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Research article

Degradation dynamics of Trifluralin in Qinghai-Tibet Plateau and screening of its degrading strains

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ABSTRACT

Trifluralin (FLL) is extensively used in rapeseed fields in the Qinghai-Tibet Plateau (QTP) region. However, the degradation kinetics of FLL in this area and its impact on environmental microbial communities are not yet known. To investigate the degradation patterns and ecological benefits of FLL, this study established a comprehensive method for detecting FLL residues and selected efficient degrading bacterial strains. Degradation experiments were conducted in two typical soil types of the QTP, and the dynamic changes in microbial communities were explored. The results indicated that FLL degradation in soils from two different regions of the QTP followed first-order kinetics, with half-lives of 25 and 39 days, respectively. The application of FLL at 173 g/ha significantly increased bacterial richness and diversity in the soils of both regions. Three efficient degrading strains were selected from soil samples: FL-3 (*Bacillus velezensis*) with a degradation rate of 80.81 %, FL-5 (*Bacillus velezensis*) at 51.18 %, and FL-6 (*Pseudomonas atacamensis*) at 49.98 %. Moreover, the optimal degradation conditions for these strains were determined, and it was verified that they had no adverse effects on the germination and seedling growth of rapeseed, wheat, and barley. The findings of this study provide important data for the environmental risk assessment of FLL and suggest potential biological resources for the rational use and environmental remediation of this herbicide. These results are significant for developing safe use and environmental management strategies for FLL in the QTP.

1. Introduction

FLL is a dinitroaniline herbicide [1], which is generally made into an emulsifiable concentrate and widely used in farmland production [2]. It is volatile and undergoes photolysis under natural conditions, while FLL in soil is fixed by soil colloids, which makes it difficult to move and volatilize [3,4]. Studies have found that after 60 days of FLL application to farmland, high residues are still detected in the soil [5]. Trifluralin has strong adsorption in soil, exists for a long time, and exists in the form of parent compounds and

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metabolites for a long time [6]. FLL is toxic to aquatic organisms and has potential genotoxicity [1,7]. The extensive use of trifluralin will inhibit the synthesis of carbonic anhydrase (CAs) in human body, and has potential effects on ovary and growth and development [8,9]. Even in some European countries, FLL has been restricted [10,11], but FLL is still a commonly used herbicide in China because of its high-cost performance and high weeding efficiency. It is often used for weed control in rapeseed fields [12]. The long-term and extensive use of FLL poses a potential threat to the soil environment, especially in the ecologically fragile QTP, which seriously affects the environment and ecological security [13,14]. Therefore, it is important to study the residual degradation of FLL for environmental protection.

The detection methods for FLL residues include electrochemical methods [15], chromatography [16], and electrochemical analysis [17]; the most commonly used analytical method is chromatography. For example, Shakerkhatibi [18] used liquid-liquid extraction and gas chromatography/mass (GC/MS) spectrometry to determine the FLL in groundwater. Chowdhury [5,19] analyzed the dissipation kinetics of FLL in soil using a GC-electron capture detector (GC-ECD). Zhu [19] used GC to detect FLL residues in tea. At present, research on the microbial degradation of FLL mainly focuses on bacteria, and it has been found that Pseudomonas fluorescens can biodegrade and accelerate its detoxification process in soil [20]. Erguven [21] isolated 11 species of bacteria and fungi in activated medium, all of which had a degradation effect on FLL. Nihaiyan [22] cloned a functional gene, PNR, encoding nitroreductase from Bacillus subtilis Y3 and verified that this functional gene can degrade FLL through nitroreduction. However, owing to the characteristics of high altitude and high ultraviolet radiation in the QTP, a degradation strain suitable for the survival of the region is needed to degrade FLL.

A few FLL-degrading strains have been screened at home and abroad [23,24]. However, there have been few studies on the degradation characteristics of FLL-degrading strains. FLL degradation is affected by many factors, including temperature, inoculation amount, and pH. Temperature mainly affects metabolism and degradation by affecting the internal environment of the microorganisms. Only by studying the most suitable degradation temperature can the highest degradation efficiency of microorganisms be achieved. The amount of inoculation directly affects the efficiency of microbial degradation. Inoculation with too little will lead to a prolonged culture time and reduced degradation time and efficiency. Excessive inoculation can easily cause insufficient dissolved oxygen and inhibit product synthesis, and the pH can directly affect the enzyme activity of microbial cells, thereby affecting the degradation efficiency of microorganisms [25]. Therefore, it is necessary to investigate the degradation conditions.

FLL is a widely used high-efficiency herbicide in the Qinghai area, and its long-term application makes the short-term residue of FLL in the soil unsuitable for digestion, which seriously affects the ecological environment and crop growth. To solve this problem, this study first clarified the degradation law of FLL in the soil and its effect on soil bacteria. The degradation effect of FLL-degrading strains was determined using the established Quick, Easy, Cheap, Effective, Rugged, and Safe - Gas Chromatography - Electron Capture Detector (QuEChERS-GC-ECD) method, and the safety and degradation characteristics of the strains were studied. Finally, we speculated that the optimal strain degraded FLL. The research results provide resources for the microbial degradation of FLL and a solid foundation for future research on the degradation mechanism of FLL.

2. Materials and methods

2.1. QuEChERS pretreatment optimization

A total 0.100 g of FLL (Purity (GC area %) $\geq$98 %) standard was weighed and diluted to 100 mL with n-hexane to obtain standard working solutions with concentrations of 0.01, 0.05, 0.1, 0.2, 0.5, and 1 mg/L. The standard curve of the solvent was prepared according to the relationship between FLL concentration and peak area.

The 10.0 g soil, 2.0 g rape roots, leaves and grains were weighed, and 5 g rape stems (the aforementioned sample does not contain FLL prior to treatment) were spiked at concentrations of 0.01 mg/L, 0.1 mg/L, and 1 mg/L, respectively. 10.0 mL Luria-Bertani (LB), modified Martin, and Gao 's No.1 were recovered at concentration of 0.01 mg/L, 0.1 mg/L and 1 mg/L, respectively. Five replicates were set for each spiked level and the recovery and relative standard deviation of each spiked level were calculated.

The pretreatment conditions of the rape plants were optimized using an orthogonal method. Primary Secondary Amine (PSA), Graphitized Carbon Black (GCB), and Octadecylsilyl (C18) were selected as the three purifying agents. The orthogonal test table of four factors and three levels was designed by optimizing the type of extracting agent, the amount of extracting agent, the type of purifying agent, and the amount of purifying agent (Tables S1 and S2).

GC conditions: The capillary column used is HP-5 (30 m $\times$ 0.25 mm, 0.25 μm), with high-purity N_2 as the carrier gas, the injection port temperature is 220 °C, using split injection with a split ratio of 5:1, and a flow rate of 1 mL/min, with an injection volume of 1 μL. The initial temperature is set at 40 °C, 0–6.0min, 40°C–240 °C.2.2 Screening and identification of degrading strains.

