitted by Freundlich equation, and curves of **B** were fitted by Langmuir equation)

hydrogen and coordination bonds, cannot be ignored (Wang et al. 2020). It was reported that the adsorption mechanism of biochar derived from herb residues at 300 °C for metolachlor included surface chemical adsorption, such as the π-π bond (Wei et al. 2020). Based on the above results, CB was selected for the subsequent experiments.

Dissipation of napropamide in the soil

The dynamic dissipation of napropamide in the control and amended soils is presented in Fig. 2. The napropamide concentration in the control group gradually decreased during the incubation period, but after 15 days, it remained constant, with a final residual percentage of 26.62%. It has been reported that indigenous soil microorganisms could degrade napropamide under optimum oxygen and moisture conditions (Pischedda et al. 2019). Compared to the control group, the addition of 10 mg kg^{-1} LMWOAs did not significantly affect the dissipation trend of napropamide and the residual napropamide amounts. After amendment with 2% CB, the decreased rate in napropamide concentration was much slower, and the amount of napropamide was 12.92% greater than that of the control group at the end of the incubation. The result indicated that 2% CB likely adsorbed a certain amount of napropamide. The entrapment and accumulation of the herbicide around the biochar particles makes degradation by soil microorganisms difficult (Deng et al. 2021b). Low bioavailable pollutants exist in the adsorbed form, which poses a low environmental risk (Zong et al. 2021).

In the treatment in which 2% CB was combined with 10 mg kg^{-1} LMWOAs, the dissipation of napropamide

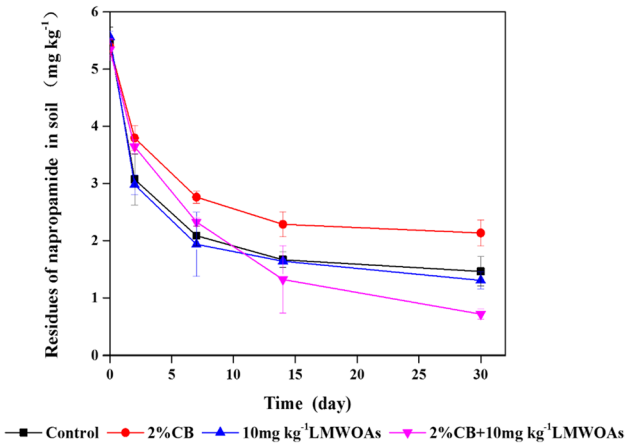

Fig. 2 Residues of napropamide in the soils during the incubation period. Control, no biochar and LMWOAs addition; 2% CB, 2% corn straw–derived biochar addition; 10 mg kg^{-1} LMWOAs, the mixture of 10 mg kg^{-1} LMWOAs (concentration ratio, acetic:glycolic:maleic:succinic acid = 1:8:6:2); 2% CB + 10 mg $kg.^{-1}$ LMWOAs, combination of 2% corn straw–derived biochar and the LMWOA mixture. Error bars indicate the standard deviation ($N = 3$)

was more complex. During the first 10 days of incubation, the amount of residual napropamide was greater in the combined treatment than that in the control soil, the biochar reduced the napropamide dissipation in the soil in the early stage of cultivation. Then the residual amount of napropamide was greatly reduced, with 86.58% of the herbicide removed at the end of the incubation period. The dissipation percentage was significantly higher than that in the control soil by 13.20%. The half-life of napropamide in the control and treatment groups varied between 2.81 and 3.21 days, with the shortest time recorded in response to the combined treatment (Table S5). Similarly, hexachlorobenzene (HCB) dissipation in biochar-amended rhizosphere was significantly enhanced (Song et al. 2016), because the (i) increased bioavailability of immobilized HCB and (ii) enhanced microbial activities, both of which were induced by oxalic acid. Likely the napropamide removal in the biochar and LMWOA treatment occurred. During the process, LMWOAs gradually released the napropamide adsorbed onto the biochar and soil particles, and more of the available napropamide would then be removed by "activated" microbial communities in the simulated rhizosphere amended with the biochar.

