Process Biochemistry 163 (2026) 105–113

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Phytodetoxification and microbial community dynamics during the bioremediation of sulfonylurea herbicide contaminated soils by esterase SulE in cross-linked gelatin/chitosan

Xiang Li [a], Xuanhe Fu [b], Xu Li [a], Zhencheng Su [a], Huiwen Zhang [a], Zhixiong Yu [b,*]

[a] Key Laboratory of Pollution Ecology and Environmental Engineering, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] Shenyang Key Laboratory of Prevention and Treatment of Systemic Important Diseases Associated with Oral Diseases, Shenyang Medical College, Shenyang 110034, China

ARTICLE INFO

Keywords:
Sulfonylurea herbicide
Immobilized enzyme
Bioremediation
Phospholipid fatty acid

ABSTRACT

Sulfonylurea herbicides exhibit persistence in alkaline soils, which consequently poses risks to both crops and soil microbial communities. This study evaluated the efficacy of gelatin/chitosan-immobilized SulE (GLT/CTS-SulE) for remediating soil contaminated tribenuron-methyl or metsulfuron-methyl, focusing on its phytoremediation effectiveness and impact on soil microbial dynamics. The results demonstrate that GLT/CTS-SulE effectively improved multiple growth indicators of maize, including plant height, main root length, fresh weight and root weight. GLT/CTS-SulE can also effectively maintain the balance of soil microorganisms. It has a mitigating effect on the inhibition of total phospholipid fatty acid (PLFA) concentration, bacterial PLFA concentration and Shannon-Wiener index, as well as the promotion of fungal PLFA concentration by sulfonylurea herbicides. Furthermore, GLT/CTS-SulE demonstrates enhanced remediation capabilities for soil contaminated with tribenuron-methyl or metsulfuron-methyl in comparison to free SulE. In summary, GLT/CTS-SulE presents a stable and efficient strategy for enhancing remediation of soils contaminated with sulfonylurea herbicides *in situ*.

1. Introduction

Sulfonylurea herbicides are extensively utilized in global agriculture due to their exceptional efficacy, low application rates, and broad-spectrum weed control [1]. However, under specific environmental conditions, such as in alkaline soil, these compounds are prone to residue and accumulation [2,3]. Even minimal residues of sulfonylurea herbicides can lead to significant phytotoxicity in sensitive rotational crops and disrupt soil microbial communities as well as animal behavior [4–7]. The long-term biological accumulation of these substances raises concerns regarding ecological safety and human health [8]. Therefore, addressing the remediation of agricultural soils contaminated with sulfonylurea herbicides remains a critical challenge.

Conventional physical-chemical remediation techniques for the degradation of sulfonylurea herbicides are often associated with high costs, complexity, and the potential for secondary contamination [9]. As a result, environmentally friendly bioremediation approaches have emerged as promising alternatives, particularly those utilizing enzymatic degradation due to their advantages in efficiency and specificity [10,11]. Esterase SulE is known to specifically hydrolyze the ester bonds present in sulfonylurea herbicides, leading to the formation of less toxic acidic metabolites. This characteristic makes it an ideal candidate for rapid in situ detoxification processes [12]. However, free enzymes are susceptible to inactivation within complex soil matrices due to factors such as pH fluctuations, temperature variations, and microbial proteases [13–16]. Enzyme immobilization technology presents an effective solution to address these issues [17,18]. Gelatin demonstrates good biocompatibility while chitosan offers excellent mechanical strength along with antibacterial properties [19,20]. The cross-linking of these materials results in a stable three-dimensional network structure that effectively encapsulates and protects free enzymes. This enhancement improves enzyme resistance against environmental stresses encountered in soil conditions, making them outstanding candidates for enzyme immobilization materials [21,22]. Consequently, the immobilization system constructed from gelatin and chitosan (GLT/CTS), owing to its excellent enzyme-protective capacity and

* Corresponding author.
E-mail addresses: xiangli@iae.ac.cn (X. Li), fuxuanhe@symc.edu.cn (X. Fu), lixu@iae.ac.cn (X. Li), zhenchengsu@iae.ac.cn (Z. Su), hwzhang@iae.ac.cn (H. Zhang), yuzhixiong@symc.edu.cn (Z. Yu).

https://doi.org/10.1016/j.procbio.2026.02.011
Received 12 September 2025; Received in revised form 26 December 2025; Accepted 10 February 2026
Available online 11 February 2026

environmental adaptability, holds considerable potential for application in the bioremediation of contaminated soil.

This study employed an environmentally compatible immobilized enzyme system, specifically gelatin/chitosan-immobilized esterase SulE (GLT/CTS-SulE). The research focused on evaluating the efficacy of GLT/CTS-SulE in remediating soil contaminated with sulfonylurea herbicides. By analyzing physiological indicators of maize, we assessed its ability to mitigate phytotoxicity. The phospholipid fatty acid (PLFA) analysis was employed to investigate the dynamic changes of total PLFA concentration, bacterial PLFA concentration, fungal PLFA concentration, and the Shannon-Wiener index. This approach aims to elucidate its role in restoring the ecological balance of microorganisms. This study aims to provide innovative technological solutions and theoretical support for the efficient and stable bioremediation of soils contaminated with sulfonylurea herbicides.

2. Materials and methods

2.1. Materials

Tribenuron-methyl (purity 96.20 %) and metsulfuron-methyl (purity 96.40 %), along with chromatographic reagents and spectroscopic analysis reagents, were procured from Sigma-Aldrich. The strain *Chenggangzhangella methanolivorans* CHL1 was successfully isolated and preserved in the laboratory.

Chitosan (CTS) was obtained from Zhuoke Biological Technology Development Co., Ltd. (Guangzhou, China). Gelatin (GLT) was sourced from Sigma (St. Louis, MO, USA). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) was acquired from J&K Chemical Ltd. (Beijing, China). Genetically modified products were purchased from TaKaRa Biotechnology (Dalian, China).

2.2. Immobilization of esterase SulE

Expression and purification of esterase SulE, along with the assessment of its enzymatic activity towards tribenuron-methyl or metsulfuron-methyl, were conducted as outlined by Yang et al. [12]. The degradation rate was utilized as an indicator of enzymatic activity. One unit (U) of enzyme activity is defined as the quantity of enzyme necessary to convert 1 μmol of either tribenuron-methyl or metsulfuron-methyl into its corresponding precursor acid per minute, using an equivalent amount of heat-inactivated enzyme as a control.

In this study, gelatin (GLT) and chitosan (CTS) were selected as immobilization carriers, with EDC serving as the cross-linker. An initial enzyme loading of 4 U was employed, along with a GLT/CTS concentration ratio of 40:1 (w/w), a cross-linking duration of 4 h, and an EDC volume of 1 mL [23]. Consequently, the immobilized gelatin/chitosan-immobilized esterase SulE (GLT/CTS-SulE) was successfully prepared.

2.3. Soil samples and experimental treatments

The soil samples were collected from an abandoned site in Shenyang, Liaoning Province, China, and are classified as aquic brown soil. The soil texture consists of approximately 35 % sand, 50 % silt, and 15 % clay. Importantly, the soil samples contained no detectable residues of sulfonylurea herbicides, ensuring minimal interference with the microcosm experiments. Its chemical properties include 402.51 mg kg^{-1} available potassium (K), 53.59 mg kg^{-1} available phosphorus (P), 109.76 mg kg^{-1} available nitrogen (N), and 57.10 g kg^{-1} organic matter, with a conductivity of 204.10 and pH of 8.15.