Preparation of soil suspension: 1.0 g of rape field soil enriched with FLL was added to sterile water (9.0 mL) and diluted with sterile water to obtain a 10^{-4}-10^{-1} soil suspension. 0.1 mL of soil suspension with different gradients was drawn and coated on LB, modified Martin, and Gao 's No.1 medium containing 1.0 mg/L FLL. Each medium was tested in triplicate and cultured at 30 °C for three days. Single colonies were picked from the plate and cultured until they appeared. Each strain was numbered and stored in solid slanted medium.

Detection of strain degradation ability: Each strain was inoculated into the corresponding liquid medium and cultured for 24 h as a seed solution. The seed liquid was inoculated with 1 % inoculation into three types of FLL liquid medium with a concentration of 1.0 mg/L and cultured at 30 °C with 180 rpm oscillation. The samples were collected on days 1, 3, 5, 7, and 14, and the medium without inoculation with the same concentration of FLL was used as the control. The samples after pretreatment were analyzed by gas chromatograph −2010 puls (Shimadzu, Japan). The degradation rate of FLL by each strain was determined and analyzed.

The degradation rate is calculated as follows:

$$Q(\%) = \frac{C_0 - C_t}{C_0} * 100\% \quad (1)$$

where Q is the degradation rate of FLL (%), C_0 is the initial concentration of FLL (mg/L), and C_t is the residual FLL content (mg/L) in the culture medium.

2.2. Seed germination test and pot experiment

The seeds of rape, wheat, and highland barley were placed in warm water at 25 °C to remove unqualified seeds, such as shriveled and diseased seeds floating on the water surface. Seeds of uniform size were selected, soaked in 1 % sodium hypochlorite solution for 30 min, and rinsed with sterile water three times. Rape, wheat, and barley seeds were used as receptors, and seven treatments were set for each crop: (1) 1×10^9 cfu/mL FL-3, (2) 1×10^9 cfu/mL FL-5, (3) 1×10^9 cfu/mL FL-6, (4) 1×10^8 cfu/mL FL-3, (5) 1×10^8 cfu/mL FL-5, (6) 1×10^8 cfu/mL FL-6, and (7) sterile water control.

Seed germination test: 15 seeds were sown in each dish, moistened with sterile distilled water, and then germinated, and 5.0 mL 1×10^9 cfu/mL bacterial solution and 1×10^8 cfu/mL bacterial solution were added to each Petri dish according to the treatment, and the same amount of sterile water was added to CK. Each treatment was set up 3 replicates, in an incubator at 28 °C constant temperature culture, light and dark for 12 h, and after 7 d, the germination rate, root length, bud length, and seedling length of each rapeseed were calculated [26].

Determination of germination index:

$$\text{percentage of germination} = \frac{\text{number of germinated seeds}}{\text{number of test seeds}} * 100\% \quad (2)$$

Indoor pot experiment: Sow 15 seeds in a flowerpot filled with sterile soil., 50 mL of 1×10^9 cfu/mL and 1×10^8 cfu/mL bacterial solution were added to each flowerpot according to the treatment, and the same amount of sterile water was added to CK. Each treatment was repeated three times, and water was added every three days during the growth period. After 7 d of culture in the greenhouse, the root length and seedling length of rape, wheat, and highland barley were calculated [27].

2.3. Single factor test and response surface optimization

The effects of temperature, pH, and amount of inoculation on the degradation ability of the strain were studied. The specific operation steps are shown in the supplementary materials: single-factor degradation characteristic operation steps. Based on a single-factor experiment, the degradation temperature (°C), pH, and inoculation amount (%) were independent variables, and the response value was the degradation rate of FLL. The Box-Behnken three-factor three-level test was designed using Design Expert 8.0.6 software. A total of 17 test points were designed to determine the optimal degradation conditions for the FLL-degrading strains.

2.4. Trifluralin digestion dynamics and high-throughput sequencing

According to the field-recommended dose of FLL (173 g a. i./hm^2), the FLL treatment area and blank control group were set up in the Xining and Menyuan rape fields. In this area, trifluralin was not used in the process of agricultural planting in previous years, and the original residue of trifluralin in soil was less than 0.001 mg/kg. Each plot area was 25.0 m^2, repeated thrice, and a protective belt was set between the plots. Rape seeds were sown 4–5 d after application. Soil samples were randomly collected at 2 h, 1, 5, 7, 14, 21, 35, 45, and 60 d to detect the degradation dynamics of FLL in the soil. The rape field soil in the Xining area of Qinghai Province was collected at 2 h and 1, 3, 7, 14, 21, 28, and 35 d, and the soil of the rape field in the Menyuan area was collected at 2 h and 1, 3, 7, 14, 21, and 28 d. Three replicates were set for each group and 16S rDNA high-throughput sequencing analysis of the bacteria was

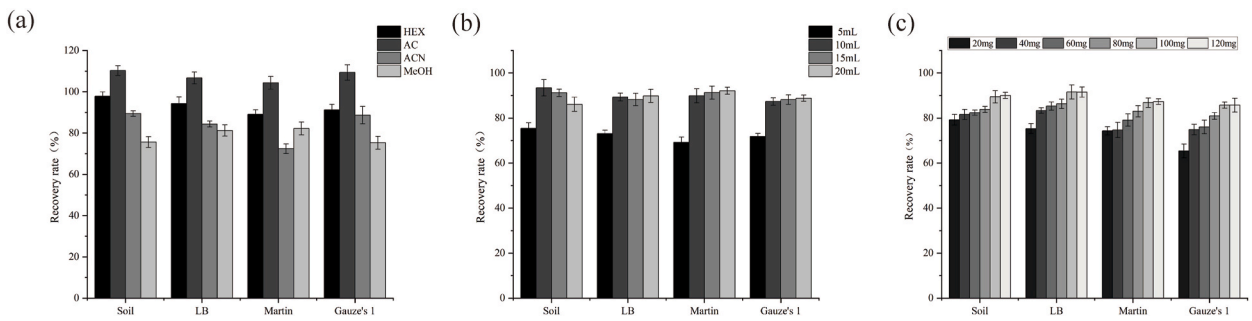

Fig. 1. Optimization of trifluralin extraction agent in soil and medium. (a) Extractant type, (b). The amount of extractant, (c). Optimization of PSA dosage.

performed.

3. Results and discussion

3.1. Construction of trifluralin detection method and optimization of pretreatment method

Finally, GC conditions were determined (Table S3). The analysis time was 11 min, with a retention time of approximately 6.5 min. No interference was observed around the target peak, indicating that the detection method could detect FLL in the matrix with high sensitivity and specificity. A standard curve was constructed by plotting the relationship between the concentration and the peak area of FLL. The standard equation was determined to be $Y = 3020242.58X + 234759.38$, with an R^2 of 0.9988. The peak area of the FLL standard sample demonstrated a satisfactory linear relationship within a concentration range of 0.01 mg/L- 1.0 mg/L.