The desorption capacity of herbicides determines their bioavailability in soil (Alvarez et al. 2021). As shown in Table S6, no obvious desorption of napropamide was detected in the control group, 2% CB treatment without LMWOAs, or 2% CB treatment with LMWOAs at a concentration of 1 mg L^{-1}. The desorption of napropamide in the 2% CB treatment was significantly increased by 10.16% and 15.64% in the presence of the 10 and 20 mg L^{-1} LMWOA solutions, respectively. Additionally, the higher the DOC concentration in the equilibrium solution (excluding the DOC concentration of LMWOAs), the higher the napropamide desorption percentage (Table. S6). The LMWOAs could disrupt the soil OM-cation-mineral linkages, leading to the release of soil OM, resulting in an increase in the DOC concentration in the soil solution (Peña 2022). The DOC concentration has been shown to be positively correlated with the desorption of polycyclic aromatic hydrocarbons, brominated diphenyl ethers, phthalates, and hexachlorocyclohexane isomers in soils or sediments (Du et al. 2020; Huang et al. 2016; Rodríguez-Garrido et al. 2020; Vázquez-Cuevas et al. 2020). It is therefore highly likely that LMWOAs disrupted the interactions of DOC with soil and biochar particles, and more DOC-bound napropamide was released into the soil solution, increasing the bioavailability of napropamide. Subsequently, changes in the biochemical processes in which microorganisms are involved after the combined application of CB and LMWOAs need to be explored to explain the rapid dissipation of the released napropamide in the simulated rhizosphere.

Shifts in microbial community composition

The activity and abundance of soil microorganisms are the main driving forces of herbicide biodegradation (Rodríguez et al. 2020; Verma et al. 2014). After 1 month of incubation, compared to the control group, the soil bacterial diversity (as indicated by the Shannon and Simpson indices) decreased in the combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs, while the fungal diversity was greatly increased (Fig. S3A, S3B, S3D, S3E). The soil bacterial richness (indicated by the Chao1 index) also decreased in response to the combined application, while the soil fungal richness increased ($p < 0.05$). Previous studies have reported the positive, neutral, and negative effects of biochar on microbial diversity and richness (Jiang et al. 2021; Meng et al. 2019; Nguyen et al. 2018). These inconsistent findings may depend on changes in soil type, biochar species, biochar application rate, and duration (Li et al. 2020). According to the clustering results and the degree of variation, the microbial diversity was not significantly different between the control group and the treatment involving 10 mg kg^{-1} LMWOAs alone (Fig. 3A and 3B), indicating that the microbial community composition in the contaminated soil was not significantly changed by the addition of 10 mg kg^{-1} LMWOAs. This may be the reason why napropamide was not effectively removed in this treatment (Fig. 2). The bacterial community was significantly separated by the combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs as well as fungal communities. The soil pH, OM, and TN concentrations were positively related to the microbial community of the combined treatment, while the NO_3^--N concentration was negatively related to the microbial community. Soil pH and nutrient concentrations are reported to be the most critical factors affecting microbial composition and functions in soils amended with biochar or root exudates (Kawasaki et al. 2016; Palansooriya et al. 2019).

The combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs greatly shifted the microbial community composition at multiple taxonomic levels. The proportion of the bacterial phyla with a relative abundance greater than 1% shifted from 96.87 to 98.60%. The proportions of the bacterial phyla whose abundance was between 0.1 and 1%, and those whose abundance was less than 0.1% decreased by 39.67% and 49.33%, respectively. Other studies have also found that bacterial phyla with low abundances were sensitive to environmental contamination (Dougal et al. 2013; Li et al. 2019). Compared to the control group, the relative abundance of Cyanobacteria in the combined treatment group increased significantly while the relative abundances of Gemmatimonadetes, Nitrospirae, Planctomycetes, and Armatimonadetes decreased (Fig. S4A). Cyanobacteria has a great potential to degrade pesticides (Srivastava et al. 2021), and the relative abundance of Gemmatimonadetes is always positively correlated with the level of soil contamination (Li et al. 2021a). Cyanobacteria, Nitrospirae, and Planctomycetes are closely related to the soil N cycle (Jia et al. 2020; Srivastava et al. 2021). The proportion of the fungal phyla whose relative abundance was greater than 1% increased slightly. The proportion of the fungal phyla whose relative abundance was between 0.1 and 1% increased from 21.15 to 78.56%. The phyla, whose relative abundance was less than 0.1% decreased by 28.72%. The relative abundances of Chytridiomycota and Ascomycota significantly increased in the combined treatment, while the relative abundances of Glomeromycota and Mucoromycota decreased (Fig. S4B). Chytridiomycota is always inhibited in response to the addition of herbicides, including acetochlor and atrazine, and in this study was strongly correlated with soil OM and N concentrations (Wang et al. 2021). The increased abundance of Ascomycota implies that soil fertility was improved (Li et al. 2021b). The richness of Mucoromycota was negatively correlated with the soil AP concentration (Zhang et al. 2019). At the bacterial genus level, as shown in Fig. 4A, the relative

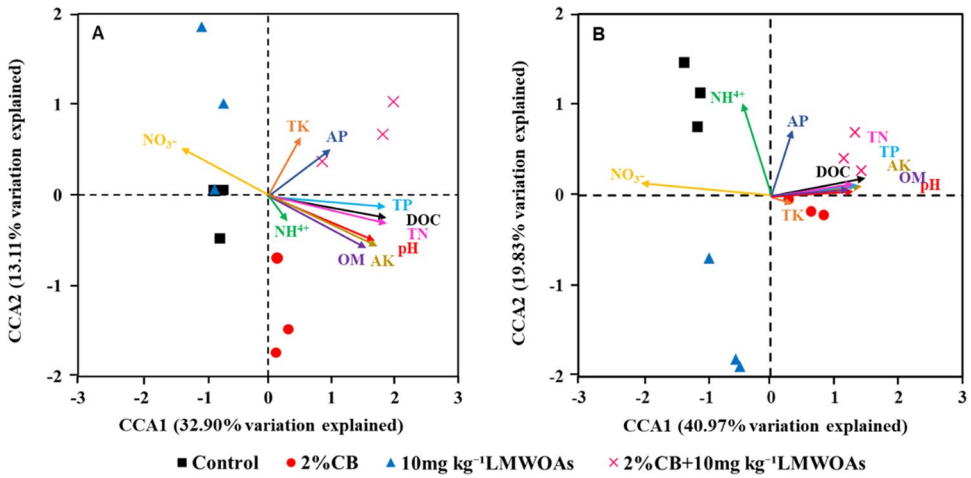

Fig. 3 Canonical correspondence analysis (CCA) of bacterial (**A**) and fungal (**B**) community structure in the soils after the incubation. Control, no biochar and LMWOAs addition; 2% CB, 2% corn straw-derived biochar addition; 10 mg kg^{-1} LMWOAs, the mixture of 10 mg kg^{-1} LMWOAs (concentration ratio, acetic:glycolic:maleic:succinic acid = 1:8:6:2); 2% CB + 10 mg kg^{-1} LMWOAs, combination of 2% corn straw-derived biochar and the LMWOA mixture

