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The herbicidal activity of pre-emergence herbicide cinmethylin and its potential risks on soil ecology: pH, enzyme activities and bacterial community

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Abstract

Background The herbicide cinmethylin, which was originally registered for use in rice fields, has the potential to control grass weeds in wheat fields before the emergence of wheat. However, its herbicidal activity against various troublesome grass weeds that infest wheat fields in China and its relationships with soil pH, soil enzymes and soil bacteria are not well known. Here, the effects of applying cinmethylin on the soil surface were tested on six grass weeds, and its impacts on soil characteristics, including the soil pH, soil enzymes and bacterial community, were evaluated.

Results *Alopecurus aequalis*, *A. japonicus* and *A. myosuroides* were highly sensitive to cinmethylin, with GR_{50} values of 78.77, 61.49 and 119.67 g a.i. ha^{-1}, respectively. The half-lives of cinmethylin at 1-, 10- and 100-fold the recommended rates were estimated at 26.46 – 52.33 d. Cinmethylin significantly increased the soil pH but decreased the activities of soil sucrase and urease. At 10- and 100-fold the recommended rate of cinmethylin, the bacterial abundance and diversity significantly decreased at 30 and 60 days after cinmethylin treatment. Cinmethylin at 100-fold the recommended rates largely promoted bacterial co-occurrence network complexity. Cinmethylin at high concentrations temporarily inhibited the abundance of the *Nitrospira* genus, as indicated by the copy numbers of the ammonia-oxidising archaea (AOA) *amoA* and ammonia-oxidising bacteria (AOB) *amoA* genes. Further analysis revealed that soil pH was negatively related to soil urease, and a significantly positive correlation was detected between soil urease and soil nitrification.

Conclusion Collectively, the application of cinmethylin at the recommended field dose had nearly no effect on the soil ecosystem, but its potential risks at high concentrations deserve further attention.

Keywords Cinmethylin, Weed control efficiency, Soil characteristics, Bacterial community, Soil nitrification, 16S rRNA illumina sequencing

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Introduction

Cinmethylin was developed by Shell Chemical Company and commercialised in 1982. It disturbs the biosynthesis of fatty acids by inhibiting thioesterase, which releases fatty acids from their carrier protein in plastids and then kills weeds [5]. Cinmethylin was originally used by mixing it with the soil in transplanted rice fields to control grass weeds, such as *Echinochloa crusgalli*, *Monochoria vaginalis* and *Cyperus rotundus* [27]. Owing to its high cost and the introduction of some new herbicides to the market at the same time, this herbicide has a small competitive edge over acetolactate synthase (ALS)-inhibiting and acetyl-CoA carboxylase (ACCase)-inhibiting herbicides on the market [8]. Recently, however, an increasing number of weed species in wheat fields have evolved resistance to ALS- and ACCase-inhibiting herbicides. Additionally, when it is applied to the soil before seedling emergence, cinmethylin has shown excellent inhibitory activity against the troublesome weeds *Lolium rigidum* and *Alopecurus myosuroides*, which infest wheat, whereas it is safe for wheat [21, 34]. Owing to its different modes of action, it is also effective in controlling the resistant grass weed *L. rigidum* [4]. Thus, cinmethylin, a preemergence herbicide, has recently been reused to selectively control annual grass weeds in wheat fields. It has been registered for use in wheat fields in Australia (http://sitem.herts.ac.uk/aeru/iupac/atoz.htm). However, whether this herbicide can also effectively control common grass weed species such as *Bromus japonicus*, *A. aequalis* and *L. perenne*, which severely infest wheat fields in China, is still unclear. Under the new application scenario where cinmethylin is directly applied to soil before the emergence of wheat, the environmental risks, particularly to the soil ecosystem, of cinmethylin must be reevaluated.

Soil plays a critical role in crop production, organic matter decomposition, pollutant degradation and groundwater protection [23, 40, 44]. Although herbicides play an important role in weed management, the active ingredients sprayed on soil have diverse impacts on soil biological properties. Soil enzymes produced by microorganisms are among the active elements responsible for biochemical processes in soil ecosystems [26]. Their activities are sensitive to the soil environment and are related to the quality and productivity of the soil as well as microbiome activity. Herbicide residues in soil can influence the activity of soil enzymes. For example, the activities of invertase and urease in rhizosphere soil are inhibited by low and high doses of the herbicide fomesafen [24]. Mesotrione significantly inhibited β-glucosidase activity throughout the experimental period, whereas urease and acid phosphatase activities were unaffected by this herbicide [13]. Soil microorganisms are also important components of the soil ecosystem and are closely related to soil function [6, 9, 38, 47]. Multiple studies have noted that the application of herbicides has diverse effects on the diversity and composition of the soil microbiome. Pyroxasulfone has been increasingly applied in recent years, and its exposure considerably alters bacterial diversity and composition after 30 days [43]. Pertile et al. [36] reported that applying the herbicides imazethapyr and flumyzin in soil influenced the microbial profile, with the potential to affect functions mediated by microbial communities. Du et al. [10] reported that the ALS-inhibiting herbicide mesosulfuron-methyl influenced the abundance, community and diversity of soil bacteria and fungi. Their abundance was decreased after exposure to mesosulfuron-methyl. Soil N cycling is a key predictor of soil ecological stability and includes three processes: N fixation, soil nitrification and soil denitrification. To date, several microbial groups have been shown to play important roles in the processes involved in producing and immobilising inorganic N [3]. For example, N_2-fixing bacteria participate in soil N fixation by reducing N_2 to NH_3. Ammonia-oxidising bacteria (AOB) and ammonia-oxidising archaea (AOA) are involved in transforming NH_4^+ to NO_3^- during the soil nitrification process. However, some studies have shown that these microbial groups are very vulnerable to herbicide pollution [12, 48].

Cinmethylin has low toxicity to various aquatic organisms, such as *Daphnia magna* and *Salmo gairdneri* [30]. Its acute mammalian toxicity is also low when it is orally administered to rats [31]. Although the responses of the soil ecosystem, including soil enzymes and the soil microbiome, to some herbicides have been well investigated, information regarding the potential risk of cinmethylin to soil ecosystems is limited.

Therefore, the objectives of this study were to (1) evaluate the herbicidal activity of cinmethylin on six grass weeds that are widespread in wheat fields in China; (2) determine the degradation dynamics of cinmethylin in soil; (3) assess the impacts of cinmethylin exposure on soil pH and soil enzymes; (4) assess the effects of cinmethylin on the ecology of soil bacteria; and (5) ascertain its impact on soil nitrification. This study helps elucidate the interactions between cinmethylin and the soil ecosystem and provides scientific guidance for the registration of the existing herbicide cinmethylin in a new scenario where it is sprayed on the soil surface before the emergence of wheat seedlings.

Materials and methods

Plant materials and cultivation

Mature seeds of the grass weeds *A. aequalis*, *A. japonicus*, *A. myosuroides*, *L. perenne*, *Avena fatua* and *Bromus japonicus* were collected from wheat fields through "Z" sampling. Cinmethylin was never used at these collection

sites where grass weeds severely infest wheat fields. The detailed geographical information for the six weed species is summarised in Table S1. The seeds of each species were germinated in Petri dishes containing two layers of filter paper [46]. When the seeds germinated, they were transferred to pots containing a mixture of soil and organic fertiliser (3:1, v/v) and organic fertiliser prepared from peat and livestock manure (Kai Yin LLC, Beijing). Germinated seeds were sown at a depth of 1 cm, and each pot included ten seeds. All the pots were subsequently placed in a growth chamber under a 14 h light/10 h dark (20 °C/15°C) photoperiod with 500 μmol $m^{-2}s^{-1}$ light intensity and 60% relative humidity (RH). The seedlings were watered every two days.

Cinmethylin application before the emergence of grass weeds

Cinmethylin (750 g L^{-1} emulsifiable concentrate, Luximax, BASF, Melbourne, Australia) at doses of 49.22, 98.44, 196.88, 393.75, 787.5 and 1575 g a.i. ha^{-1} was sprayed on the pots 24 h after grass weed sowing. The dose of 393.75 g a.i. ha^{-1} was the recommended field rate. Distilled water was used as a control. Herbicide spraying was conducted with an ASS-3 Walking Spray Tower (450 L ha^{-1}; National Engineering Research Centre for Information Technology, Beijing, China). All the plants were subsequently cultivated under the above conditions. The aboveground tissue of each plant was cut at 21 days after treatment (DAT); afterwards, the fresh weights of the seedlings were determined to calculate the herbicide dose resulting in a 50% reduction in weed growth (GR_{50}). Each treatment included four pots, and the experiment was independently repeated three times.