The effects of different types (n-hexane, ethanol, acetonitrile, and acetone) and different amounts (5, 10, 15, and 20 mL) of extractants on FLL were compared with the addition of 1 mg/L FLL. It can be seen from the results of Fig. 1a that the extraction effect of n-hexane was the best. When extracted with 10 mL n-hexane (Fig. 1b), the average recovery rates of FLL in the soil, LB medium, modified Martin medium, and Gao 's No.1 medium were 93.45 %, 89.34 %, 89.91 %, and 87.31 %, respectively. Considering the extraction effect and cost, 10 mL n-hexane was selected to extract FLL from the soil and three media.

The effects of PSA dosages of 20, 40, 60, 80, 100, and 120 mg on the recovery of FLL in different matrices were compared. It can be seen from Fig. 1c that when PSA 100 mg, the average recovery rates of FLL in soil, LB, modified Martin and Gao 's No.1 medium were 89.44 %, 91.59 %, 87.29 % and 85.72 %, respectively. Considering the purification effect and cost, PSA 100 mg was selected as the purifying agent. There are few pigments in soil and medium, and PSA can meet the basic requirements of pretreatment. The key to ensure the purification effect in QuEChERS method is not only the type of purifying agent, but also the amount of purifying agent.

The results of orthogonal test of rape plants were shown in Tables S4–S5. The recovery rate of trifluralin in rape roots was employed as the evaluation index. The order of the influencing factors of the extraction effect was A > D > B > C, and the optimal pretreatment combination was $A_3B_1C_2D_1$. The order of the factors affecting the extraction effect of rape stems was A > D > B > C, and the optimal pretreatment combination was $A_3B_3C_1D_1$. The order of factors affecting the extraction effect of rape leaves was A > D > C > B, and the best pretreatment combination was $A_3B_3C_2D_1$. The order of factors affecting the extraction effect of rape seeds was A > D > C > B, and the best pretreatment combination was $A_2B_2C_3D_2$. Rape plants have a higher pigment content, and rapeseed grains contain a significant amount of oil. The color of methanol and acetonitrile extracts is darker, while that of the n-hexane extract is the lightest and the oil content is the least (Fig. S1 A-D). The pigment adsorption effect of GCB in the purifying agent is the most effective, and the oil content of the sample purified by C_{18} is significantly the least, and the sample is clearer (Fig. S1 E-H). The results of the orthogonal test were combined with those of the recovery rate to determine that 2 g of rape root was extracted with 10 mL of n-hexane and purified with 20 mg of GCB. Five grams of rape stem were extracted with 20 mL n-hexane and purified by PSA 20 mg. Two grams of rapeseed leaves were extracted with 20 mL n-hexane and purified with 20 mg GCB, while 2 g of rapeseed grains were extracted with 15 mL acetonitrile and purified with 50 mg C_{18}.

The endogenous components in the plant and the exogenous components in the sample pretreatment process will affect the extraction efficiency, resulting in a matrix effect [28]. The blank matrix solution was obtained by pretreatment of soil, LB, modified Martin and Gao 's No.1 medium, and the matrix standard curve was made by the relationship between the concentration and peak area of FLL. The linear regression equations of FLL in soil, LB, modified Martin, Gao 's No.1, rape root, stem, leaf and grain are shown in Table 1, and the correlation coefficients R2 are 0.9979, 0.9928, 0.9905, 0.9967, 0.9987, 0.9989, 0.9937 and 0.9983, respectively, indicating that the mass concentration and peak area of FLL have a good linear relationship in the concentration range of 0.01–1 mg/L. The LOQ of FLL in these eight matrices was 0.01 mg/kg. The linear equation and detection limit met the requirements of pesticide residue detection (NY/T788-2018). In addition, the absolute value of ME in the modified Martin medium was greater than 20 %, indicating that there may be factors affecting the extraction efficiency of FLL in the modified Martin medium. Finally, the matrix matching standard curve was used for quantitative analysis to offset the matrix effect. The absolute values of ME in soil, LB, Gao 's No.1, rape roots, stems, leaves and grains were less than 20 %, indicating that the matrix effect was weak, and the solvent standard curve could be used for quantitative analysis. The matrix standard curve of FLL in soil, three media and rapeseed plants are shown in Fig. 2.

Table 1
The regression equation, correlation coefficient, detection limit and matrix effect of trifluralin in 8 kinds of matrix were analyzed.

matrix	Regression equation	R^2	LOQ (mg/kg)	Range (mg/kg)	Matrix effect (%)
Soil	y = 3161151.67x+209410.89	0.9979	0.01	0.01–1	4.67
LB	y = 3111521.54x+214544.57	0.9928	0.01	0.01–1	3.02
Improved Martin	y = 2078173.84x+214117.03	0.9905	0.01	0.01–1	−31.19
high number 1	y = 2810353.83x+135256.81	0.9967	0.01	0.01–1	−6.95
Root	y = 2897060.36x+200736.96	0.9987	0.01	0.01–1	−4.08
Stem	y = 3353746.34x+287235.14	0.9989	0.01	0.01–1	11.04
Leaf	y = 3506778.14x+220622.94	0.9937	0.01	0.01–1	16.11
kernel	y = 3174878.65x+223191.45	0.9983	0.01	0.01–1	5.12

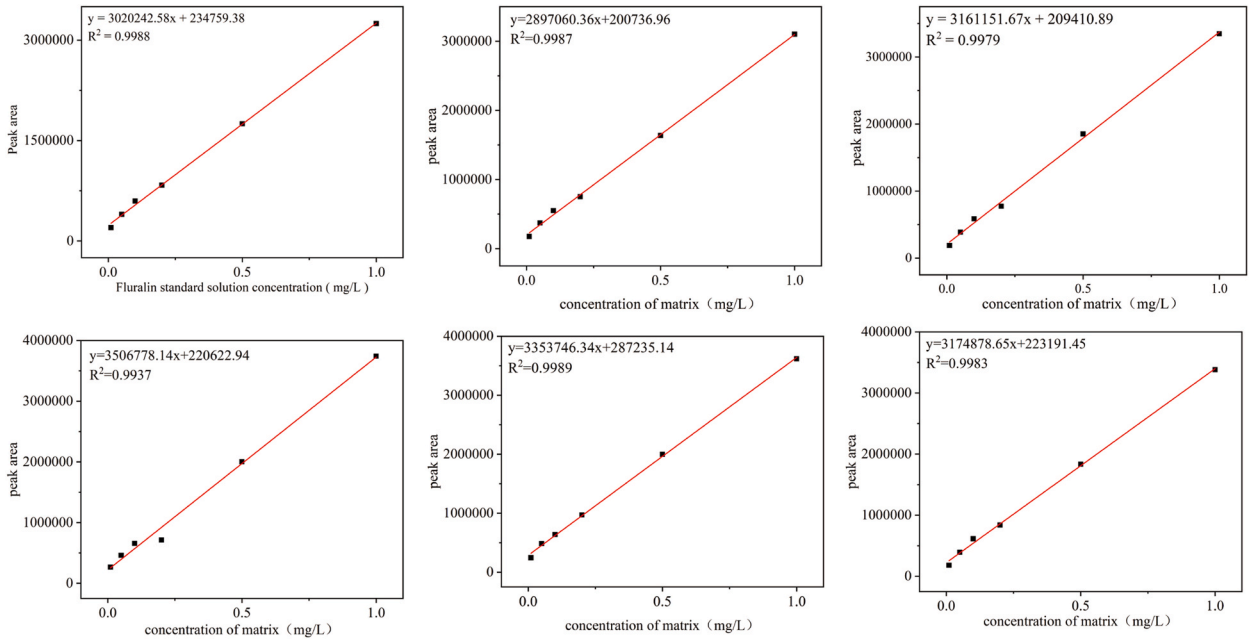

Fig. 2. Standard curve of trifluralin in each substance.

