

Residue dynamics of florporauxifen-benzyl and its effects on bacterial community structure in paddy soil of Northeast China



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ARTICLE INFO

Edited by Dr G Liu

Keywords:

Bacterial community
Degradation
Florporauxifen-benzyl
Herbicide
Paddy soil

ABSTRACT

Florporauxifen-benzyl is an herbicide that has been developed in recent years. Its degradation mode in paddy soil environments is not clear. In this study, the degradation dynamics in paddy soil and water were studied by ultrahigh-performance liquid chromatography. Microbial degradation was the main degradation pathway. Using third-generation high-throughput sequencing technology, the changes in the soil bacterial community structure were studied. After 30 days of application, compared with the control group (F0), the abundance of *Sphingomonas*, *Lysobacter*, and *Flavisolibacter* in the recommended and repeated application groups (F1, F5 and F10) increased significantly, and uncultured bacterium and *Terrimonas* decreased significantly. Compared with the F0 and F1 groups, the species diversity of the F0 and F1 groups showed a significant increase over time. The species diversity of the F5 and F10 groups decreased significantly on Days 5 and 15. On Day 30, the recovery even exceeded that of the control group. *Luteimonas* and five other genera were positively correlated with herbicide residues, and *Pseudolabrys* and two other genera were negatively correlated. Repeated application showed a significant effect on the structure of the soil bacterial community, mainly showing a trend of a significant decrease in the initial stage and gradual recovery in the later stage. The results will guide the safe and rational use of florporauxifen-benzyl and provide a scientific basis for florporauxifen-benzyl dynamic supervision of environmental pollution and protection of black soil in Northeast China.

1. Introduction

Florporauxifen-benzyl, similar to other SAHs, mimics indole-3-acetic acid (IAA), acting as a “molecular glue” between the receptor protein complex SCF^{TIR/AFB} (Skp1-cullin-F-box protein) and the corepressor protein Aux/IAA at the plant cell nucleus, promoting the degradation of Aux/IAA by the ubiquitin—proteasome pathway (26 S proteasome) (Velásquez et al., 2021). It is a synthetic hormone herbicide newly developed by Corteva Agriscience of the United States, which is used to prevent and eliminate annual impurities in rice direct seeding fields or rice transplanting fields. It has a new arylpyridine formate structure, which interferes with the normal physiological and biochemical functions of plants by binding with hormone receptors in plants, resulting in the death of sensitive plants (Epp et al., 2016). Florporauxifen-benzyl is different from the receptor of traditional synthetic hormone herbicides

and has a stronger affinity for the receptor. It can be used to manage herbicide-resistant weeds with other mechanisms and will become an important tool in the field of weed resistance control. To date, it has been registered in China, the United States, South Korea, Chile and Australia (Sun et al., 2018). Florporauxifen-benzyl is safe for most subsequent crops, but some sensitive broad-leaved crops need a certain interval. In addition, its toxicity to aquatic animals is low, but some adverse reactions of mysid shrimp have been observed (Netherland et al., 2016; Buczek et al., 2020). The recommended application of florporauxifen-benzyl is 15–30 g/hm². Increasing the application or repeated application without authorization will affect the growth of crops (Miller and Norsworthy, 2018a, 2018b). After the application of herbicides, some volatilize into the air, and some fall on plant leaves (Sharkey et al., 2020; Takeshita et al., 2021). Most of them enter the soil and groundwater with the leaching of rainwater and are absorbed by

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soil, plant roots or fed by small animals (Kadiyala et al., 2020; Carneiro et al., 2020; Šuk et al., 2021; Delhomme et al., 2021). The herbicides in the soil environment gradually decrease mainly through chemical hydrolysis, microbial degradation and a small amount of photochemical decomposition (J. Liu et al., 2021; G.Y. Liu et al., 2021; Siripattanakul et al., 2009; Gruber et al., 2021). While ensuring an increase in grain yield and income, long-term large residues of herbicides will cause changes in the soil ecological environment (Zhao et al., 2022).

Soil microorganisms are the power of material transformation in soil, which can promote the decomposition of soil organic matter and the transformation of nutrients and is one of the key indicators for soil health assessment. The study of soil microbial diversity can realize the monitoring of soil quality and effective response to pollution, which is of great significance to soil health and sustainable agricultural development. Researchers have used different methods to study the microbial diversity of different pollutants (F.G. Wang et al., 2020; L. Wang et al., 2020; L.Y. Wang et al., 2020; M. Wang et al., 2020; Kuang et al., 2021). Soil contains an enormous number of microorganisms, especially bacteria. Most bacteria are decomposers and participate in the formation of humus and complete mineralization of organic matter together with other soil microorganisms. Currently, high-throughput sequencing and bioinformatics are the main technologies, such as third-generation high-throughput sequencing technology (Pacbio/Nanopore) marked by single molecule real-time sequencing and nanopore. The study of bacterial diversity mainly focused on the 16 S region of the nucleic acid sequence encoding ribosomal RNA. There were both conservative regions and variable regions in these sequences, which reflect the genetic relationship and differences between species, respectively (Chen et al., 2018).