Soil treatment with cinmethylin

Soil at a depth of 0–15 cm was collected from an experimental station located in Shangzhuang, Haidian, Beijing. Pesticides were not used at this sampling site for ten years. According to the chemical and physical properties, the soil sample was a silty clay loam (Table S2). The soil that passed through a 2 mm sieve was used in the experiment. Before treatment with cinmethylin, the soil was placed under controlled conditions at 25 °C and 50% maximum water-holding capacity for two weeks to restore soil microorganisms [10].

Cinmethylin (97%) was obtained from Beijing Qinchengyixin Technology Development Co., Ltd. (Beijing, China) and dissolved in acetone. Cinmethylin was dissolved in acetone because it is insoluble in water. Cinmethylin solution and 50 g of soil were added to 1 L brown bottles and then mixed adequately [11]. After being spread out completely in the dark for 24 h to volatilise the acetone, each mixture was mixed with 200 g of soil to reach final concentrations of 0.2625 (C1), 2.625 (C10) and 26.25 mg kg^{-1} (active ingredients per soil dry weight, a.i./dw, C100) [10]. The cinmethylin doses for the soil treatments were set according to GB/T31270.16-2014. C1 is the recommended field dose, assuming that the soil layer depth is 10 cm with a bulk density of 1.5 g cm^{-3} [19]. Soil mixed with the same amount of acetone was regarded as the control (C0), and its soil treatment procedure was conducted as described earlier. The water-holding capacity of the soil was subsequently adjusted to 50% by adding sterile water. All the plants were placed into a dark growth chamber at 25 °C. Sterile water was added to maintain the soil moisture at 50% of the water-holding capacity throughout the experimental period. The soil samples used for pH and enzyme activity assays, cinmethylin residue detection and DNA extraction were collected at 1, 7, 15, 30 and 60 DAT and then stored at -80 °C. Each treatment included four independent replicates.

Extraction and detection of cinmethylin

A total of 5 g of soil was added to 20 mL of 90% acetonitrile aqueous solution and vortexed for 5 min. NaCl (2.0 g) and $(Mg)_2SO_4$ (3.0 g) were added to the mixture before vortexing for 2 min and sonicating for 10 min. The supernatant (1 mL) was evaporated until dry with a gentle stream of nitrogen after centrifugation at 8000 ×*g* for 5 min and subsequently redissolved in 1 mL of hexane. The reconstituted solutions were filtered through a 0.22-μm organic membrane and used to detect cinmethylin with gas chromatography–mass spectrometry (GC–MS).

Cinmethylin was quantified via a QP2010 SE GC–MS system (Shimadzu, Japan) according to a previous study with some minor modifications [50]. Cinmethylin was separated on an Rtx-5MS capillary column (30-m length, 0.25-μm inner diameter (i.d.), 0.25-μm film thickness; Shimadzu, Japan) with helium (99.999%) as the carrier gas, and the injection volume was 2 μL. The gas chromatographic operation conditions were set as follows: the temperature program was started at 60 °C (hold time 1 min), increased to 180 °C at a rate of 30 °C min^{-1} and subsequently increased to 250 °C at a rate of 10 °C min^{-1} (hold time 5 min). The oven and injector port temperatures were set to 50 °C and 250 °C, respectively. For the mass spectrometer conditions, the ion source and MS interface temperatures were 230 °C and 280 °C, respectively. The solvent delay was 4 min. Cinmethylin was monitored in single ion monitoring (SIM) mode (105 *m*/*z*, 123 *m*/*z* and 169 *m*/*z*). The ion at 105 *m*/*z* was used to quantify the amount of cinmethylin.

To verify the extraction method for cinmethylin in soil, a recovery experiment was performed with spiked concentrations of 0.5, 1.0 and 10 mg L^{-1}. Five replicates were included for each spiked concentration. The detailed results are described in Table S3 and indicate that the

procedure was suitable for extracting cinmethylin from the soil.

Soil pH assay

The soil pH was assayed following the methods of García-Pérez et al. [18], with some modifications. A total of 10 g of soil was mixed with 25 mL of deionised water to remove carbon dioxide. This mixture was vortexed for 2 min and then allowed to stand for 30 min. The pH of the supernatant was detected with a FiveEasy Plus pH meter (Shanghai Mettler-Toledo Instrument Co., Ltd., Shanghai, China).

Soil enzyme activity assay

The activities of soil catalase, urease and sucrase were determined following the manufacturers' instructions for the Soil Catalase Activity Assay Kit, Soil Urease Activity Assay Kit and Soil Sucrase Activity Assay Kit (Beijing Solarbio Science and Technology Co., Ltd., Beijing, China), respectively.

DNA extraction, PCR amplification and high-throughput sequencing

The soil microbial DNA was isolated with a MagicPure Stool and Soil Genomic DNA Kit (TransGen Biotech, Beijing, China). The quality and concentration of the DNA were assessed via 1% agarose gel electrophoresis and a NanoDrop 2000 instrument (Thermo Scientific, Massachusetts, USA), respectively. A pair of primers (338-F/806-R) was used to amplify the bacterial 16 S rRNA gene to study the soil bacterial communities (Table S4). PCR amplification was conducted in a total volume of 20 μL containing 10 ng of DNA, 4 μL of 5× FastPfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of forwards and reverse primers (1.0×10^{-8} mol L^{-1}), 0.4 μL of FastPfu Polymerase (TransGen Biotech) and a variable volume of double-distilled water (ddH_2O). PCR began with a predenaturation step at 95 °C for 3 min, followed by 30 cycles of 30 s at 95 °C, 30 s at 55 °C and 45 s at 72 °C, and a final extension at 72 °C for 10 min. PCR products were visually evaluated via 2% agarose gel electrophoresis and then purified with an *EasyPure*® Quick Gel Extraction Kit (TransGen Biotech). Purified PCR products were sequenced with an Illumina MiSeq PE300 (Santiago, CA, USA).

The paired-end reads were merged according to the overlap relationship. Strict quality control was conducted on the raw reads following previously reported criteria [10]. The optimised sequences were used for operational taxonomic unit (OTU) cluster analysis at a 97% similarity level to obtain representative OTU sequences. The RDP classifier (version 2.11) was employed to clarify the representative OTU sequences against the SILVA database at a confidence threshold of 0.7. Sequences annotated as chloroplasts or mitochondria or not classified as bacterial were removed. The obtained sequences were used for further analysis.

Gene copy number assay

The bacterial 16 S rRNA, AOA *amoA* and AOB *amoA* genes were amplified with the primers described in Table S4. PCR was performed in a total volume of 25 μL, which comprised 600 ng of DNA, 12.5 μL of 2× Taq PCR Master Mix (Tiangen, Beijing, China), 0.5 μL of forward primer and reverse primer (1.0×10^{-8} mol L^{-1}), and 10.5 μL of ddH_2O. The amplification cycling conditions were set as follows: 4 min at 95 °C; 38 cycles of 30 s at 95 °C, 30 s at the annealing temperature of each gene and 60 s at 72 °C; and a final extension step at 72 °C for 10 min. The evaluation and purification of the PCR products were performed as described in Sect. DNA extraction, PCR amplification and high-throughput sequencing. The PCR products of each gene were subsequently cloned and inserted into a T1 vector with a *pEASY*®-T1 Cloning Kit (TransGen Biotech). The positive clones were subjected to plasmid extraction following the instructions of the EasyPure Plasmid MiniPrep Kit (TransGen Biotech). The plasmid DNA concentration was determined with a NanoDrop 2000. Tenfold dilution of the positive plasmid was conducted with DNase-free water, and a total of seven serial dilutions were obtained and considered plasmid standards. qPCR was performed on the plasmid standards via an ABI 7500 Fast sequencer as reported by Yu et al. [45] [45]. The cycle threshold (CT) values of each standard plasmid dilution (y-axis) and the corresponding log of its copy number (x-axis) were used to establish a standard curve. The copy number of the plasmid was obtained on the basis of its concentration [20]. The primers used for qPCR are listed in Table S4.

The DNA used for high-throughput sequencing was also used for the copy number assay. qPCR was performed on all the DNA samples described above. The copy number of each gene was calculated according to its standard curve and CT values.