3.2. Screening, identification and safety evaluation of FLL-degrading bacteria

After continuous screening and enrichment, the degradation rates of 3 FLL-degrading strains FL-3, FL-5 and FL-6 were 80.81 %, 51.18 % and 49.98 %, respectively. The concentration of FLL decreased gradually with the prolongation of treatment time (Table S6). Compared with other screened degrading strains, this strain is more suitable for the environment of Qinghai Plateau and has faster degradation efficiency [18,29,30]. Further enriched the biological resources of trifluralin degradation.

The FL-3 colony was light yellow, irregular, rough and wrinkled (Fig. 3A and D). The FL-5 colony was grayish white, irregular, rough, and wrinkled (Fig. 3B and E); the FL-6 colony was light green, the surface was smooth and moist, and the edges were neat

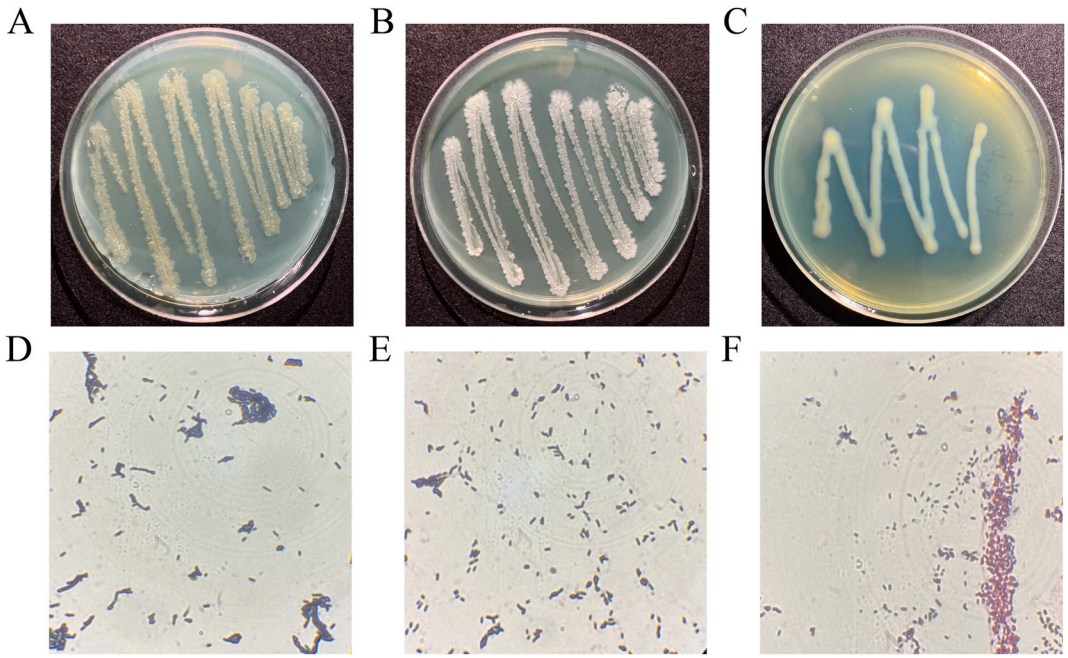

Fig. 3. Degradation strain colony morphology and microscopic morphology. (a, d) FL-3, (b, e) FL-5, (c, f) FL-6.

(Fig. 3C and F). The morphology of the three bacteria was short rod-shaped. The Gram staining results of FL-3 and FL-5 were purple, and FL-6 was red, indicating that FL-3 and FL-5 were Gram-positive bacteria, and FL-6 was Gram-negative bacteria (Fig. 3).

The bacterial universal primers 16SrDNA, gyrA, gyrB and rpoB (Table S7) were used to amplify and compare the strains FL-3, FL-5 and FL-6. The obtained 16S rRNA sequences were used to identify the strain. First, the closest relatives were determined based on the similarity of their 16S rRNA sequences obtained through direct BLAST searches of NCBI GenBank. A phylogenetic tree was then constructed. The results showed that FL-3 (accession numbers: PP001204, PP032080, PP032082, PP032085) and FL-5 (accession numbers: PP001205, PP032081, PP032083, PP032086) were clustered into the same branch with *Bacillus velezensis* Q6 (OQ181207.1). FL-6 (accession number: PP001206, PP032084, PP032087, PP032088) and *Pseudomonas atacamensis* Y58 (OM802502.1) were clustered into the same branch (Fig. S2). Combined with morphological and molecular biological identification, FL-3 and FL-5 were identified as *Bacillus velezensis*, FL-6 was identified as *Pseudomonas atacamensis*. This is different from the previously screened strains. The strains that have been screened include strains such as *Seudomonas fluorescens*, *Chitinophaga* sp. And *Dyadobacter* sp [31,32]. And it has been determined that molecules can be partially degraded by dealkylation or nitro reduction [33,34]. The degradation mechanism of the three new degradation strains selected in this paper needs to be further explored.

The effects of FL-3, FL-5 and FL-6 on seed germination and seedling growth of rapeseed, wheat and highland barley were shown in Table S8 and Fig. 4. After treatment with FL-3, FL-5 and FL-6 at a concentration of 1×10^9 cfu/mL, the germination rate of rapeseed, wheat and highland barley was significantly lower than that of the control treatment. The seedling length and root length of rapeseed were significantly lower than those of the control (Fig. 4A–C), and the bud length and root length of wheat and highland barley were significantly lower than those of the control (Fig. 4D–I). After treatment with FL-3, FL-5 and FL-6 at a concentration of 1×10^8 cfu/mL. The germination rate, seedling length and root length of rape, wheat and barley were not significantly different from those of the control treatment. In conclusion, the strains FL-3, FL-5 and FL-6 with a concentration of 1×10^8 cfu/mL were safe for the growth of rapeseed, wheat and barley. Pesticide residues in soil not only destroy the biodiversity of soil, but also may enter the human body through the food chain, posing a threat to human health [35]. According to statistics, only about 30 % of the pesticides applied in farmland are attached to crops, and most of the rest are diffused into soil and atmosphere, resulting in an increase in pesticide residues and their derivatives in soil. In addition, pesticide residues can also lead to soil acidification, nutrient loss, porosity and other issues, further reducing soil quality [36]. Most lost pesticides eventually become adsorbed into the soil, posing a potential threat to the ecosystem, and currently, the remediation of pesticide residues is primarily carried out through bioremediation methods. The degradation of target pesticides by degrading strains is beyond doubt, but the safety of crop production cannot be ignored [37,38], The