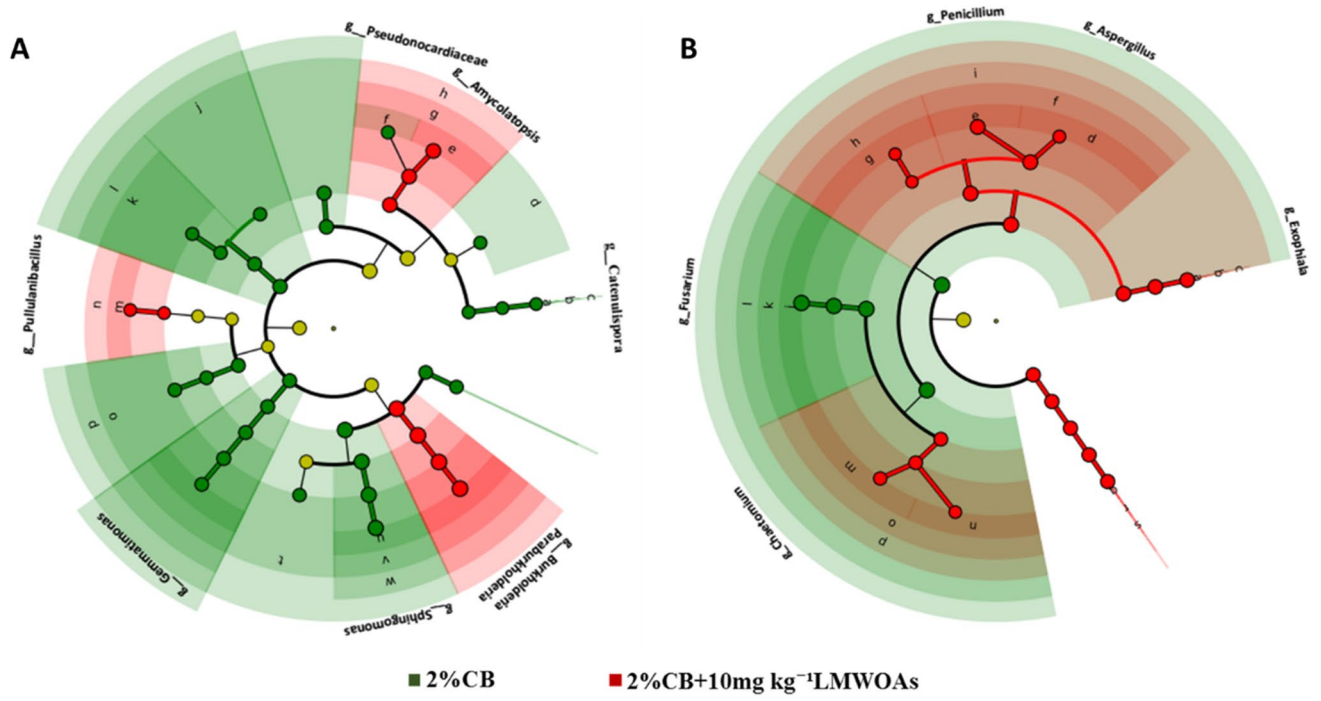

Fig. 4 Linear discriminant effect size analysis cladogram of comparison results at bacterial (**A**) and fungal (**B**) genus levels between the control and the combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs after the incubation. 2% CB, 2% corn straw–derived biochar addition; 2% CB + 10 mg kg^{-1} LMWOAs, vombination of 2% corn straw–derived biochar and the LMWOA mixture

abundances of *Catenulispora*, *Pseudonocardia*, *and Gemmatimonas* significantly decreased in response to the combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs, whereas the relative abundances of *Pullulanibacillus, Amycolatopsis* and *Paraburkholderia* increased. *Pullulanibacillus* is reportedly closely related to the soil OM and NO_3^- concentrations (Ding et al. 2021). *Amycolatopsis* plays a crucial role in the degradation of acetochlor and metolachlor in soil (Han et al. 2022), and *Paraburkholderia* is beneficial for plant growth and fitness (Esmaeel et al. 2018). The relative abundances of the fungal genera *Chaetomium*, *Penicillium*, *Exophiala*, and *Aspergillus* significantly increased, while the relative abundance of *Fusarium* decreased, compared to the control group (Fig. 4B). *Penicillium* and *Aspergillus* have previously performed well in terms of the remediation of pesticide-polluted rhizospheres (Asemoloye et al. 2019; Zhang et al. 2020). *Chaetomium*, which is a beneficial rhizosphere-inhabiting fungus, has shown potential biocontrol effects on many plant pathogens (Gao et al. 2019), and *Exophiala* can promote plant growth via hormone production and P absorption under abiotic stress (Xu et al. 2018). *Fusarium* can produce toxins under certain environmental conditions, causing poisoning in humans and domestic animals (Antonissen et al. 2014). It is therefore likely that a large amount of microbial species, which are associated with herbicide degradation and involved in soil nutrient cycling (especially C and N), emerge in the simulated rhizosphere environment.