The soil samples were meticulously homogenized and subsequently passed through a 2-mm sieve. Subsequently, the soil samples were categorized into 15 distinct groups, including a control group, soils contaminated with either low or high concentrations of herbicide, and these herbicide-contaminated soils supplemented with free SulE or GLT/CTS-SulE, respectively. More details are presented in Table 1.

Table 1
Experimental design and treatments.

Treatment code	Tribenuron-methyl (μg kg^{-1})	Metsulfuron-methyl (μg kg^{-1})	Free-SulE (U kg^{-1})	GLT/CTS-SulE (U kg^{-1})
CK0	0	0	0	0
CKf	0	0	10	0
CKg	0	0	0	10
ML0	0	4	0	0
MLf	0	4	10	0
MLg	0	4	0	10
MH0	0	20	0	0
MHf	0	20	10	0
MHg	0	20	0	10
TL0	4	0	0	0
TLf	4	0	10	0
TLg	4	0	0	10
TH0	8	0	0	0
THf	8	0	10	0
THg	8	0	0	10

"CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

2.4. Microcosm experiment

Each treatment consisted of three pots (3 kg soil/pot). The soil moisture was maintained at 25 % (±5 %) by adding sterilized deionized water daily. Uniformly sized maize seeds with plump kernels were selected and sown into the treated soils at a density of 15 seeds per pot. The pots were incubated at room temperature for 7 days. After the incubation period, several growth parameters were measured: plant height, root length, fresh weight, and root weight. Soil samples (300 g) were collected on days 0, 1, 3, 5, and 7 to determine the residues of tribenuron-methyl or metsulfuron-methyl and the concentrations of phospholipid fatty acids (PLFAs). The residual concentrations of tribenuron-methyl or metsulfuron-methyl in soils have been measured and published, as shown in Supplementary Material Table S1 [23].

2.5. Analysis of soil microbial community structure

PLFA analysis was utilized to characterize the structure of the soil microbial community [24,25]. The detailed procedure is outlined as follows: Freeze-dried soil samples (8 g) were placed in culture tubes and extracted twice with 30.4 mL of extraction buffer composed of chloroform, methanol, and citrate buffer (pH = 4) in a ratio of 1:2:0.8 (v/v/v). The chloroform layer was collected and evaporated under nitrogen gas. The total lipid extract was then fractionated into neutral lipids, glycolipids, and polar lipids using a Cleanert Silica column. Polar lipids underwent methylation to produce fatty acid methyl esters (FAMEs), which were subsequently analyzed by gas chromatography (GC) utilizing the Sherlock system (MIDI Inc., Newark, DE, USA). Fatty acid quantification was conducted by comparing peak areas against an internal standard (19:0) [26].

The samples were injected at a split ratio of 30:1, and fatty acids ranging from 10 to 24 carbon atoms were identified. The total amount of phospholipid fatty acids (PLFA) was quantified to estimate the overall microbial biomass. Specific fatty acids used as bacterial markers included i14:0, i15:0, 15:0, a15:0, i16:0, 16:1ω7, i17:0, a17:0, cy17:0, 17:0, and cy19:0 [27]. Additionally, specific fatty acids 18:2ω6 and 18:1ω9c served as indicators for fungal biomarkers. The Shannon-Wiener indices were calculated to evaluate microbial species richness and evenness [28].

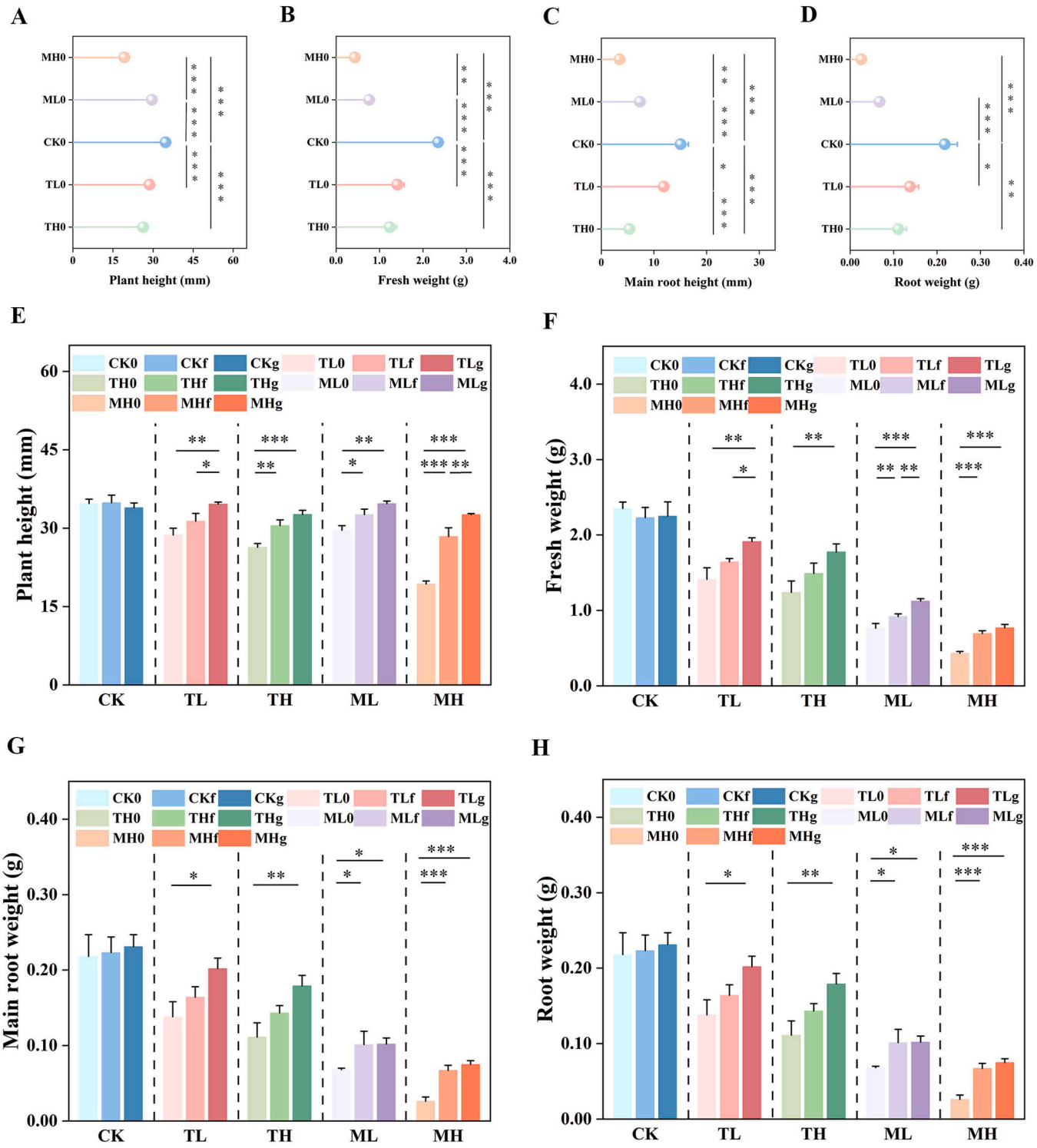

Fig. 1. The growth parameters of maize under different treatments. Statistically significant differences are indicated by * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$). "CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

To minimize potential interference from plant-derived fatty acids, visible root residues were carefully removed from soil samples prior to freeze-drying and lipid extraction. In addition, PLFA biomarkers used for microbial community analysis were restricted to well-established microbial-specific phospholipid fatty acids, which are considered to have negligible contributions from higher plants.