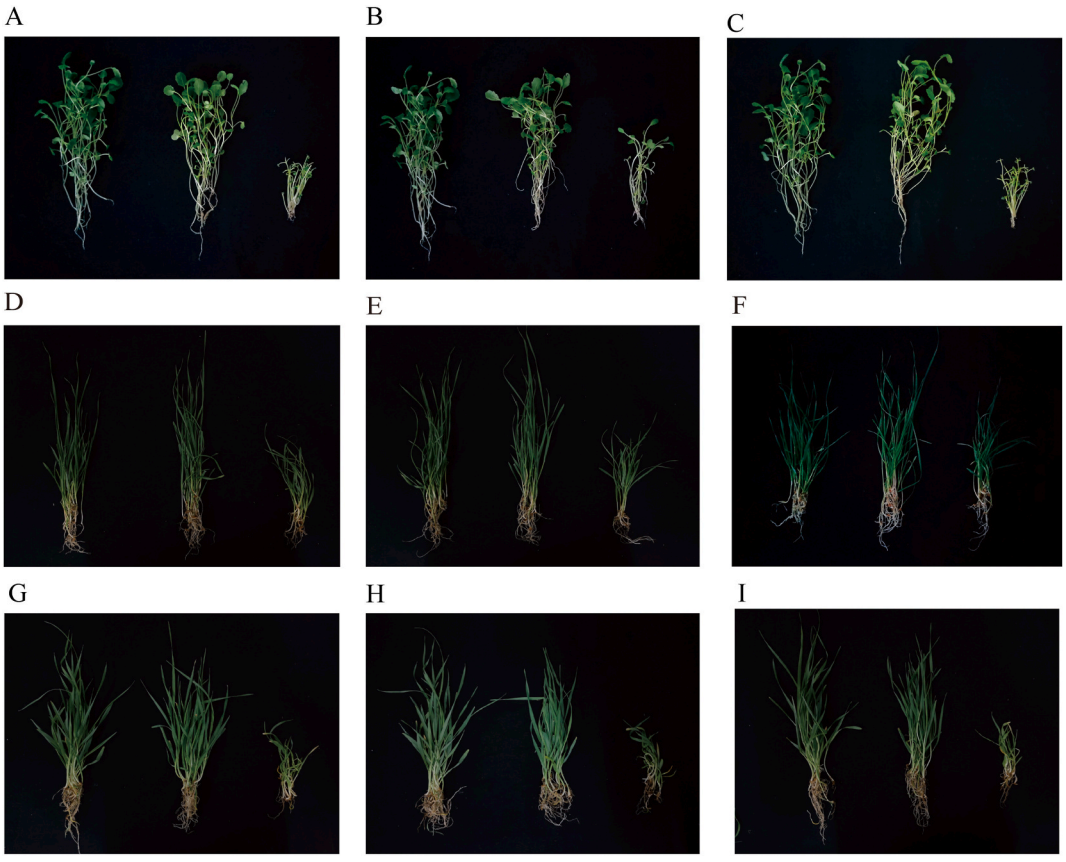

Fig. 4. Effects of Degrading Bacteria on Crop Growth. (a - c) rape, (d - f) wheat, (g - i) barley.

use of unsafe strains can easily affect the root nutrition of crops. The screening of this degrading strain is particularly important for the protection of the ecological environment of the QTP. It provides biological resources for rapidly reducing the concentration of FLL in the soil, and also makes preliminary preparations for the study of FLL degradation products.

3.3. Study on degradation characteristics of FLL-degrading strains

Firstly, the temperature, pH, and inoculation amount of FL-3, FL-5, and FL-6 were studied by a single factor (Figs. S3–S5). The results demonstrated that the degradation rate of FLL by the three strains increased with the increase in temperature, pH, and inoculation amount. The degradation effect of the three strains was most pronounced at 35 °C, while FL-3 and FL-5 exhibited the most pronounced degradation effect at pH 7 and 8, and FL-6 exhibited the most pronounced degradation effect at pH 8 and 9. Based on the outcomes of the preceding single-factor experiments, a Box-Behnken experiment was designed to obtain the experimental design results for the response values of FL-3, FL-5, and FL-6 (Table S9). The degradation rate of FLL was then fitted. The regression model of FLL degradation conditions for the three strains exhibited a nonlinear relationship, indicating that the factors involved in the degradation process interacted with one another.

The regression equations of FL-3, FL-5, and FL-6 were subjected to variance analysis (Tables S10–S12). The P values of the FL-3, FL-5, and FL-6 models were all less than 0.0001, indicating that the three models were reliable. The P values of the first term (A, B, C) and the second term (A^2, B^2, C^2), as well as the interaction term (AB, AC, BC) of the three models, were all less than 0.05, indicating that each factor in the experiment can significantly affect the response value results. The determination coefficients R^2 of the three bacterial degradation regression equations were 0.9788, 0.9781, and 0.9805, respectively, and the adjusted R^2 was 0.9516, 0.9500, and 0.9554, respectively, indicating that the three response regression equations were well-fitted. The Adequate Precision value was greater than 4, and the predicted R^2 and adjusted R^2 values were higher (Table 2). These results indicated that the three response surface models were reasonable and reliable, and that the precision was also high.

The interaction of the three factors can be presented by 3D curves (Fig. 5). The response surface slopes of the three strains were large. The response surfaces of FL-3 and FL-6 were convex, with a maximum value in the range, while the response surface of FL-5 was concave, with a minimum value in the range. When the inoculation amount of FL-3 was 5 %, the degradation rate of FLL exhibited an increase with the temperature rise, while a decrease was observed with the increase in pH (Fig. 5A). At a pH of 7, the degradation rate of FLL exhibited a gradual increase with the rise in inoculation amount and temperature (Fig. 5B). At a temperature of 30 °C, an increase in the inoculation amount resulted in a gradual increase in the degradation rate of FLL. Conversely, an increase in pH led to a first increase and then a gradual decrease in the degradation rate of FLL (Fig. 5C). The degradation rate of FLL was observed to decrease initially and then increase gradually with an increase in temperature when the inoculation amount of FL-5 was 5 %. Conversely, the degradation rate of FLL increased gradually with increased Ph (Fig. 5D). When the pH was 7, the degradation rate of FLL decreased initially and then increased with an increase in inoculation amount and temperature (Fig. 5E). At a temperature of 30 °C, the degradation rate of FLL increased gradually with the increase of inoculation amount and Ph (Fig. 5F). At an inoculation amount of FL-6 of 5 %, the degradation rate of FLL increased with temperature and pH (Fig. 5G). At a pH of 7, the inoculation amount had no significant effect on the degradation rate of FLL. As the temperature increased, the degradation rate of FLL exhibited a gradual increase (Fig. 5H). At a temperature of 30 °C, the inoculation amount had no significant effect on the degradation rate of FLL, while the degradation rate of FLL exhibited a gradual increase with an increase in pH (Fig. 5I).