Data analysis

The data from all repetitive experiments were pooled for further analysis. The fresh weight relative to the control group was fitted to a four-parameter log-logistic nonlinear regression model via SigmaPlot software (version 12.5). The herbicide concentration required for 50% growth inhibition (GR_{50}) was estimated according to the obtained log-logistic curve [39]. The residue of cinmethylin in the soil was fit to a first-order kinetic model as follows:

$$y = y_0 e^{-kt}$$

where y is the cinmethylin concentration at time t, y_0 represents the cinmethylin concentration at the initial time, and k represents the first-order rate constant. The half-life ($t_{1/2}$) represents the time needed to degrade to 50% of the initial concentration of cinmethylin and is calculated on the basis of the formula $t_{1/2}=(\ln 2)/k$. Principal coordinate analysis (PCoA) was used to visualise the bacterial community structure. FAPROTAX was employed to further analyse the bacterial functional groups. For bacterial co-occurrence network analysis, the Spearman correlation matrix was obtained on the basis of the relative abundance of OTUs. The significant correlations of each pairwise OTU with $r>0.60$ and $P<0.05$ were used to generate the network. This network was plotted with Gephi (version 0.92). Analysis of variance (ANOVA) and Duncan's test were conducted via SPSS software (version 21.0) to evaluate the significant differences ($P<0.05$) among the different treatments. Pearson's correlation analysis was performed with SPSS software to determine the relationships between cinmethylin, soil characteristics, soil bacterial diversity and soil nitrifying.

Results

Cinmethylin effectively inhibited the growth of A. Aequalis, A. japonicus, and A. myosuroides

To evaluate the potential of the preemergence herbicide cinmethylin to control grass weeds that infest wheat, the sensitivities of six troublesome grass weeds to this herbicide were assessed. The tested grass weeds presented diverse growth responses to cinmethylin (Fig. 1A). At 21 DAT, more than 80% of the *A. aequalis* and *A. japonicus* plants were dead at the recommended field rate of cinmethylin, with GR_{50} values of 78.77 and 61.49 g a.i. ha^{-1}, respectively. Compared with that of the control group, the fresh weight of *A. myosuroides* decreased with increasing cinmethylin dose. Its growth was inhibited by

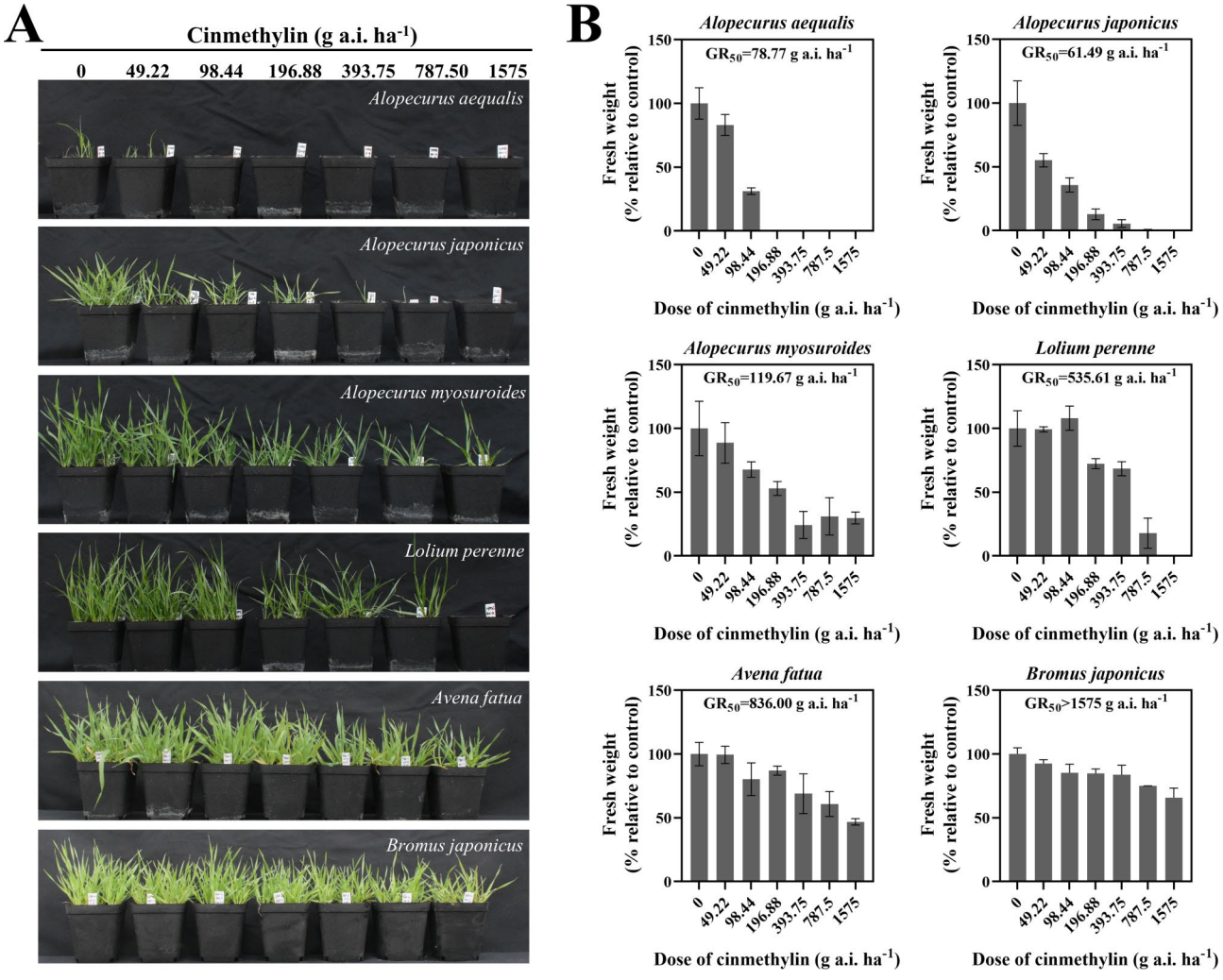


Fig. 1 Herbicidal effects of cinmethylin on six grass weed species infesting wheat. (**A**) Growth status of the six grass weed species at 21 days after treatment (DAT) with cinmethylin. (**B**) Growth dose responses of the six grass weed species to cinmethylin at 21 DAT. GR_{50} represents the cinmethylin dose leading to 50% growth inhibition relative to the control

an estimated 50% at a dose of 119.67 g a.i. ha^{-1}. Approximately 25% of *L. perenne* plants were killed by cinmethylin at a dose of 393.75 g a.i. ha^{-1}, and the GR_{50} value was estimated at 535.61 g a.i. ha^{-1}. However, no *(A) fatua* or *(B) japonicus* plants died at the recommended field concentration of cinmethylin, with GR_{50} values over 787.50 g a.i. ha^{-1} (twofold greater than the recommended field rate) (Fig. 1B). Clearly, *A. aequalis*, *A. japonicus* and *A. myosuroides* were more sensitive to cinmethylin, *L. perenne* was moderately sensitive to cinmethylin, and *(A) fatua* and *(B) japonicus* were less sensitive to this herbicide.

Degradation of cinmethylin in soil

To ascertain the dissipation dynamics of cinmethylin in soil, its residues in soil were measured at different times (Fig. 2A). The concentration of cinmethylin gradually decreased with time in the soil. At 60 DAT, approximately 22.27%, 29.43% and 44.52% of the initial cinmethylin amount was maintained in the soil of the 0.2625, 2.625 and 26.25 mg kg^{-1} treatments, respectively. Its half-life in the C1, C10 and C100 treatments was 26.46, 28.74 and 52.33 days (Fig. 2B), respectively. Overall, cinmethylin degraded slowly in the soils treated with high amounts of cinmethylin.