2.6. Data analysis and statistics

All values are presented as mean ± standard deviation. The differences among the variables were evaluated by analysis of variance (ANOVA), and the differences were tested by least significant difference (LSD) using SPSS 16.0 (SPSS, Chicago, IL, USA). A P value < 0.05 was considered statistically significant.

Table 2
Multivariate analysis of variance by a three-way ANOVA of the total PLFA concentration, bacterial PLFA concentration, fungal PLFA concentration, and Shannon index.

Herbicide	Factor	Total PLFA	Bacteria	Fungi	Shannon index
Tribenuron-methyl	Treatment	32.015***	78.625***	40.724***	195.476***
	Concentration	368.427***	605.909***	200.146***	654.677***
	Time	87.704***	208.076***	82.006***	524.354***
	Treatment*Concentration	5.589***	14.359***	19.486***	40.746***
	Treatment*Time	7.850 ***	18.481***	11.371***	69.126***
	Concentration*Time	34.554 ***	67.206***	36.034***	137.105***
	Treatment*Concentration*Time	2.488**	4.861***	3.488***	17.316***
Metsulfuron-methyl	Treatment	23.210***	58.951***	22.741***	125.488***
	Concentration	434.717***	579.256***	129.364***	284.889***
	Time	73.657***	102.644***	52.847***	125.488**
	Treatment*Concentration	2.743**	10.211***	15.096***	31.792***
	Treatment*Time	3.506***	9.045***	6.490***	23.471***
	Concentration*Time	31.114***	44.911***	25.604***	43.649***
	Treatment*Concentration*Time	1.194**	2.608***	3.494***	11.349***

The categorical factors are treatment (without SulE, with free SulE, and with GLT/CTS-SulE), concentration (without sulfonylurea herbicide, low concentration of sulfonylurea herbicide; high concentration of sulfonylurea herbicide), and time (0, 1, 3, 5, and 7 days). Presented are the F-values with the level of significance ($P < 0.05$*, $P < 0.01$**, and $P < 0.001$***).

3. Results and discussion

3.1. Effects of phytodetoxification

After 7 days, the maize growth parameters were measured across all experimental groups (Fig. 1). Compared with group CK0, the plant height, fresh weight, main root length, and root weight of maize in groups TL0, TH0, ML0 and MH0 with added tribenuron-methyl or metsulfuron-methyl were significantly inhibited ($P < 0.05$) (Fig. 1A-1D). Furthermore, the inhibition of root weight by tribenuron-methyl exhibited a concentration-dependent relationship. Similarly, metsulfuron-methyl demonstrated a concentration-dependent inhibitory effect on plant height, root weight, and fresh weight ($P < 0.05$).

As shown in the Fig. 1E-1H, no significant differences in the growth parameters of maize were observed following the addition of free SulE or GLT/CTS-SulE alone ($P > 0.05$). GLT/CTS-SulE demonstrated a significantly superior ability to mitigate the inhibitory effects of herbicides on maize compared to free SulE. In the low concentration metsulfuron-

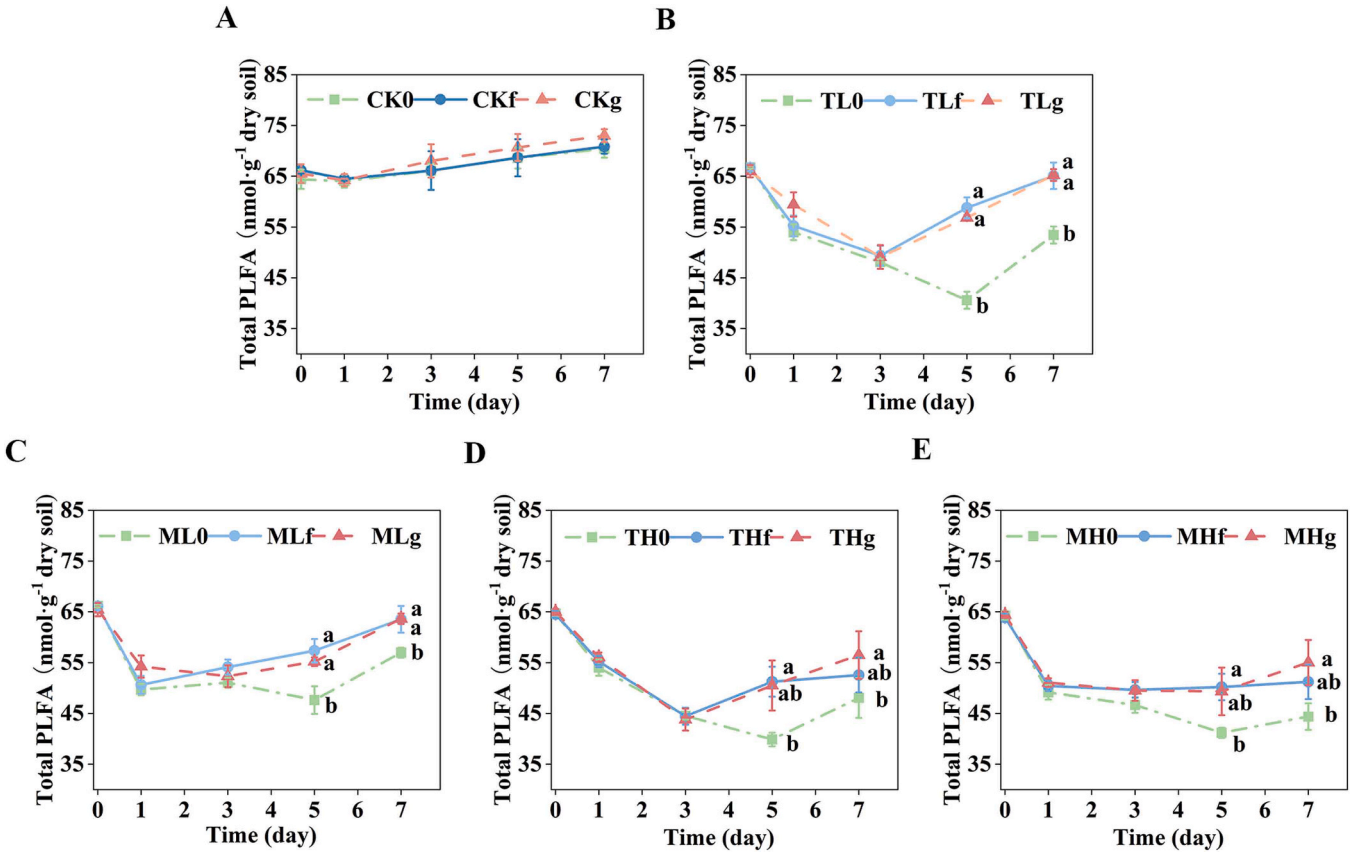

Fig. 2. The total PLFA concentration in soils. Different lowercase letters indicate significant differences among different treatment groups on the same sampling day, respectively ($P < 0.05$). "CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