The analysis of the response surface model revealed that the optimal conditions for the degradation of FLL by FL-3 were as follows: 35 °C, pH 8.1093, inoculation amount 5 %, with a predicted degradation rate of 82.12 %. The optimal conditions for the degradation of FLL by FL-5 were as follows: The optimal conditions for the degradation of FLL by FL-6 were determined to be 35 °C, pH 9, and an inoculum amount of 3 %. The predicted degradation rate was 69.51 %. To facilitate the actual operation, the degradation conditions of FL-3 were revised to 35 °C, pH 8, and inoculation amount of 5 %; the degradation conditions of FL-5 were revised to 35 °C, pH 7, and inoculation amount of 5 %. The degradation conditions of FL-6 were revised to 35 °C, pH 9, inoculation amount 5 %, and the degradation time of the three strains was 14 days according to the aforementioned degradation conditions. The actual degradation rates of FLL were 82.59 %, 81.97 %, and 69.82 %, respectively, which were in close alignment with the predicted values.

3.4. Degradation of FLL in soil and its effect on soil bacteria

The residual amount of FLL in Xining and Menyuan soils exhibited a gradual decrease over time, with the degradation trend initially exhibiting a fast decline and subsequently a slower rate. After 60 days of application, the dissipation rates of FLL in the soils of the two regions were 83.46 % and 60.58 %, respectively, with the final residues being 0.1633 mg/kg and 0.3453 mg/kg, respectively (Fig. 6). The degradation kinetic equation of FLL in Xining soil is $C_t = 0.95384e\text{-}0.02745t$ ($R^2 = 0.9908$), while the equation for

Table 2
Response data fitting results.

strain	Std.Dev	Mean	C.V. %	R^2	Adjusted R^2	Predicted R^2	Adeq Precision
FL-3	1.81	77.02	2.35	0.9788	0.9516	0.8691	17.4822
FL-5	1.56	72.03	2.17	0.9781	0.9500	0.7249	17.9965
FL-6	0.8353	63.43	1.32	0.9805	0.9554	0.9299	23.0733

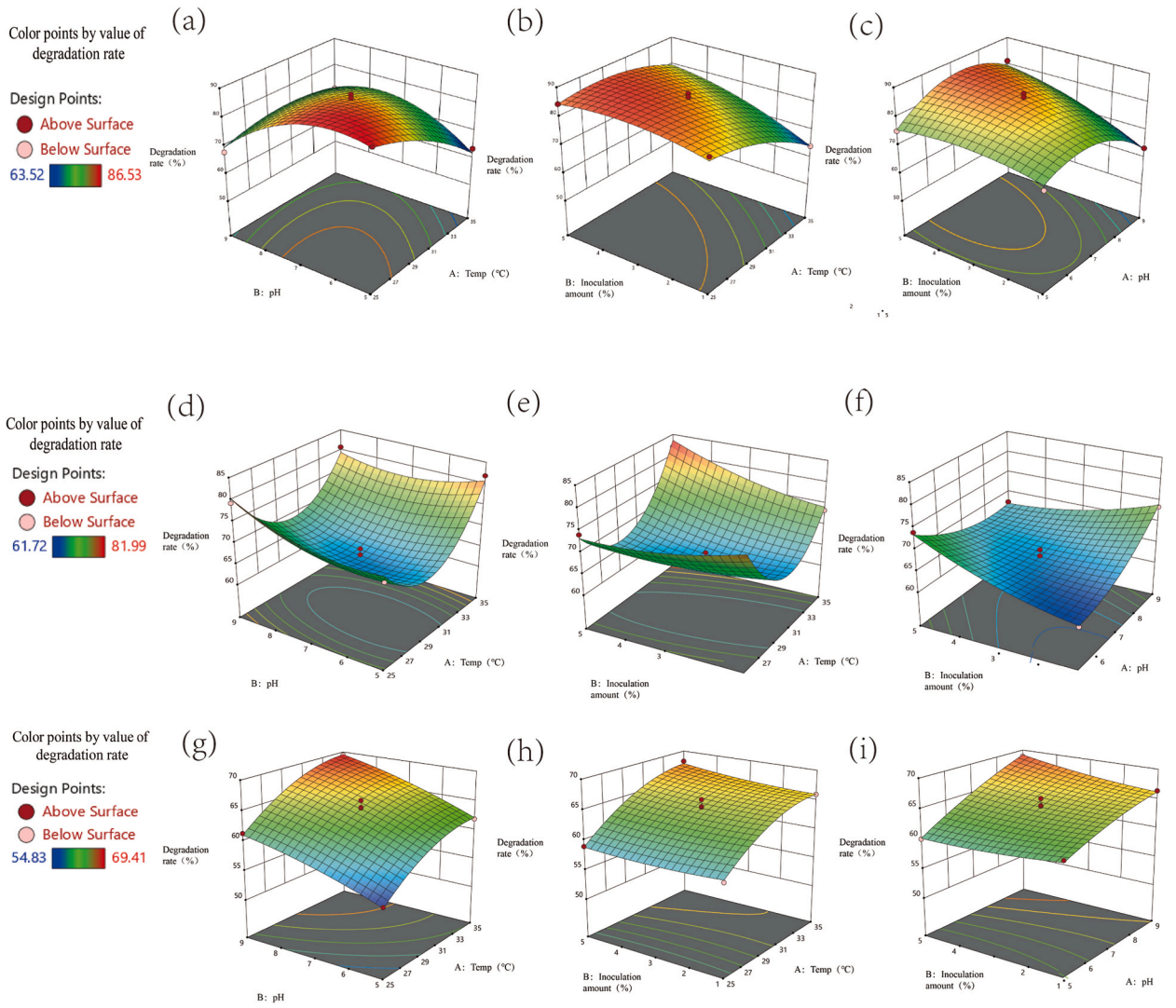

Fig. 5. Response surface diagram of the interaction of FL-3, FL-5, FL-6 factors on the degradation rate of FLL. (a, d, g) pH-temperature interaction term; (b, e, h) Inoculation amount-temperature interaction term; (c, f, i) Inoculation amount-pH interaction term.

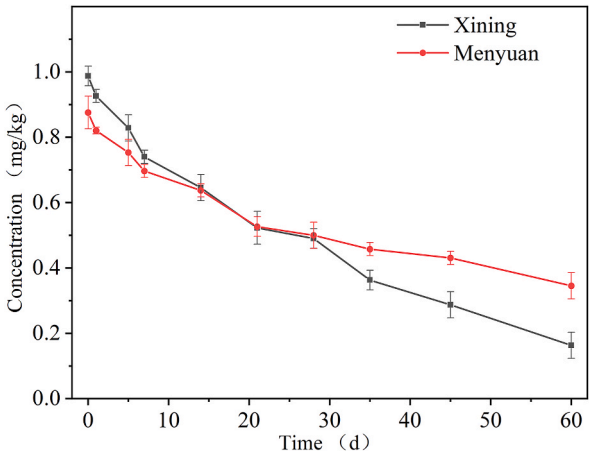

Fig. 6. Degradation rule of FLL in the soil of two areas.