Cinmethylin increases soil pH and decreases sucrase and urease activities

To determine whether cinmethylin affects soil physicochemical properties, the responses of soil pH, sucrase, urease and catalase to this herbicide were determined. Compared with that of the control group, the change in soil pH was relatively stable after treatment with cinmethylin. Throughout the experimental period, the pH of the soils treated with cinmethylin was significantly greater than that of the control soils, especially at 1 DAT ($P<0.0001$) and 30 DAT ($P<0.0001$) (Fig. 3A). Therefore, cinmethylin increased the alkalinity of the soil. The activity of soil sucrase was obviously inhibited by cinmethylin at 30 DAT ($P=0.006$), with 7.47%, 13.57% and 11.66% reductions in the C1, C10 and C100 groups, respectively. A similar result was also detected at 60 DAT ($P<0.0001$), with an approximately 15.00% reduction in the treatment groups compared with the control group (Fig. 3B). The activity of soil urease in the control group was 1101.90 U g^{-1} at 15 DAT, which was 1.12-, 1.12- and 1.13-fold

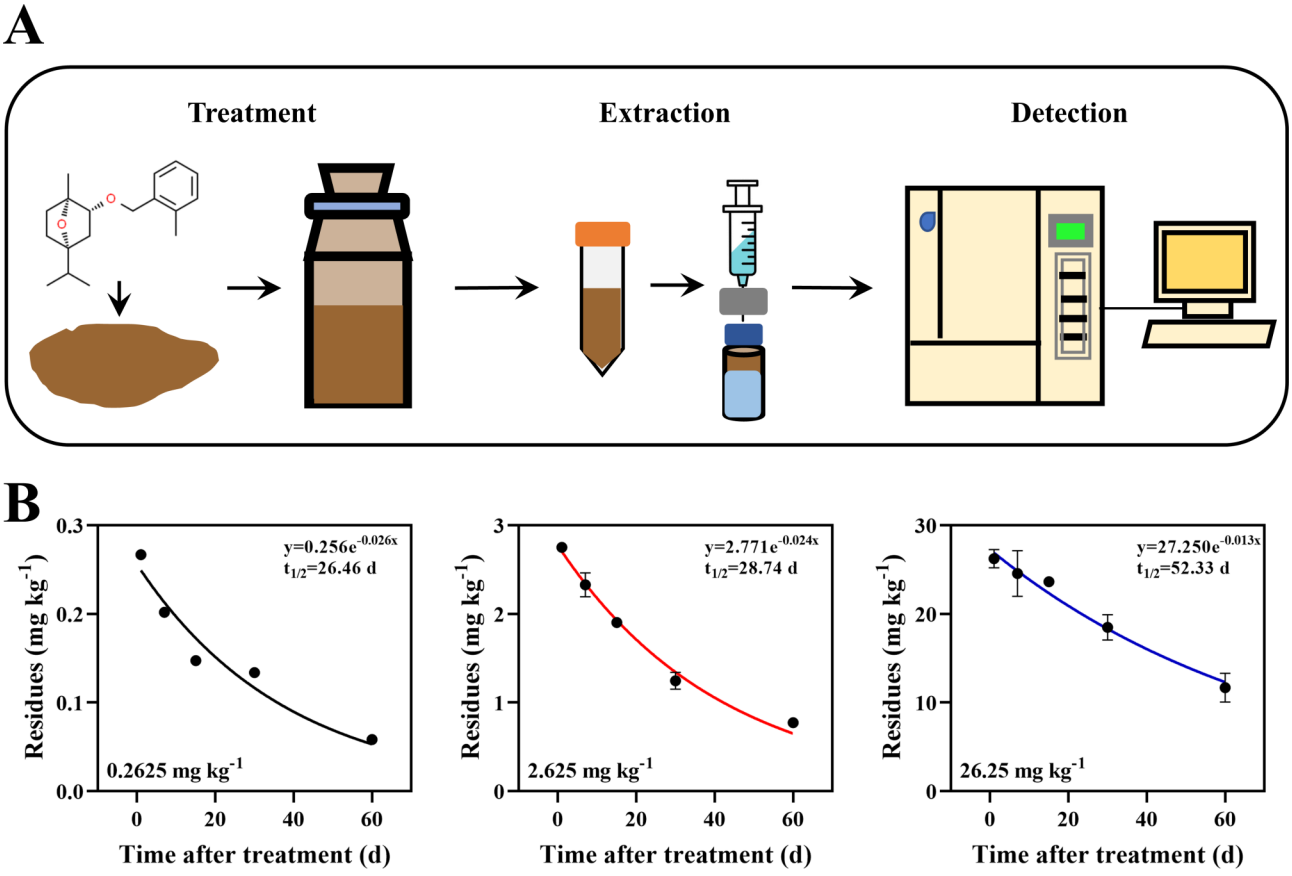


Fig. 2 Dissipation dynamics of cinmethylin in soil. (**A**) Procedure for cinmethylin extraction and detection. (**B**) Residues of cinmethylin in soil at different times after treatment with cinmethylin

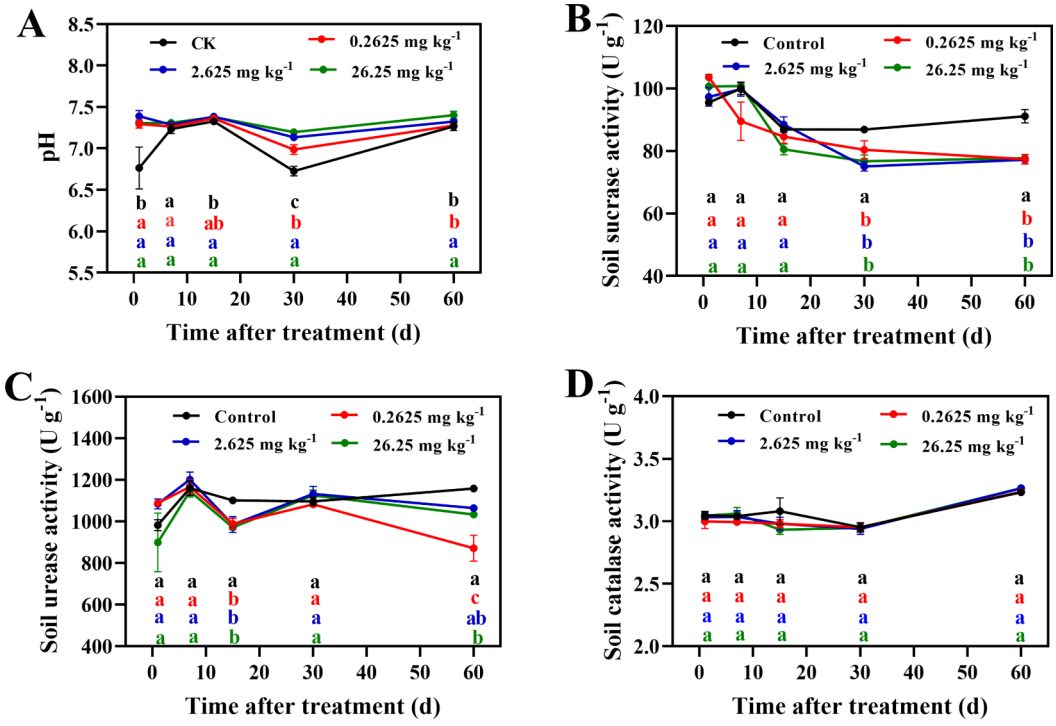


Fig. 3 Effects of cinmethylin on soil pH (**A**), sucrase activity (**B**), urease activity (**C**) and catalase activity (**D**). Different letters represent significant differences among different concentrations of cinmethylin at the same time ($P<0.05$)

greater than that in the C1, C10 and C100 groups, respectively. At 60 DAT, its activity in the control group was also distinctly greater than that in the cinmethylin treatment groups ($P=0.001$) (Fig. 3C). However, no significant difference was observed in soil catalase activity between the control and treatment groups (Fig. 3D). In total, cinmethylin had a significantly inhibitory effect on the activities of soil sucrase and urease, while its impact on soil catalase was slight.

Cinmethylin influences bacterial abundance, diversity and composition

To evaluate the relationship between cinmethylin and soil bacteria, the bacterial communities in the control and cinmethylin-treated soils were studied via 16 S rRNA Illumina sequencing. The copy number of the 16 S rRNA gene determined by absolute quantitative real-time PCR was used to estimate bacterial abundance. Throughout the experimental period, the bacterial abundance initially remained stable (1, 7 and 15 DAT), then sharply increased (30 DAT) and then decreased (60 DAT). No significant ($P=0.981$, $P=0.938$ and $P=0.974$ at 1, 7 and 15 DAT, respectively) difference was observed in bacterial abundance until 30 DAT. Compared with that in the control soil, the bacterial abundance in the C10- and C100-treated soils was obviously lower at 30 DAT ($P=0.027$) and 60 DAT ($P=0.009$) (Fig. 4A). Cinmethylin at high concentrations clearly affected bacterial abundance in the late period.