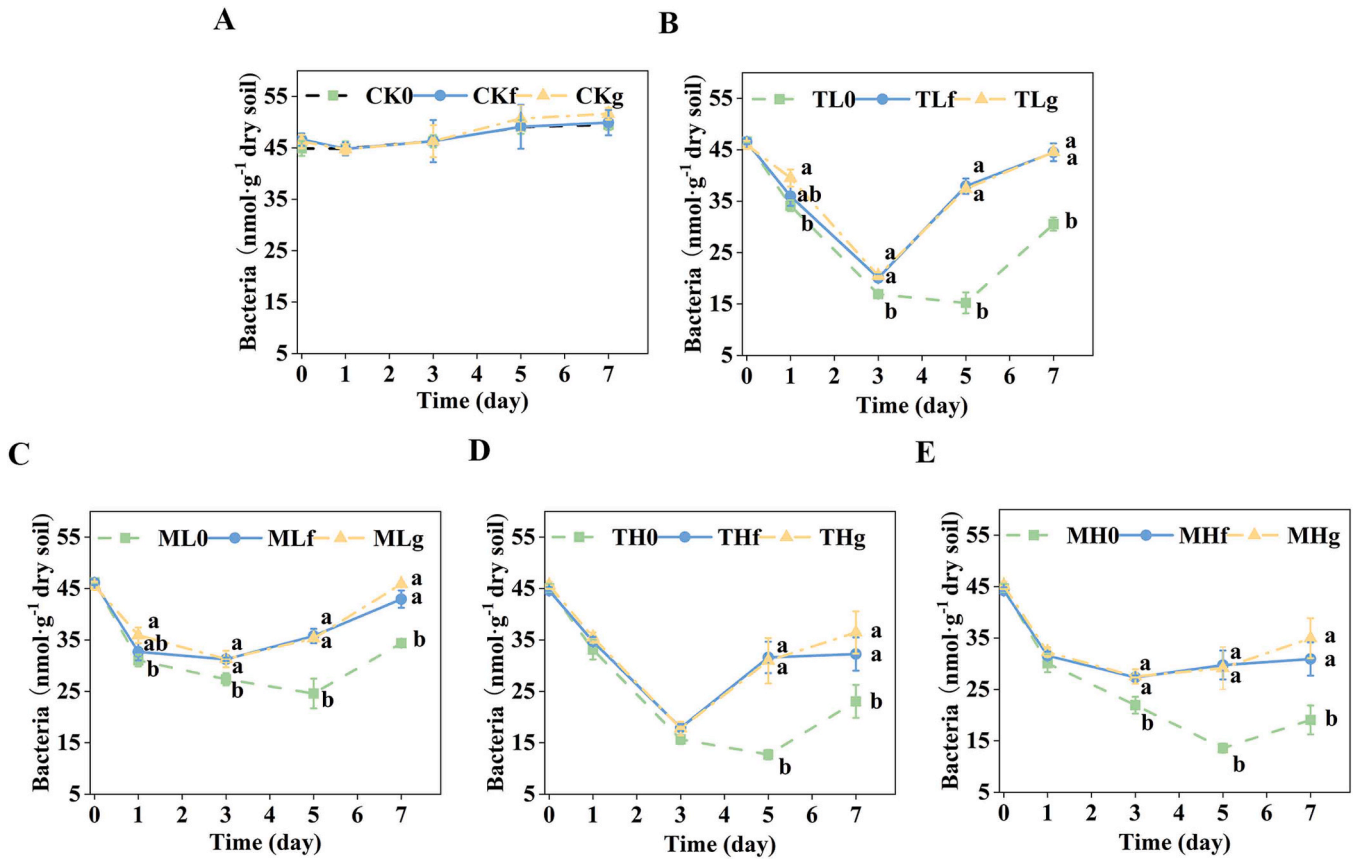

Fig. 3. The bacterial PLFA concentration in soils. Different lowercase letters indicate significant differences among different treatment groups on the same sampling day, respectively ($P < 0.05$). "CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

methyl group ML0, GLT/CTS-SulE exhibited detoxification efficiencies for the plant height, fresh weight, main root length and root weight of maize of 96.41 %, 24.52 %, 56.82 %, and 21.81 %, respectively. In contrast, free SulE achieved only 56.33 %, 10.36 %, 38.36 %, and 20.74 % in these parameters. Similarly, a comparable trend was observed in the high-concentration metsulfuron-methyl treatment groups MHf and MHg. In the tribenuron-methyl treatment groups TLf, TLg, THf and THg, the detoxification efficiency of GLT/CTS-SulE for four parameter indicators of maize ranged from 53.27 % to 96.04 %. This was significantly higher than that of free SulE (24.93–54.90 %). Overall, the GLT/CTS-SulE exhibited a superior detoxification capacity across all maize indicators, particularly with respect to plant height and root growth. Furthermore, GLT/CTS-SulE alleviated the inhibitory effects on a broader range of maize growth parameters than free SulE, particularly under high concentrations of tribenuron-methyl pollution.

The observed improvement in plant growth reflects an overall mitigation of herbicide-induced stress in soil plant systems. Although plant growth parameters provide an integrative indicator of phytotoxicity, future studies incorporating herbicide residues in plant tissues, antioxidant enzyme activities, and photosynthetic characteristics will be necessary to elucidate the direct physiological mechanisms underlying enzyme-mediated detoxification.

3.2. Analysis of phospholipid fatty acid (PLFA) concentrations in soils

Three factors of treatment, concentration and time, as well as their interactions, significantly influenced the total PLFA concentration, bacterial PLFA concentration, fungal PLFA concentration, and the Shannon-Wiener index ($P < 0.05$), as detailed in Table 2. It should be noted that PLFA analysis in planted soil systems reflects an integrated response of soil microorganisms under rhizosphere conditions. Although strict sample preparation and the use of microbial-specific biomarkers were applied to minimize plant-derived interference, minor contributions from root exudates cannot be completely excluded.

3.3. Dynamics of the total PLFA concentration in soils

Free SulE and GLT/CTS-SulE had no significant effect on the total PLFA concentration in soils ($P > 0.05$) (Fig. 2A). As illustrated in Fig. 2B-2E, within 5 days, the total PLFA concentration in soils from groups TL0, ML0, or TH0, MH0 with only tribenuron-methyl or metsulfuron-methyl added was almost completely inhibited.

On day 5 and day 7, the total PLFA concentration in soils of groups TLf or TLg were significantly higher than that group TL0 ($P < 0.05$) with no significant difference observed between the two treatment groups (Fig. 2B). The same result was achieved for the low concentration of metsulfuron-methyl (Fig. 2C). These results suggest that both free SulE and GLT/CTS-SulE can mitigate the inhibitory effects of low concentrations of tribenuron-methyl or metsulfuron-methyl on the total PLFA concentration in soils, demonstrating comparable efficacy and nearly restoring levels to baseline conditions.