Responses of microbial community functions

Soil microbial functions associated with microbial metabolism were investigated by PICRUSt gene prediction. Bacterial functional genes in the control and all the treatment groups after 1 month of incubation were classified into five categories, i.e., metabolism (52.49–53.93%), environmental information processing (13.44–14.54%), genetic information processing (13.72–15.37%), organismal systems (8.35–8.66%), and unclassified (12.63–13.04%). Compared to the control group, the 2% CB combined with 10 mg kg^{-1} LMWOAs treatment led to an enrichment of functional genes related to the first two categories (data not shown). It has been reported that upregulated signaling molecule expression associated with environmental information processing could enhance microbial metabolism (Korenblum et al. 2020). A total of 146 bacterial functional genes were predicted to be related to metabolism, including processes involving carbohydrates, amino acids, lipids, and terpenoids and polyketides, glycan biosynthesis, biodegradation of xenobiotics, cofactors and vitamins, and nucleotides (Fig. S5). Compared to the control group, the metabolism of carbohydrates, lipids, and terpenoids, and polyketides were significantly enhanced in the combined treatment (Fig. 5). Carbohydrate metabolic processes can generate the energy needed for the operation of soil ecosystems and help to maintain the stability of the soil environment (Ruf et al.

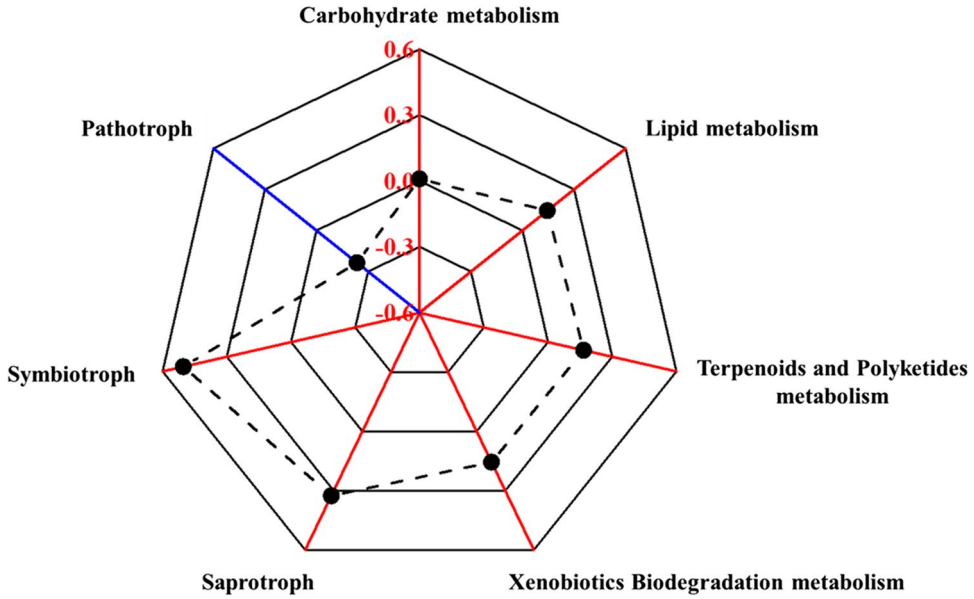

Fig. 5 Changed soil bacterial metabolic function and fungal metabolic function by the combined treatment of 2% CB and 10 mg kg^{-1} LMWOAs after the incubation. The data of black dots were calculated by Log_{10} (the relative abundances of microbial metabolic functions in the combined treatment/ those in the control). The red axis corresponds to significantly upregulated microbial functions, and the blue axis corresponds to significantly downregulated microbial functions. Control: no biochar and LMWOAs addition. 2% CB + 10 mg $kg.^{-1}$ LMWOAs: vombination of 2% corn straw–derived biochar and the LMWOA mixture (concentration ratio, acetic:glycolic:maleic:succinic acid = 1:8:6:2)