The Sobs index, Shannon index and PD index were used to describe the diversity of bacteria. In the C1-treated soil, the Sobs index first decreased but then increased to the same level as that initially observed. Nevertheless, it gradually declined with time for the soils treated with C10 and C100. The Sobs index was significantly inhibited by C100 treatment at 1 DAT ($P=0.04$) and 30 DAT ($P=0.012$) (Fig. 4B). The responses of the Shannon index and PD index to the C1 treatment were similar to those of the Sobs index throughout the experimental period. Compared with that of the control treatment, the Shannon index distinctly decreased in the soils treated with C10 and C100 at 30 DAT ($P<0.0001$) and 60 DAT ($P=0.019$). The PD index was significantly lower in the C100 treatment group than in the control group at 1 DAT ($P=0.003$). Similar results were also observed at 30 DAT and 60 DAT, but the differences were not significant (Fig. 4C–D). Bacterial diversity was inhibited by cinmethylin at the 10-fold and 100-fold recommended rates, especially during the late period of cinmethylin degradation. In the PCoA, the differences in bacterial community structure throughout the experimental period were caused mainly by time but not by the concentration of cinmethylin. The bacterial community was clearly divided into five groups according to the sampling time

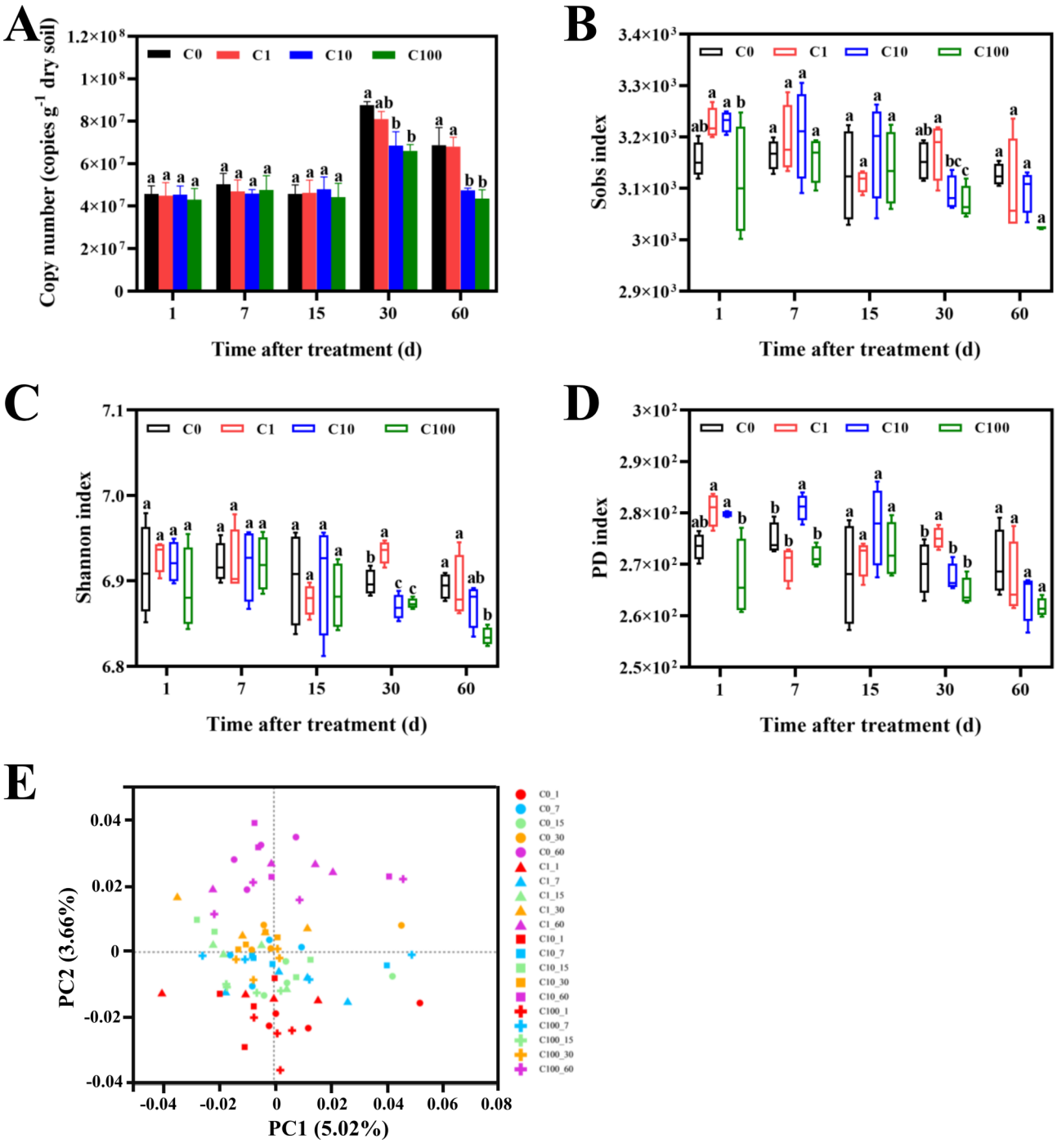


Fig. 4 Effects of cinurine on bacterial abundance, diversity and community composition. (**A**) Copy number of the 16 S rRNA gene under exposure to different cinmethylin application doses. (**B**-**D**) Sobs index, Shannon index and PD index for 16 S rRNA bacteria over time after cinmethylin treatment. (**E**) Principal coordinate analysis of soil bacteria. Different letters represent significant differences among different concentrations of cinmethylin at the same time ($P < 0.05$). C0, C1, C10 and C100 represent cinmethylin treatment at doses of 0, 0.2625, 2.625 and 26.25 mg kg^{-1}, respectively. C0_1, C0_7, C0_15, C0_30 and C0_60 represent samples collected at 1, 7, 15, 30 and 60 days after cinmethylin treatment, respectively

points (Fig. 4E). The samples collected at 60 DAT were clearly separate from those collected at other time points.

Cinmethylin did not alter the composition of bacteria at the phylum or genus level; however, it changed their relative abundances. At the phylum level, Actinobacteria, Proteobacteria, Acidobacteria and Chloroflexi were the top four dominant phyla, accounting for 80% of the bacterial community abundance (Fig. 5A). At the genus level, *norank_f_Vicinamibacteraceae*, *norank_f_norank_o_Vicinamibacterales*, *norank_f_JG30-KF-CM45*,