In the high concentration groups, only groups THg and MHg exhibited significantly increased the total PLFA concentration in soils than groups TH0 and MH0 ($P < 0.05$) (Fig. 2D, 2E). The findings suggested that GLT/CTS-SulE is more effective than free SulE in mitigating the inhibitory effect of high concentrations of tribenuron-methyl or metsulfuron-methyl on the total PLFA concentration in soils. Although a complete recovery to initial levels was not achieved, the alleviation

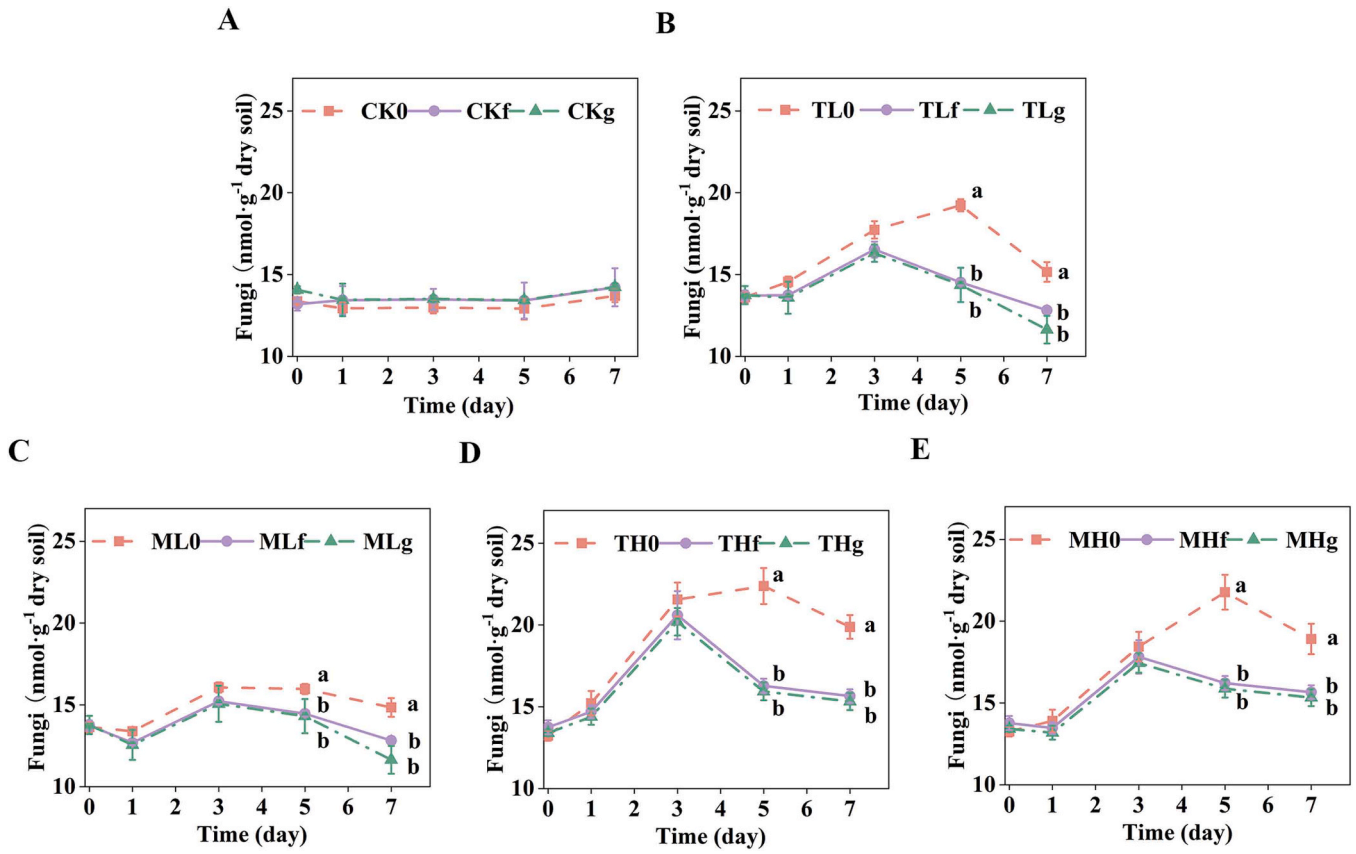

Fig. 4. The fungal PLFA concentration in soils. Different lowercase letters indicate significant differences among different treatment groups on the same sampling day, respectively ($P < 0.05$). "CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

effect was significant. This may be attributed to the enhanced stress resistance of GLT/CTS-SulE relative to free SulE in practical applications [29].

3.4. Dynamics of the bacterial PLFA concentration in soils

The results presented in Fig. 3A indicate that neither free SulE nor GLT/CTS-SulE exerted a significant impact on the bacterial PLFA concentration in soils ($P > 0.05$). The bacterial PLFA concentration in soils of groups TL0, TH0, ML0, MH0 decreased continuously within 5 days (Fig. 3B-3E). This reduction may be attributed to the inhibitory effects of tribenuron-methyl or metsulfuron-methyl on acetolactate synthase (ALS) in bacteria, which hinders their ability to synthesize essential branched-chain amino acids and ultimately leads to a decrease in bacterial biomass within the soil [30,31].

Compared to groups TL0 and ML0, the bacterial PLFA concentration in soils of groups TLg and MLg showed significant differences as early as 1 day, whereas groups TLf and MLf required 3 days ($P < 0.05$) (Fig. 3B, 3C). The results indicated that, compared with free SulE, GLT/CTS-SulE was more effective than free SulE in mitigating the inhibitory effects of low-concentration of tribenuron-methyl or metsulfuron-methyl on the bacterial PLFA concentration in soils. The initial advantage of GLT/CTS-SulE can be attributed to substrate enrichment, enhanced stability, and optimized microenvironments. In contrast, the convergence of effects between free SulE and GLT/CTS-SulE observed in the later stages may result from diffusion limitations, product accumulation, and irreversible inhibition, among other factors [32]. As illustrated in Fig. 3B-3E, on day 7, both free SulE and GLT/CTS-SulE significantly mitigated the inhibitory effects of low or high concentrations of tribenuron-methyl and metsulfuron-methyl on the bacterial PLFA concentration in soils. However, no significant difference was observed between the two treatments.

3.5. Dynamics of fungal PLFA concentration in soils

Neither free SulE nor GLT/CTS-SulE exhibited a significant effect on the fungal PLFA concentration in soils ($P > 0.05$) (Fig. 4A). However, the fungal PLFA concentration in soils from groups TL0, TH0, ML0, MH0 increased continuously within 5 days ($P < 0.05$) (Fig. 4B-4E). It is suggested that tribenuron-methyl or metsulfuron-methyl may enhance the growth of fungal PLFA concentration in soils by selectively inhibiting bacterial populations and mitigating the competitive suppression exerted by bacteria on fungi [33].

Compared to group TL0, the fungal PLFA concentration in groups TLg and TLf had significant differences on days 5 and 7. However, no obvious distinction was found between the two (Fig. 4B). For the low concentration of metsulfuron-methyl and the high concentrations of tribenuron-methyl or metsulfuron-methyl, we obtained consistent results (Fig. 4C-4E). The results indicated that both free SulE and GLT/CTS-SulE could mitigate the stimulatory effects of low or high concentrations of tribenuron-methyl or metsulfuron-methyl on the fungal PLFA concentration, thereby contributing to the maintenance of dynamic balance within the soil community. Further analysis revealed that after the addition of free SulE and GLT/CTS-SulE, the fungal PLFA concentration in the low concentration groups essentially returned to control levels. In contrast, while there was no complete recovery in the high concentration groups, a significant alleviation was observed ($P < 0.05$).