2019). Promoted terpenoid and polyketide metabolic process improve soil health and mitigate continuous cropping obstacles, including infestation and crop quality declination (Li et al. 2021c). The predicted fungal functional genes were classified into three categories (pathotrophic, symbiotrophic, and saprotrophic) based on trophic mode (Fig. 5). After the addition of 2% CB combined with 10 mg kg^{-1} LMWOAs, pathotrophic metabolism in the soil decreased, while symbiotrophic and saprotrophic metabolism increased. Symbiotrophic fungal function in conjunction with plant roots to absorb nutrients improved crop quality (Pang et al. 2019). Saprotrophic fungi can successfully transform organic pollutants through enzymatic degradation and favor biochemical and physical immobilization (Ceci et al. 2019).

There were 6003 microbial functional genes (encoding enzymes) detected in the soils after 1 month of incubation. The combined treatment also led to the upregulated expression of genes that encode functional enzymes associated with xenobiotic metabolism (Fig. 5). It has been found that different biodegradation pathways of napropamide in the soil involve several key reactions, such as demethylation, ether bond fracture, and benzene ring lysis (Huang et al. 2019; Waterman and Lepesheva 2005). In this study, the relative abundances of cytochrome P450, family 51 (sterol 14-demethylase), soluble epoxide hydrolase, limonene-1,2-epoxide hydrolase, 2-chlorobenzoate 1,2-dioxygenase, 4-hydroxyphenylpyruvate dioxygenase, protocatechuate 4,5-dioxygenase, and alpha chain, which played key roles in the stages of biodegradation, significantly increased by 30.27–126.29% after the application of 2% CB combined with 10 mg kg^{-1} LMWOAs (Table S8). Thus, in the simulated rhizosphere, amendment with 2% CB could enrich the microbial functional genes related to napropamide biodegradation and enhance the microbial metabolism associated with soil health and crop growth, while reducing the environmental risks presented by soil pathogens.

Conclusion and outlook

The removal efficiency of residual napropamide in the soil after the combined application of 2% CB and LMWOAs reached 86.58%. Napropamide sorption on CB particles and its desorption from the CB-amended soil by LMWOAs exuded from tobacco roots were successively observed. Moreover, the microbes (e.g., *Amycolatopsis*, *Penicillium*, and *Aspergillus*) and functional genes (e.g., soluble epoxide hydrolase, limonene-1,2-epoxide hydrolase, and 2-chlorobenzoate 1,2-dioxygenase) associated with napropamide biodegradation became significantly enriched. In particular, a stable and healthy soil ecosystem was established with a greater abundance of advantageous keystone species, verifying the environmental friendliness of biochar application in agricultural systems. Therefore, the results of this study

provided a "multiwin" strategy for the safe utilization and sustainable development of residual herbicide-contaminated soils. Biochar, whose application represents an important method by which waste resources can be used, has been investigated for its effectiveness in remediating contaminated soils. Moreover, a stable farmland ecosystem is conducive to sustainable agriculture. In this study, the application of low-temperature-pyrolyzed straw biochar at a rate of 60 t ha^{-1} was found to directly or indirectly participate in the cycling of nutrients, e.g., C and N, and improve microbial community composition, providing a new perspective for the restoration of ecosystems in amide herbicide-contaminated soils. Future exploration of microbial metabolic pathways and the manipulation of the abundance of keystone species via metagenomic and stable isotope tracer techniques are important for remediating and managing the soils contaminated with amide herbicides.

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Declarations

Competing interests The authors declare no competing interests.

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