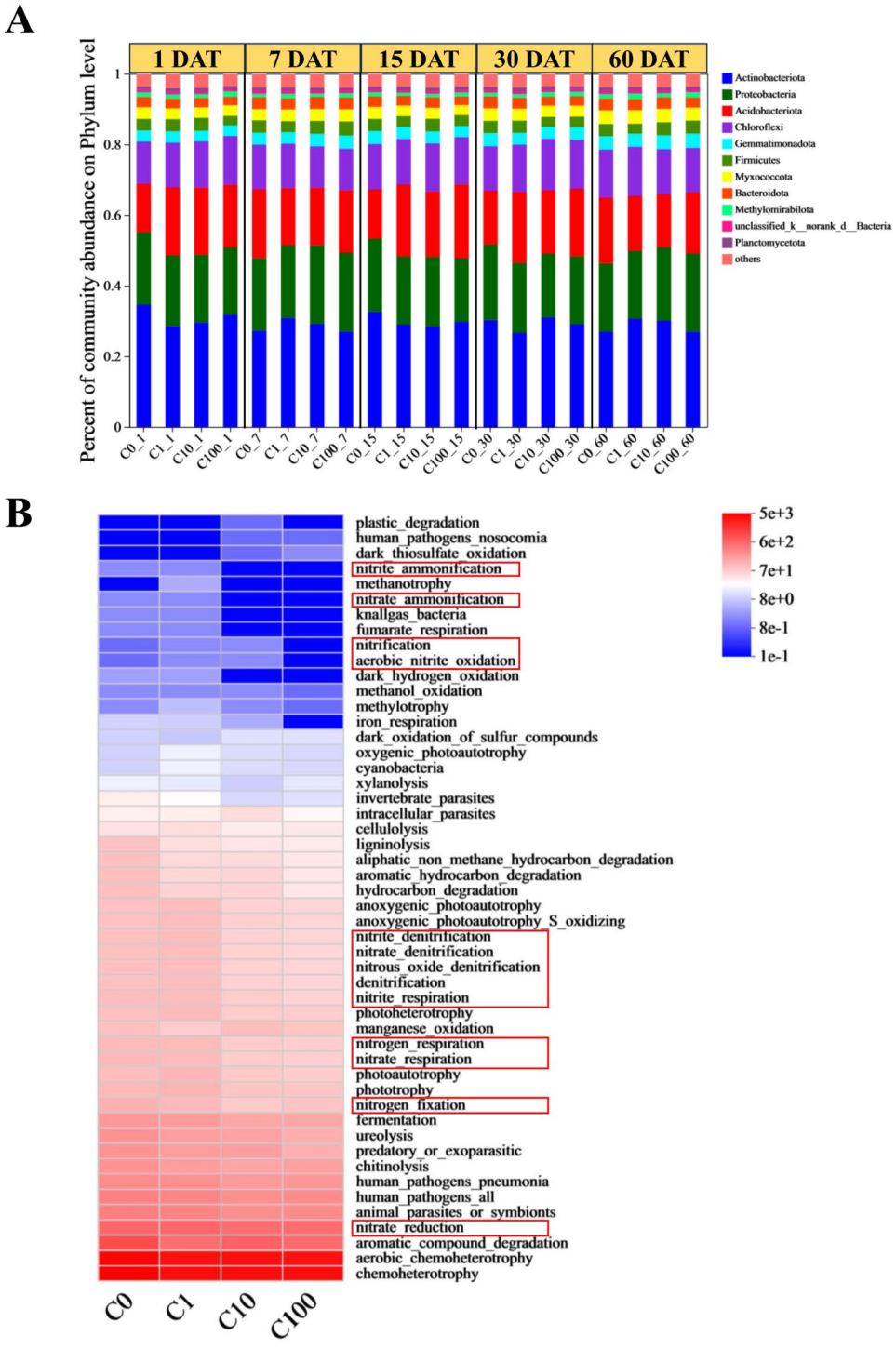


Fig. 5 (**A**) Relative abundance of 16 S rRNA bacteria in different samples at the phylum level. (**B**) Heatmap of the top 50 dominant functional groups. The functional groups marked with boxes are the groups associated with the soil nitrogen cycle. C0, C1, C10 and C100 represent cinmethylin treatment at doses of 0, 0.2625, 2.625 and 26.25 mg kg^{-1}, respectively. C0_1, C0_7, C0_15, C0_30 and C0_60 represent samples collected at 1, 7, 15, 30 and 60 days after cinmethylin treatment, respectively

noranl_f_norank_o_Gaiellales and *Gaiella* were the dominant species in the bacterial communities of each group (Figure S1). Among these dominant genera, cinmethylin had significant effects on the abundances of *norank_f_Vicinamibacteraceae*, *norank_f_norank_o_Vicinamibacterales* and *noranl_f_norank_o_Gaiellales*. The abundances of *norank_f_Vicinamibacteraceae* and *norank_f_norank_o_Vicinamibacterales* were greater in

the cinmethylin-treated soils than in the control soils, and their abundances were positively related to the cinmethylin concentration, suggesting that these two species have the potential to degrade cinmethylin. However, cinmethylin had an inhibitory effect on the abundance of *noranl_f_norank_o_Gaiellales* (Figure S2).

Bacterial community functions

The most abundant functional groups were those associated with chemoheterotrophy, aerobic chemoheterotrophy, aromatic compound degradation and nitrate reduction. The relative abundances of genes related to chemoheterotrophy, aerobic chemoheterotrophy, aromatic compound degradation and nitrate reduction were comparable among the different treatments (C0, C1, C10 and C100). Among the top 50 dominant functional groups, 13 were associated with the soil nitrogen cycle. In the C10 and C100 groups, nitrite ammonification, nitrate ammonification, nitrite denitrification, nitrate denitrification, nitrous oxide denitrification, denitrification, nitrite respiration, nitrogen respiration, nitrate respiration and nitrogen fixation, which are involved in the soil nitrogen cycle, presented relatively low relative abundances. The lowest relative abundances of nitrification and aerobic nitrite oxidation were observed in the C100 treatment (Fig. 5B).

Cinmethylin at high concentrations promotes bacterial network complexity

To clarify the effects of cinmethylin on the bacterial community network, co-occurrence networks were established under different treatments. As shown in Fig. 6, the bacterial networks formed differed among the treatments. The number of nodes in the bacterial network was comparable among the different groups. Compared with that in the control group, the clustering coefficient of the bacterial network formed was greater in the C1 treatment group, slightly lower in the C10 treatment group and obviously greater in the C100 treatment group. Similar tendencies were also detected for the network density, average degree and number of edges (Fig. 6C). In each network, the composition of the modules was different (Fig. 6A and B). In the C0 network, Acidobacteria, Proteobacteria and Actinobacteria dominated in Module I, Module II and Module IV, respectively. Acidobacteriota mainly co-occurred with Actinobacteria, and Proteobacteria primarily co-occurred with Chloroflexi. In the C1 network, Actinobacteria, Proteobacteria and Acidobacteria were the dominant members of Module I, Module II and Module IV, respectively. Acidobacteriota mainly co-occurred with Actinobacteria. In the C10 network, Module II and Module VI were composed mainly of Acidobacteriota and Actinobacteriota, respectively. Proteobacteria was the dominant member in Module VII. Proteobacteria primarily co-occurred with Chloroflexi. In the C100 network, Acidobacteriota dominated primarily in Module I. Module II consisted mainly of Actinobacteriota, Proteobacteria and Chloroflexi. Collectively, cinmethylin at 1- and 10-fold the recommended rate had a relatively slight effect on the bacterial network, whereas cinmethylin at 100-fold the recommended rate largely promoted bacterial complexity.

Effects of cinmethylin on soil nitrification

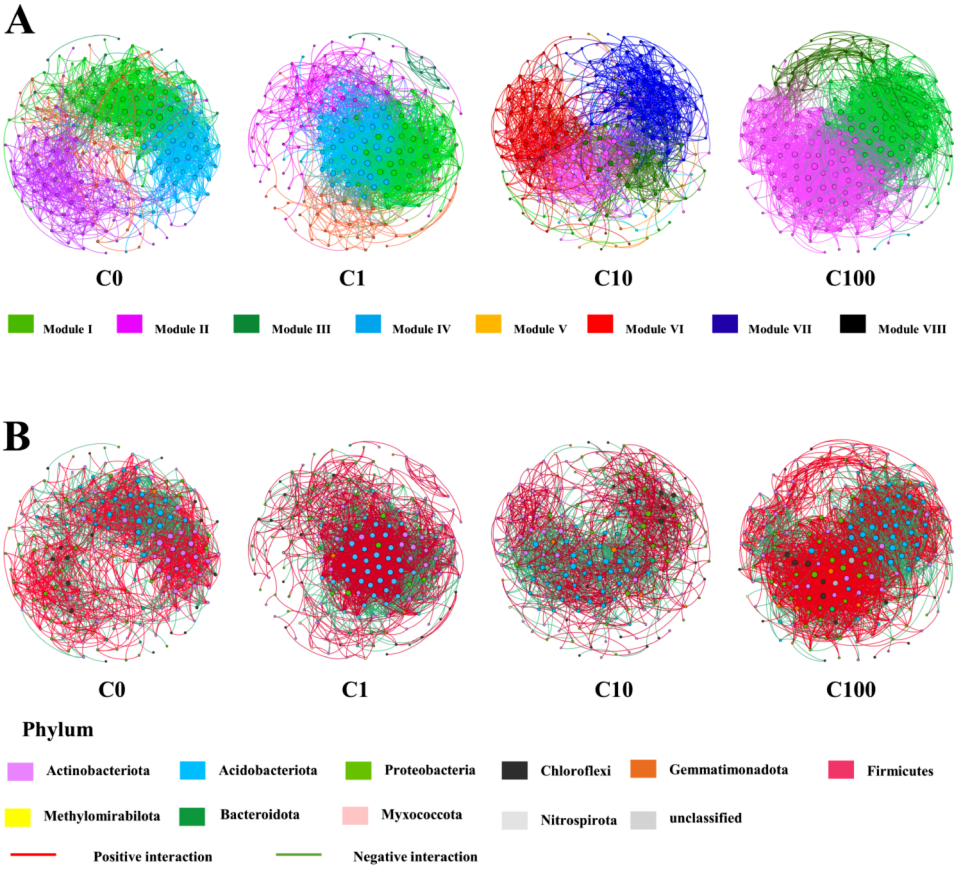
To determine the influence of cinmethylin on the N-cycling function of soil, the abundance of bacteria involved in soil nitrification was assessed under different treatments. The abundance of nitrifying bacteria *Nitrospira* assayed by 16 S rRNA Illumina sequencing was significantly influenced by cinmethylin at 1 DAT ($P=0.012$), 15 DAT ($P=0.02$) and 30 DAT ($P=0.048$) (Fig. 7A). At these time points, it was obviously lower in the soil treated with 100-fold the recommended rate of cinmethylin than in the control group, suggesting that cinmethylin at high concentrations inhibited soil nitrification. To verify this result, the copy numbers of AOA *amoA* and AOB *amoA* functional genes involved in soil nitrification were assayed. In contrast with the results of the control group, a visible decrease in the AOA *amoA* and AOB *amoA* copy numbers was detected in the cinmethylin-treated soil at 1 DAT, especially at high concentrations. However, at 60 DAT, their copy numbers increased to the maximum numbers, which were significantly greater than that of the control group (Fig. 7B-C). Clearly, cinmethylin at high concentrations temporarily inhibited the soil nitrification process.