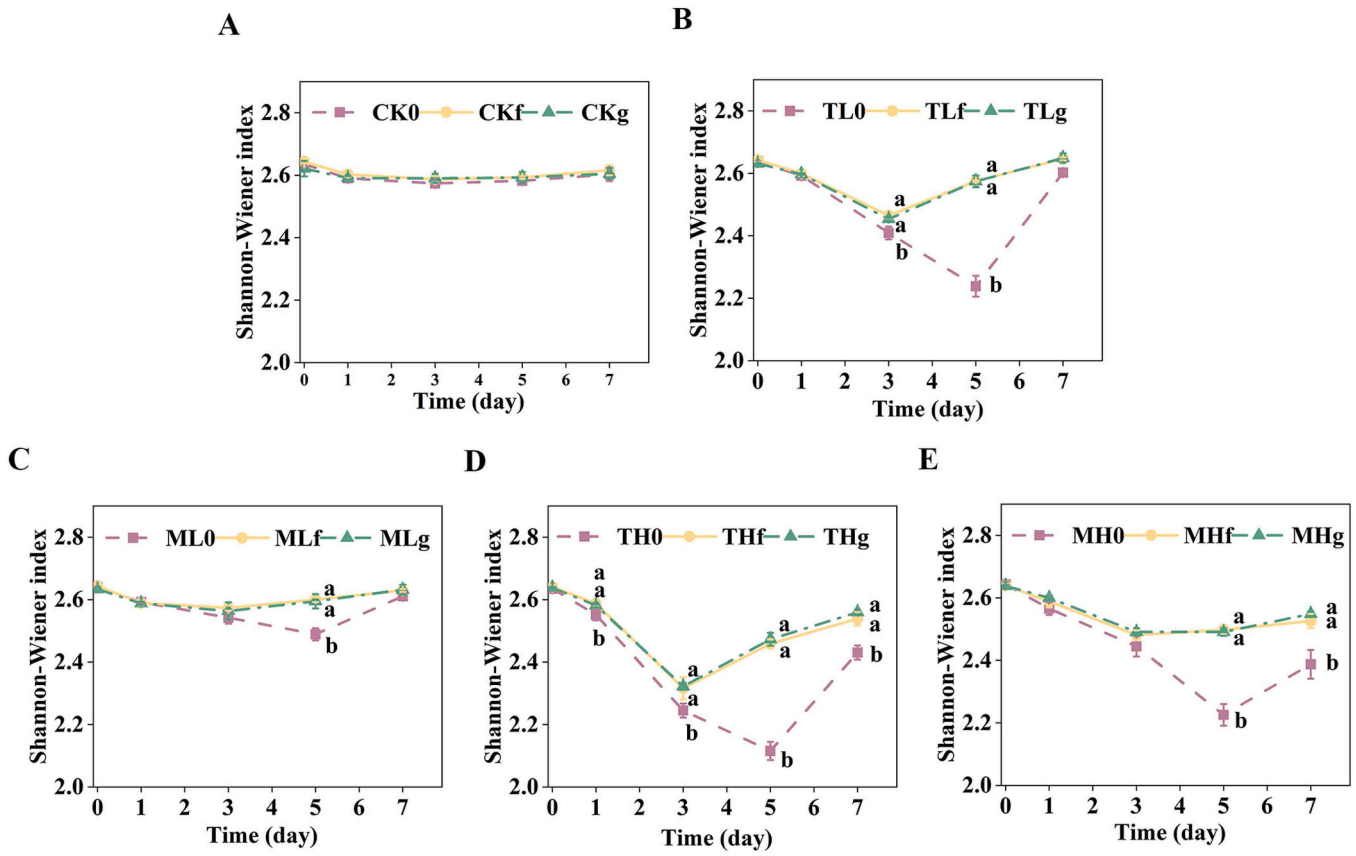

Fig. 5. The Shannon-Wiener index in soils. Different lowercase letters indicate significant differences among different treatment groups on the same sampling day, respectively ($P < 0.05$). "CK" indicates control treatment, "M" indicates metsulfuron-methyl treatment, "T" indicates tribenuron-methyl treatment, "L" indicates low concentration of sulfonylurea herbicide treatment, "H" indicates high concentration of sulfonylurea herbicide treatment, "0" indicates without SulE treatment, "f" indicates free SulE treatment, "g" indicates GLT/CTS-SulE treatment.

3.6. Dynamics of Shannon-Wiener index in soils

The results presented in Fig. 5A indicate that neither free SulE nor GLT/CTS-SulE significantly affected the Shannon-Wiener index in soils ($P > 0.05$). The Shannon-Wiener index in soils of groups TL0, TH0, ML0, MH0 consistently decreased within 5 days (Fig. 5B-5E), suggesting that tribenuron-methyl or metsulfuron-methyl may reduce the Shannon-Wiener index in soils, thereby simplifying the microbial community structure and disrupting its dynamic balance [34].

On days 3 and 5, the Shannon-Wiener index in soils of group TLf and group TLg showed significant differences ($P < 0.05$) compared to group TL0 (Fig. 5B). Similarly, in soils contaminated with a low concentration of metsulfuron-methyl, a significant difference was observed on day 5 ($P < 0.05$) (Fig. 5C). However, on day 7, the Shannon-Wiener index in soils of groups TL0 and ML0 demonstrated a tendency to recover, showing no significant differences ($P > 0.05$) when compared to other enzyme treatment groups TLf, MLf, TLg, and MLg (Fig. 5B, 5C). This result may be due to the dynamic balance of the soil microbial community itself [35].

On the 7th day, relative to group TH0, the Shannon-Wiener index in soils of groups THf and TLg both showed significant differences ($P < 0.05$). However, no significant difference was observed between these two groups ($P > 0.05$) (Fig. 5D). Furthermore, similar results were obtained in soils contaminated with the high concentration of metsulfuron-methyl (Fig. 5E). The research results indicate that both the free SulE and GLT/CTS-SulE can significantly mitigate the inhibitory effect of high concentration tribenuron-methyl or metsulfuron-methyl on the Shannon-Wiener index in soils, with comparable efficacy observed for both treatments.

To further contextualize the performance of GLT/CTS-SulE, we compared its repair efficacy with that of other reported immobilized enzyme systems. Immobilized enzymes on single-component or rigid inorganic carriers often face limited mass transfer, reduced substrate accessibility, and rapid activity loss under alkaline soil conditions [36, 37]. In contrast, the gelatin/chitosan composite carrier used in this study provides a biocompatible, porous, and mechanically stable microenvironment that enhances enzyme stability and preserves catalytic efficiency [38]. This synergy enhances SulE's resistance to environmental stress and prolongs catalytic activity. Chitosan-based supports and composite hydrogels have been shown to markedly improve enzyme resistance to pH and temperature stress, enhancing functional stability relative to free enzymes [39,40]. As a result, GLT/CTS-SulE exhibits sustained detoxification performance across a range of herbicide concentrations, outperforming free enzymes and less robust carriers, particularly under high-stress conditions. This highlights the advantages of gelatin/chitosan as an immobilization matrix, supporting both enzyme longevity and functional resilience in complex soil systems.

4. Conclusion

This study demonstrates that the immobilization of esterase SulE on gelatin/chitosan (GLT/CTS-SulE) effectively remediates soil contaminated with sulfonylurea herbicides by simultaneously mitigating phytotoxicity and restoring the balance of soil microbial communities. GLT/CTS-SulE has shown superior performance compared to free SulE, particularly under conditions of high herbicide stress. This highlights the significant advantages of the immobilization strategy in enhancing

enzyme efficacy and stability within complex soil environments.

Exposure to tribenuron-methyl or metsulfuron-methyl significantly inhibited maize height, fresh weight, main root length, and root weight. The application of GLT/CTS-SulE effectively mitigated this inhibition, demonstrating a significantly greater efficacy compared to free SulE, particularly under conditions of high concentration of tribenuron-methyl or metsulfuron-methyl pollution. Microbial community analysis revealed that pollution from tribenuron-methyl or metsulfuron-methyl significantly decreased the total PLFA concentration, bacterial PLFA concentration and Shannon-Wiener index, while simultaneously promoting fungal PLFA concentration. The application of GLT/CTS-SulE effectively sustains the dynamic balance of soil microbial community structure in soils contaminated with tribenuron-methyl or metsulfuron-methyl. Furthermore, the inhibitory effect of GLT/CTS-SulE on total PLFA concentration in soil contaminated by high concentrations of tribenuron-methyl or metsulfuron-methyl is markedly superior to that observed with free SulE.