Menyuan soil is $C_t = 0.85098e\text{-}0.01768t$ ($R^2 = 0.9171$). The degradation of FLL in the soil of the two regions is consistent with the first-order kinetic equation (Table 3). The half-life of FLL in Xining soil is 25 days, while in the Menyuan area, it is 39 days. According to previous studies, the degradation rate of herbicides is affected by environmental factors, such as temperature, ultraviolet light, soil properties, human activities and so on [39–41], the residual degradation dynamics of the same compound in different regions are also different [42], the observed difference in half-life between the two regions may be influenced by temperature and ultraviolet light.

The dynamic changes of soil bacterial communities in Xining and Menyuan following the application of FLL were investigated. The annotation results of ASVs and the characteristic table of each sample yielded a total of 31 phyla, 80 classes, 169 orders, 248 families, 414 genera, and 96 species in the Xining soil. A total of 31 phyla, 83 classes, 173 orders, 254 families, 411 genera, and 92 species were identified in Menyuan soil. When the sequencing amount of Xining and Menyuan soil samples reached 46, The dilution curve exhibited a flat trend with the increase in the number of sequenced sequences, with values of 192 and 48,142, respectively (Fig. 7A and B). This indicated that the sequencing depth of the experiment was sufficient, and that the coverage of the sequencing data had reached saturation. It can be concluded that this test can reflect the community and structure of bacteria in each soil sample and that the sequencing depth and data volume are appropriate.

The effect of FLL on the alpha diversity of bacteria in the soil of the two regions (Fig. 8a and b) should be analyzed. Overall, the species richness index, Chao1 index, ACE index, Shannon index, Invsimpson index, Pielou index, and goods coverage index were found to be higher in the Xining area than in the CK group. The Simpson index did not differ significantly from the CK group. The PD whole tree index was observed to be generally lower than the CK group. All indexes in the soil at different sampling times were found to be significantly higher than the CK group. The results demonstrated that the richness and diversity of bacterial communities in the soil of the two regions were significantly enhanced following the application of FLL, this conclusion is not the same as previous studies. Most of the addition of pesticides can significantly reduce the microbial activity and quantity of soil bacteria, fungi and actinomycetes [43], however, studies have found that pesticides do not have a wide range of toxicity to soil microbial communities [44,45]. FLL will not cause damage to all microorganisms in the region and has less impact on soil microorganisms.

PCoA was conducted using Weighted Unifrac and distance to gain further insight into the impact of FLL on the soil bacterial community structure in the two regions. The contribution rates of principal component PCoA1 and principal component PCoA2 to the variation of soil bacterial community in Xining were 12.6 % and 11 %, respectively, with the corresponding contribution rates to the source being 22.6 % and 10.3 %, respectively **(Fig, 9a)**. Before the application of FLL in Xining soil for 7 days, the spatial distance of the soil sample community was relatively small. As time progressed, the spatial distance between soil samples increased, accompanied by a reduction in the similarity of bacterial communities. However, the similarity of bacterial communities in Menyuan soil increased over time following the application of FLL (Fig. 9b). From the perspective of soil types, the dispersion of bacterial communities in Menyuan soil following the application of FLL was greater than that in Xining soil. It can be observed that the application of FLL has a more pronounced effect on the bacterial community structure of Menyuan soil samples.

4. Conclusions

A complete set of FLL detection method was established by orthogonal experiment, which was used to detect the content of FLL in soil, culture medium, rape plant and grain. The method was comprehensive, high precision, fast, environmentally friendly and economical. It was also found that the natural degradation rates of FLL in Xining and Menyuan soils for 60 days were 83.46 % and 60.58 %, respectively, and the half-lives were 25 d and 39 d, respectively. And FLL can significantly increase the soil bacterial richness and diversity in the two regions and has a greater impact on the bacterial community structure of Menyuan soil. The degradation strains of FLL screened from the soil were *Bacillus velezensis*, *Bacillus velezensis* and *Pseudomonas atacamensis*, and the three strains were safe for seed germination and seedling growth of rapeseed, wheat and highland barley. The degradation of FLL in the QTP provides biological resources. Through single factor and response surface optimization experiments, the optimal conditions for the degradation of FLL by the three strains were finally obtained. The degradation rates of 14 d were 82.59 %, 81.97 % and 69.82 %, respectively, which provided a theoretical basis for the biodegradation of FLL.

CRediT authorship contribution statement

Dong Zhao: Writing – original draft, Software, Formal analysis, Data curation, Conceptualization. **Lei Wang:** Writing – original draft, Software, Investigation, Conceptualization. **Shuo Shen:** Project administration, Formal analysis. **Enyu Lu:** Software, Investigation. **Junlong Feng:** Investigation, Conceptualization. **Nima Bai:** Methodology, Investigation. **Hongyu Chen:** Supervision, Project administration. **Wei Li:** Writing – review & editing, Resources, Project administration, Funding acquisition.

Table 3
Degradation kinetic parameters of FLL in soil of two sites.

parameter	Xining	Menyuan
A	0.95384	0.85098
k	0.02745	0.01768
$t_{1/2}$(d)	25	39
R^2	0.9908	0.9171
C_t	0.95384e-0.02745t	0.85098e-0.01768t

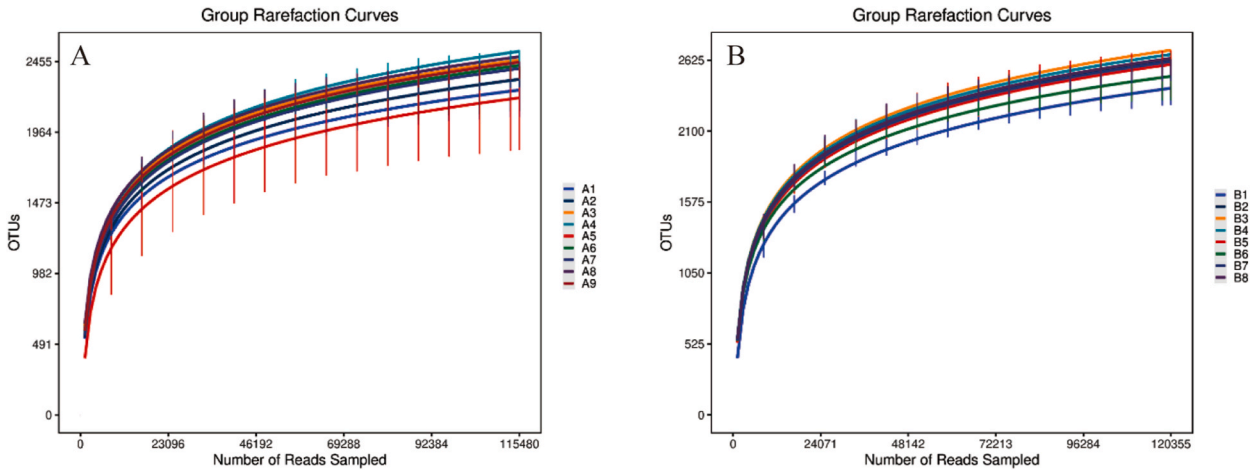

Fig. 7. Sparse curve (A) Xining area; (B) Menyuan area.