Relationships among cinmethylin, soil characteristics, bacterial diversity and soil nitrifying

To illuminate the relationships among soil characteristics, the soil bacterial community and soil nitrification, Pearson correlation analysis was performed in this study. The results revealed that cinmethylin residues in the soil were negatively related to the soil pH and the relative abundance of *Nitrospirota*. Soil pH was positively correlated with soil catalase but negatively correlated with soil urease. Soil urease and sucrase activities were positively related to bacterial diversity, whereas soil catalase activity was negatively related to bacterial diversity. The relative abundance of *Nitrospirota* was positively related to soil urease and sucrase. The copy numbers of AOA *amoA* and AOB *amoA* were positively related to soil catalase (Fig. 8).

Discussion

Currently, ACC-inhibiting herbicides such as clodinafop-propargyl and fenoxaprop-p-ethyl and ALS-inhibiting herbicides such as mesosulfuron-methyl and pyroxsulam



C

Parameter	C0	C1	C10	C100
Clustering coefficient	0.468	0.559	0.431	0.59
Network density	0.095	0.131	0.084	0.171
Average degree	18.155	24.54	16.104	32.471
Number of nodes	193	189	193	191
Number of edges	1752	2319	1554	3101

Fig. 6 Effects of cinmethylin on the complexity of the bacterial co-occurrence network. (**A**-**B**) Visual co-occurrence network of 16 S rRNA bacteria under different cinmethylin application rates. (**A**) Nodes are coloured by module. The bacterial modules I-VIII were the eight clusters of closely interconnected nodes. (**B**) Nodes are coloured according to bacterial phyla. The red and green edges represent positive and negative interactions between two nodes, respectively. The sizes of the nodes in A and B are proportional to the number of connections (degree). (**C**) Parameters for the co-occurrence network of 16 S rRNA bacteria. C0, C1, C10 and C100 represent cinmethylin treatment at doses of 0, 0.2625, 2.625 and 26.25 mg kg^{-1}, respectively

are widely used for grass weed control in wheat fields in China. Owing to their excessive use for a long period, various levels of resistance to these herbicides have evolved in some grass weed species, such as *A. aequalis*, which is resistant to mesosulfuron-methyl and fenoxaprop-P-ethyl [49]; *Beckmannia syzigachne*, which is resistant to mesosulfuron-methyl [41]d *japonicus*, which is resistant to flucarbazone-sodium [29]. The prevalence of weed resistance poses a serious threat to wheat yield. Recently, the commercialised herbicide cinmethylin, which is registered for use in rice fields, has the potential to control grass weeds in wheat fields before the emergence of crops. In this study, cinmethylin had good control effects on the troublesome weeds *A. aequalis*, *A. japonicus*, *A. myosuroides* and *L. perenne*. Owing to its unique mode of action, cinmethylin may be a good alternative herbicide to solve resistance problems in *A. aequalis*, *A. japonicus*, *A. myosuroides* and *L. perenne*. Although cinmethylin did not kill *(A) fatua* or *(B) japonicus* plants at the recommended field rate, it had an inhibitory effect on the growth of these weed species. Cinmethylin application before the emergence of wheat is a good strategy to synergize the control effect of postemergence herbicides on *(A) fatua* and *(B) japonicus*.

In the field, the half-life of cinmethylin was approximately 22.4 days (http://sitem.herts.ac.uk/aeru/iupac/

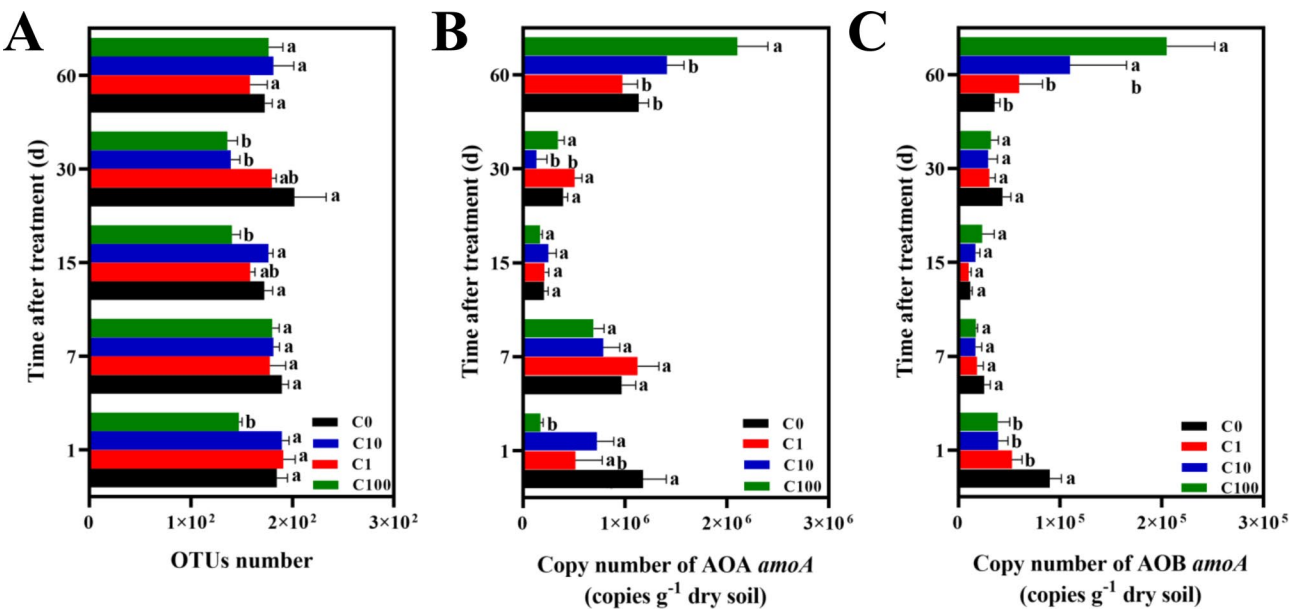


Fig. 7 Effects of cinmethylin on soil nitrification. (**A**) Changes in the abundance of the *Nitrospira* genus following different cinmethylin application rates over time. (**B**-**C**). Copy numbers of the AOA *amoA* and AOB *amoA* genes in soils treated with different concentrations of cinmethylin over time. Different letters represent significant differences among different concentrations of cinmethylin at the same time ($P < 0.05$). C0, C1, C10 and C100 represent cinmethylin treatment at doses of 0, 0.2625, 2.625 and 26.25 mg kg^{-1}, respectively

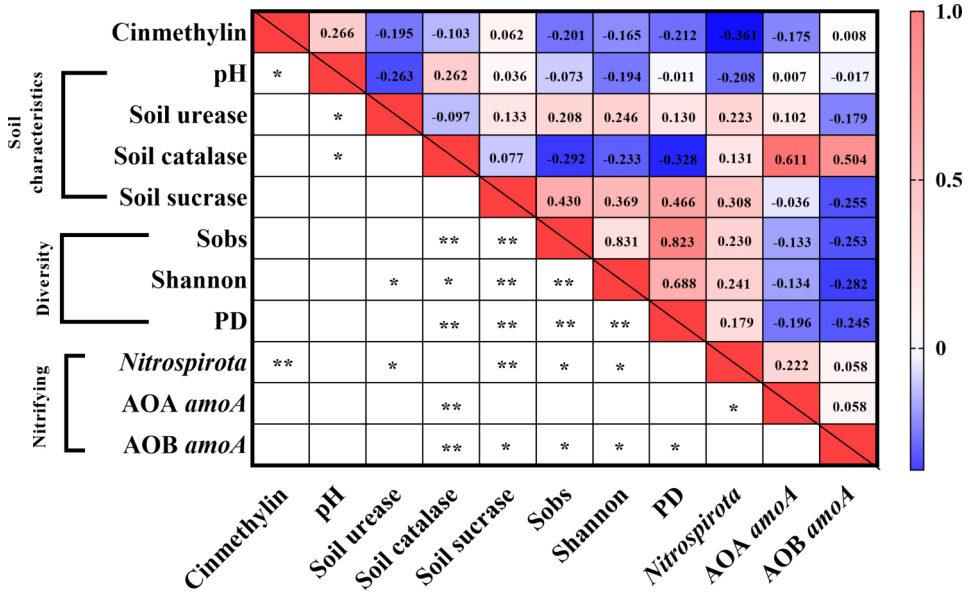


Fig. 8 Pearson correlations among cinmethylin residues, soil characteristics, bacterial diversity and soil nitrification. The values in the upper right represent the correlation coefficient. * and ** in the lower left represent significant correlations at $P < 0.05$ and $P < 0.01$, respectively