In conclusion, GLT/CTS-SulE represents a reliable and efficient bioremediation technology strategy for soil contaminated with sulfonylurea herbicides. This approach effectively catalyzes the degradation of residual herbicides, significantly mitigating their physiological toxicity to plants while also facilitating the recovery process of soil microbial communities. Future research should focus on evaluating the long-term stability and efficacy of GLT/CTS-SulE under field conditions, as well as exploring its degradation potential against a broader spectrum of sulfonylurea herbicides.

CRediT authorship contribution statement

Xuanhe Fu: Visualization, Formal analysis. **Xiang Li:** Writing – original draft, Formal analysis, Conceptualization. **Zhixiong Yu:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Formal analysis, Conceptualization. **Huiwen Zhang:** Funding acquisition, Conceptualization. **Zhencheng Su:** Investigation. **Xu Li:** Formal analysis.

Declaration of Competing Interest

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

Acknowledgements

The work was supported by Strategic Priority Research Program of the Chinese Academy of Sciences (Grant No. XDA28010503), and Doctoral Start-up Foundation of Liaoning Province (Grant No. 2025-BS-0866).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2026.02.011.

Data availability

Data will be made available on request.

References

[1] Q. Lei, J. Zhong, S. Chen, S. Wu, Y. Huang, P. Guo, S. Mishra, K. Bhatt, S. Chen, Microbial degradation as a powerful weapon in the removal of sulfonylurea herbicides, Environ. Res. 235 (2023) 116570, https://doi.org/10.1016/j.envres.2023.116570.

[2] Z. He, T. Zhang, X. Lu, R. Li, W. Peng, F. Ding, Assessing the environmental risks of sulfonylurea pollutants: insights into the risk priority and structure-toxicity relationships, Ecotoxicol. Environ. Saf. 292 (2025) 117973, https://doi.org/10.1016/j.ecoenv.2025.117973.

[3] M. Zheng, Y. Li, Q. Zhang, W. Wang, Enzymatic degradation toward herbicides: the case of the sulfonylureas, Environ. Sci. Technol. 58 (2024) 20049–20059, https://doi.org/10.1021/acs.est.4c04929.

[4] D. Chetverikova, M. Bakaeva, S. Starikov, A. Kendjieva, S. Chetverikov, The influence of plant growth-stimulating bacteria on the glutathione-S-transferase activity and the toxic effect of the herbicide metsulfuron-methyl in wheat and canola plants, Toxics 12 (2024) 886, https://doi.org/10.3390/toxics12120886.

[5] A.B. Chowdhary, J. Singh, J. Quadar, S. Singh, A. Singh, R. Dutta, D. Angmo, A. P. Vig, Metsulfuron-methyl induced physiological, behavioural and biochemical changes in exotic (Eisenia fetida) and indigenous (Metaphire posthuma) earthworm species: toxicity and molecular docking studies, Pestic. Biochem. Physiol. 188 (2022) 105276, https://doi.org/10.1016/j.pestbp.2022.105276.

[6] X. Song, J. Cao, S. Guo, H. Wang, Q. Dong, P. Guo, X. Yuan, Effect of brassinolide on soil microorganisms in millet field polluted by tribenuron-methyl, Microorganisms 11 (2023) 1829, https://doi.org/10.3390/microorganisms11071829.

[7] H. Zhang, H. Yu, Y. Xiang, H. Wang, Y. Qian, X. Huang, Enhanced bioremediation of bensulfuron-methyl contaminated soil by Hansschlegelia zhihuaiae S113: metabolic pathways and bacterial community structure, J. Hazard. Mater. 480 (2024) 136471, https://doi.org/10.1016/j.jhazmat.2024.136471.

[8] C. Zhou, D. Li, P. Miao, H. Cheng, H. Zhang, X. Wan, H. Yu, Y. Jia, Q. Dong, C. Pan, Bensulfuron methyl induced multiple stress responses in the field wheat plants: microbial community and metabolic network disturbance, J. Hazard. Mater. 476 (2024) 134874, https://doi.org/10.1016/j.jhazmat.2024.134874.

[9] B. Liu, W. Tian, M. Chu, Z. Lu, M. Zou, Z. Chen, R. Zhang, Removal of sulfonylurea herbicides with g-C_3N_4-based photocatalysts: a review, Chemosphere 354 (2024) 141742, https://doi.org/10.1016/j.chemosphere.2024.141742.

[10] Z. Gao, C. Gu, X. Fan, L. Shen, Z. Jin, F. Wang, X. Jiang, Biochemical insights into the biodegradation mechanism of typical sulfonylureas herbicides and association with active enzymes and physiological response of fungal microbes: a multi-omics approach, Environ. Int. 190 (2024) 108906, https://doi.org/10.1016/j.envint.2024.108906.

[11] Z. Yu, W. Gu, Y. Yang, X. Li, X. Li, T. Li, J. Wang, Z. Su, X. Li, Y. Dai, M. Xu, H. Zhang, Whole-genome sequencing of a chlorimuron-ethyl-degrading strain: Chenggangzhangella methanolivorans CHL1 and its degrading enzymes, Microbiol. Spectr. 10 (2022) e0182222, https://doi.org/10.1128/spectrum.01822-22.

[12] L. Yang, X. Li, X. Li, Z. Su, C. Zhang, M. Xu, Improved stability and enhanced efficiency to degrade chlorimuron-ethyl by the entrapment of esterase SulE in cross-linked poly (γ-glutamic acid)/gelatin hydrogel, J. Hazard. Mater. 287 (2015) 287–295, https://doi.org/10.1016/j.jhazmat.2015.01.056.

[13] P.R. Yaashikaa, M.K. Devi, P.S. Kumar, Advances in the application of immobilized enzyme for the remediation of hazardous pollutant: a review, Chemosphere 299 (2022) 134390, https://doi.org/10.1016/j.chemosphere.2022.134390.

[14] X. Liu, M. Qiu, Y. Zhang, J. Pan, W. Zhang, L. Guo, X. Zhang, Y. Jiang, Enzyme immobilization based on reticular framework materials: strategy, food applications, and prospect, Adv. Colloid Interface Sci. 344 (2025) 103589, https://doi.org/10.1016/j.cis.2025.103589.

[15] H. Hu, W. Lu, S. Li, X. Zhou, C. Zhu, X. Wang, H. Dai, H. Geng, Hydrogel-based materials for microbial/enzyme immobilization: advanced applications in wastewater treatment, Chem. Eng. J. 511 (2025) 161878, https://doi.org/10.1016/j.cej.2025.161878.

[16] S. Zhang, Z. Wang, S. Huang, J. Zhang, J. Wu, S. Wu, Degradation mechanism of PGNa by the immobilized degrading enzymes from magnetic fermentation residue biochar, Sci. Total Environ. 963 (2025) 178432, https://doi.org/10.1016/j.scitotenv.2025.178432.

[17] R.R. Magalhães, P.G. Abrantes, P.G. Abrantes, I.F. Costa, E.E.S. Teotonio, J.A. Vale, Chitosan functionalized with EDTA as a new support for enzyme immobilization and its application on enzymatic resolution of (R,S)-1-phenylethanol, Process Biochem. 149 (2025) 128–136, https://doi.org/10.1016/j.procbio.2024.12.004.