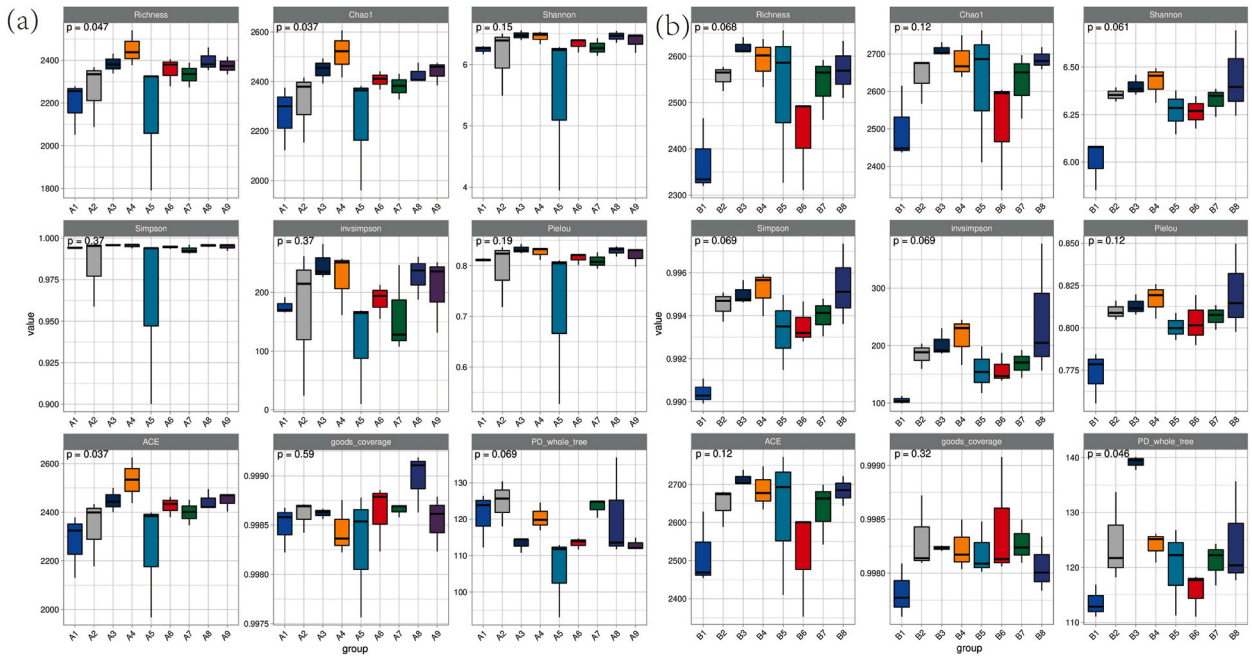

Fig. 8. α diversity index of bacterial community structure in Xining (a) and Menyuan (b) soil samples.

Data availability statement

All data generated or analyzed during this study are included in the article/supplementary material.

Ethical approval

Not applicable.

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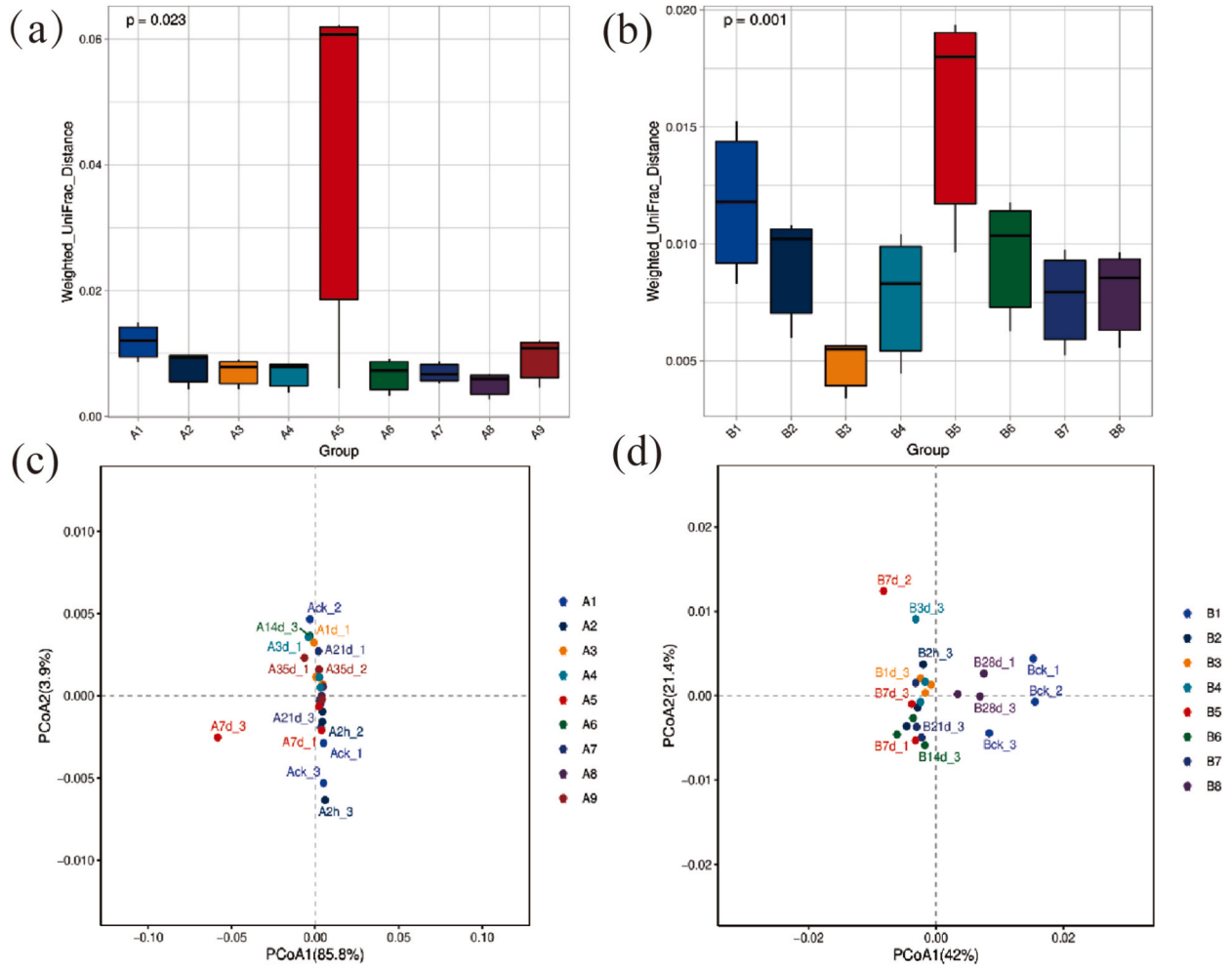

Fig. 9. Two regional soil samples based on unifrac distance box plot. (a, c) xining, (b, d) mengyuan.

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.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e41770.

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