Reports/1021.htm), which was lower than our observations in the C1 (26.46 d), C10 (28.74 d) and C100 (52.33 d) treatments. Pesticide degradation in soil is associated with the soil type. In soils containing high levels of organic matter, slow degradation was observed for myclobutanil, clomazone and diazinon [12, 28, 33]. The differences in the cinmethylin degradation rates may be due to the different soil types used in this study and previous studies [11, 22]. However, one soil type was used in this research, which is limited to evaluating the dynamics of cinmethylin degradation in soil. Thus, more experimental data regarding cinmethylin dissipation in different soil types are needed in the future. The degradation rate of cinmethylin in the C1 treatment was comparable to that in the C10 treatment, which was obviously greater than that observed in the C100 treatment. Like cinmethylin, some herbicides, such as mesosulfuron-methyl and clomazone, also degrade slowly at high concentrations [10, 12]. In the

present study, the richness of *Sphingomonas* significantly decreased in the C100-contaminated soil compared with that in the control soil, whereas no significant difference was observed between the C1 and C10 treatment groups (Figure S2). *Sphingomonas* can degrade some pesticides, such as chlorpyrifos and *ortho*-phenylphenol [15, 16, 35]. Therefore, this phenomenon might be due to the adverse effects that high amounts of cinmethylin have on the growth of bacteria related to agrochemical degradation, such as *Sphingomonas*, ultimately resulting in a slow dissipation rate of cinmethylin in the soil. The bacterial diversity was distinctly decreased in the C100 treatment group, which also suggested that some bacterial groups related to cinmethylin degradation were inhibited under exposure to high amounts of cinmethylin.

Soil acidity–alkalinity is an important soil physiochemical property that directly influences crop growth and soil microbiome activities. In this study, cinmethylin increased the alkalinity of the soil, which differed from the decreased pH in soil contaminated with the herbicide glyphosate [18]. Cinmethylin can be degraded into hydroxylated metabolites by hydroxylation of the isopropyl moiety in soil (Woodward et al., 1986). The release of hydroxyl ions from hydroxylated metabolites might increase the soil pH. Soil functional enzymes are also closely related to the retention of soil function. Soil urease can hydrolyse urea in soil to generate NH_3 and carbonic acid [1]. The activity of soil urease is an important indicator reflecting the condition of soil nitrogen. The NH_4^+ ions yielded by soil urease can be rapidly transformed into NO_3^- ions and H^+ ions through the nitrification process, and NO_3^- can leach down with water [1]. A decrease in urease activity in soil caused by cinmethylin could reduce urea hydrolysis and then inhibit nitrification that leads to the production of NO_3^- ion and H^+ ion, which indirectly increased soil pH. In addition, cinmethylin obviously increased the alkalinity of the soil but decreased the soil urease activity. In the correlation analysis, a significantly negative relationship was detected between soil pH and soil urease activity. Soil pH is an important factor influencing soil urease activity [7, 14]. In galaxolide-contaminated soil, *Solanum nigrum* affects soil urease activity by regulating the soil pH [32]. Therefore, it was speculated that cinmethylin contamination inhibited soil urease activity by increasing the soil pH.

Real-time quantitative PCR, 16 S rRNA Illumina sequencing and bioinformatics were used to determine the effects of cinmethylin exposure on the bacterial community. The abundance and diversity of bacteria distinctly decreased in response to high cinmethylin concentrations in the late period of degradation. Several herbicides, including pyroxasulfone [43], imazethapyr [36], and fomesafen [24], have also been reported to affect microbial community structure. Additionally, different bacterial genera responded differently to cinmethylin pollution, indicating that bacterial species might have different levels of sensitivity to the environmental stress caused by cinmethylin. At the genus level, the abundances of *norank_f_Vicinamibacteraceae* and *norank_f_norank_o_Vicinamibacterales* were significantly greater in the cinmethylin-treated soil than in the control group. The *Vicinamibacteraceae* family was first identified in 2018, and some members belonging to this family can degrade chemicals with complex structures [25]. Thus, *norank_f_Vicinamibacteraceae* might participate in the biodegradation of cinmethylin. On the basis of an obvious reduction in soil urease activity caused by cinmethylin and the well-established knowledge regarding the relationships between soil urease and soil nitrification as described earlier [1], we speculated that this herbicide had a decreased effect on the abundance of nitrifiers. To validate these findings, the abundance of the genus *Nitrospira* and the copy numbers of the AOA *amoA* and AOB *amoA* genes were studied via 16 S rRNA Illumina sequencing and qPCR after exposure to cinmethylin, respectively. According to the results of the abundance of *Nitrospira*, AOA *amoA* and AOB *amoA*, cinmethylin imposed a transient decrease in soil nitrification, which was supported by a decrease in the relative abundance of predicted functional groups related to the soil nitrogen cycle under exposure to cinmethylin. The inhibition of soil urease activity by cinmethylin would result in less ammonium being released from organic nitrogen to feed the ammonia oxidisers for nitrification. The soil urease activity was positively correlated with the relative abundance of the *Nitrospirota* genus. Additionally, the inhibition of nitrifiers by cinmethylin was transient. Therefore, cinmethylin might have an indirect rather than direct toxic effect on soil nitrification. Cinmethylin might impede the soil nitrification process by inhibiting soil urease. Actually, there were many biological factors controlling soil nitrification. For example, the fungal community could also influence the soil nitrification. However, in current study, the response of soil nitrification to cinmethylin were analysed in view of soil bacteria. In the future, it is necessary to evaluate the effect of cinmethylin on soil nitrification based on more experimental data.

Co-occurrence patterns are powerful tools for studying changes in microbial community structure and discovering potential microbiome interactions [2, 37]. The microorganisms in one module have strong interactions and similar habitat preferences. As shown in Fig. 5, the complexity of the bacterial network was obviously promoted by cinmethylin at high concentrations. This finding is inconsistent with previous results showing a decline in microbial complexity in soils treated with the insecticide thiamethoxam [47], the fungicides fosety-Al and propamocarb-hydrochloride [17], or the herbicide atrazine

[42]. The increased complexity of the bacterial community in the soil treated with a high dose of cinmethylin might be due to close cooperation among more bacterial species to resist the increasing environmental stress caused by a high dose of cinmethylin.

Conclusion

Overall, the herbicide cinmethylin effectively controlled the grass weeds *A. aequalis*, *A. japonicus* and *A. myosuroides* when applied before the emergence of crops and weeds, suggesting its potential to control grass weeds in wheat fields as a preemergence herbicide. Given the responses of soil pH, soil enzyme activity, the bacterial community and soil nitrification to cinmethylin, the herbicide at the recommended rate hardly influenced the soil ecosystem. However, the potential risks to the soil ecosystem caused by high amounts of cinmethylin deserve further attention. Cinmethylin at high concentrations inhibited soil urease activity by increasing the soil pH; reduced soil urease activity was most likely to cause a temporary decline in soil nitrification. These findings will provide scientific guidance for the use of the existing herbicide cinmethylin in a new scenario where it is directly applied to the soil surface and will help further understand the relationships between cinmethylin and the soil ecosystem.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40793-024-00608-y.

Supplementary Material 1

Author contributions

H.Y wrote the main manuscript text and conducted the experiments; H.L. and J.C. analyzed the data; X.J. designed the experiments and reviewed the manuscript.

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Data availability

The raw sequence data has been deposited in the NCBI Sequence Read Archive (SRA) database with accession number of PRJNA1141104.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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