[18] K. Garg, R. Sehgal, D. Sharma, R. Gupta, Polyhydroxyalkanoates as matrices for enzyme immobilization: in vivo and in vitro approaches, Process Biochem. 147 (2024) 530–542, https://doi.org/10.1016/j.procbio.2024.10.016.

[19] Y. Huang, T. Chen, C. Ren, B. Bao, R. Huang, Y. Sun, C. Yu, Y. Yang, W.T. Wong, Q. Zeng, L. Jiang, T. Liu, Q. Lin, L. Zhu, Y. Liao, High-strength gelatin hydrogel scaffold with drug loading remodels the inflammatory microenvironment to enhance osteoporotic bone repair, Adv. Mater. 37 (2025) e2501051, https://doi.org/10.1002/adma.202501051.

[20] Z. Pang, Q. Li, K. Liu, X. Wu, H. Xu, Z. Chen, H. Dai, Efficacy of melanin-loaded lipoic acid-modified chitosan hydrogel in diabetic wound healing, Carbohydr. Polym. 340 (2024) 122215, https://doi.org/10.1016/j.carbpol.2024.122215.

[21] S. Sethi, Medha, B.S. Kaith, A review on chitosan-gelatin nanocomposites: synthesis, characterization and biomedical applications, React. Funct. Polym. 179 (2022) 105362, https://doi.org/10.1016/j.reactfunctpolym.2022.105362.

[22] N. Maslamani, E.M. Bakhsh, R. Almutairi, Y. Alrasheedi, R. Hammad, M. Aljohani, K. Akhtar, Y.O. Al-Ghamdi, S.B. Khan, M. Alharbi, Metal nanocomposite supported on gelatin chitosan hydrogel as a catalyst for the removal of toxins, Sci. Rep. 5 (2025) 24084, https://doi.org/10.1038/s41598-025-08503-3.

[23] Z. Yu, H. Zhang, X. Fu, X. Li, Q. Guo, T. Yang, X. Li, Immobilization of esterase SulE in cross-linked gelatin/chitosan and its application in remediating soils polluted with tribenuron-methyl and metsulfuron-methyl, Process Biochem. 98 (2020) 217–223, https://doi.org/10.1016/j.procbio.2020.08.014.

[24] A. Frostegård, E. Bååth, The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil, Biol. Fertil. Soils 22 (1996) 59–65, https://doi.org/10.1007/BF00384433.

[25] J.H. Hwang, T.R. Choi, S. Kim, Y. Lee, Y. Shin, S. Choi, J. Oh, S.H. Kim, J.H. Park, S.K. Bhatia, Y.H. Yang, Evaluation of simplified ester-linked fatty acid analysis (ELFA) for phospholipid fatty acid (PLFA) analysis of bacterial population, Anal. Biochem. 695 (2024) 115638, https://doi.org/10.1016/j.ab.2024.115638.
[26] D.A. Bossio, K.M. Scow, N. Gunapala, K.J. Graham, Determinants of soil microbial communities: effects of agricultural management, season, and soil type on phospholipid fatty acid profiles, Microb. Ecol. 36 (1998) 1–12, https://doi.org/10.1007/s002489900087.
[27] L. Zelles, Q.Y. Bai, R.X. Ma, R. Rackwitz, K. Winter, F. Beese, Microbial biomass, metabolic activity and nutritional status determined from fatty acid patterns and poly-hydroxybutyrate in agriculturally-managed soils, Soil Biol. Biochem. 26 (1994) 439–446, https://doi.org/10.1016/0038-0717(94)90175-9.
[28] R.F. Herrmann, J.F. Shann, Microbial community changes during the composting of municipal solid waste, Microb. Ecol. 33 (1997) 78–85, https://doi.org/10.1007/s002489900010.
[29] P.R. Yaashikaa, M.K. Devi, P.S. Kumar, Advances in the application of immobilized enzyme for the remediation of hazardous pollutant: a review, Chemosphere 299 (2022) 134390, https://doi.org/10.1016/j.chemosphere.2022.134390.
[30] W. Weng, X. Yao, M. Zhao, Z. Fang, S. Yang, J. Ruan, Novel mutations in acetolactate synthase confer high levels of resistance to tribenuron-methyl in Fagopyrum tataricum, Pestic. Biochem. Physiol. 204 (2024) 106039, https://doi.org/10.1016/j.pestbp.2024.106039.
[31] M. Bakaeva, S. Chetverikov, S. Starikov, A. Kendjieva, G. Khudaygulov, D. Chetverikova, Effect of plant growth-promoting bacteria on antioxidant status, acetolactate synthase activity, and growth of common wheat and canola exposed to metsulfuron-methyl, J. Xenobiot. 14 (2024) 79–95, https://doi.org/10.3390/jox14010005.
[32] Y. Bai, Z. Jing, R. Ma, X. Wan, J. Liu, W. Huang, A critical review of enzymes immobilized on chitosan composites: characterization and applications, Bioprocess Biosyst. Eng. 46 (2023) 1539–1567, https://doi.org/10.1007/s00449-023-02914-0.
[33] L. Radivojevic, D. Jovičić, L. Šantrić, S. Gasić, J. Gajić-Umiljendić, Effects of metsulfuron-methyl on soil microbial activity, Arch. Tech. Sci. 11 (2014) 77–87, https://doi.org/10.7251/afts.2014.0611.077R.
[34] X. Li, D. Meng, J. Li, H. Yin, H. Liu, X. Liu, C. Cheng, Y. Xiao, Z. Liu, M. Yan, Response of soil microbial communities and microbial interactions to long-term heavy metal contamination, Environ. Pollut. 231 (2017) 908–917, https://doi.org/10.1016/j.envpol.2017.08.057.
[35] L. Philippot, C. Chenu, A. Kappler, M.C. Rillig, N. Fierer, The interplay between microbial communities and soil properties, Nat. Rev. Microbiol. 22 (2024) 226–239, https://doi.org/10.1038/s41579-023-00980-5.
[36] S.A. Nour, E.A. Sakr, H. Kandil, Enhanced enzyme activity and stability through immobilization of recombinant chitinase on sodium alginate-modified rice husk beads for efficient decolorization of synthetic dyes, J. Biol. Eng. 19 (2025) 78, https://doi.org/10.1186/s13036-025-00546-4.
[37] G. Peta, R. Avrahami, E. Zussman, J.S. Rokem, C. Greenblatt, Immobilization of enzymes for synergy in polymers to produce lactose free milk, Sci. Rep. 15 (2025) 17618, https://doi.org/10.1038/s41598-025-00172-6.
[38] M. Tadesse, Y. Liu, Recent advances in enzyme immobilization: the role of artificial intelligence, novel nanomaterials, and dynamic carrier systems, Catalysts 15 (2025) 571, https://doi.org/10.3390/catal15060571.
[39] H. Zhang, X. Zhang, L. Wang, B. Wang, X. Zeng, B. Ren, Functionalized chitosan and alginate composite hydrogel-immobilized laccase with sustainable biocatalysts for the effective removal of organic pollutant bisphenol A, Catalysts 14 (2024) 304, https://doi.org/10.3390/catal14050304.
[40] V. Atiroğlu, A. Atiroğlu, A. Atiroğlu, A.S. Al-Hajri, M. Özacar, Green immobilization: enhancing enzyme stability and reusability on eco-friendly support, Food Chem. 448 (2024) 138978, https://doi.org/10.1016/j.foodchem.2024.138978.