The herbicide has been registered and officially listed in China, but the degradation mode of the herbicide in paddy soil environments after application is not clear. Researchers at home and abroad mainly focus on weed control effects, herbicide compatibility, safety of current and subsequent crops, sensitivity of soybean, soil adsorption and transfer, response of aquatic plants, etc. (Netherland et al., 2016; Buczek et al., 2020; Miller and Norsworthy, 2018a, 2018b; Duy et al., 2018; Takale et al., 2020). However, the degradation mode of herbicides in the rice soil environment and its impact on microbial diversity have not been reported. Hydrolysis, photolysis and microbial degradation tests of the herbicide were used to explore the main degradation mode of the herbicide. The degradation law of florporauxifen-benzyl in paddy soil and water was monitored by ultrahigh-performance liquid chromatography (UPLC). The changes in soil bacterial community structure after florporauxifen-benzyl application were studied using third-generation 16 S high-throughput sequencing technology.

Three questions were raised in this study: (i) What was the dynamic change rule of florporauxifen-benzyl degradation in paddy soil and water? (ii) Which was the main degradation mode of florporauxifen-benzyl in paddy soil? Hydrolysis, photolysis or microbial degradation? (iii) Did repeated application of florporauxifen-benzyl significantly affect bacterial community structure and diversity in paddy soil? If so, what were the major impacts? The research results will guide the safe and rational use of florporauxifen-benzyl and provide a scientific basis for the dynamic monitoring of florporauxifen-benzyl environmental pollution and black soil protection in Northeast China.

2. Materials and methods

2.1. Test site and sample collection

Soil samples were collected from paddy fields (46.00 N latitude and 126.64 E longitude) in Harbin, Heilongjiang Province, China. This area is located in the second accumulated temperate zone, with an annual precipitation of 800 mm. The crop was rice (Wuyou rice No. 1) for the previous 3–5 years. Soil samples were randomly collected and mixed and then sub packed in pots for pot experiments. Rice plants at the 4-leaf

stage were treated with florporauxifen-benzyl (purchased from Corteva Co., Ltd.). In accordance with the commercial recommended farmland application dose, 3% Rinskor was diluted and sprayed at 900 ml/hm² using a sprayer (F1). Additionally, five and ten times the recommended dose was also applied in the field as different treatments (F5 and F10). The nonapplication group was set as the control group (F0). Soil samples were collected at the following time points: the day of spraying (Day 1) and the days after spraying (Day 5, Day 15 and Day 30). The soil was collected from the tillage layer at a depth of 5–15 cm using a three-point method. Three repetitions were set at each sampling time in each group. For example, the first day of sampling in the control group is represented by F0D1a, F0D1b and F0D1c. Roots, leaves, weeds and other visible debris were removed from the soil. A portion of the soil sample was dried at room temperature and then sieved (sieve size: 250 µm). Additionally, the water on the sludge is also collected for the determination of florporauxifen-benzyl residues.

2.2. Determination of florporauxifen-benzyl residue in rice pots

2.2.1. Extraction method of herbicide residues from soil and water

Florporauxifen-benzyl was extracted from the soil as follows: a soil sample was added to a 50 ml centrifuge tube, and 15 ml extraction solvent (hexane:acetone 1:1) was added. The suspension was then sonicated for 5 min and centrifuged for 5 min at 5000 r/min. The supernatant was removed into a new glass tube and flushed with nitrogen for later use. The solvent extraction process was repeated four times in total, and the supernatants were pooled and dried under nitrogen after each step. Then, 2 ml of methanol was added to the glass tube, and the collected supernatant was sonicated for 30 s and then passed through a 0.22 µm filter membrane and C₁₈ purification column (X. Yang et al., 2021; F.S. Yang et al., 2021). This method has been improved.

Florporauxifen-benzyl was extracted from water as follows: Take 1 ml culture medium and put it into 10 ml centrifuge tube, add 0.25 g NaCl, add 5 ml CH₂Cl₂, shake on vortex for 1 min, and stand for stratification; Take out the upper aqueous phase and discard it, add 0.2 g anhydrous Na₂SO₄ to the lower organic phase, shake, remove a small amount of water from the organic phase, place the upper liquid in a 10 ml volumetric flask, place the volumetric flask in a constant temperature water bath at 50 °C, and evaporate CH₂Cl₂; Fix the volume to 10 ml with chromatographic pure methanol, vibrate with ultrasonic for 30 min, pass through 0.45 µm organic filter membrane, place it in a centrifuge tube, and put it into a 4 °C refrigerator for detection at the first time (Liu et al., 2016). This method has been improved.

2.2.2. Detection conditions of herbicide residues and added recovery rate by UPLC

Florporauxifen-benzyl was determined by ultra-performance-liquid chromatography (UPLC, WATERS ACQUITY H UPLC CLASS) (L. Wang et al., 2020; L.Y. Wang et al., 2020; M. Wang et al., 2020; F.G. Wang et al., 2020). The best chromatographic conditions were as follows: chromatographic column, WATERS ACQUITY UPLC BEH C₁₈ 1.7 µm, 2.1 × 50 mm; column temperature, 40 °C; mobile phase, acetonitrile and water; detection wave, 230 nm; injection volume, 3 µL; and flow rate, 0.3 ml/min. The elution conditions were as follows: time (min): 0, 1.5, 3, 4.5, 6; acetonitrile (%): 10, 90, 90, 10, 10; water (%): 90, 10, 10, 90, 90.

To validate the reliability of the soil extraction method and chromatographic conditions, florporauxifen-benzyl was added to the soil at three different concentrations (0.05 mg/kg, 0.5 mg/kg, 5.0 mg/kg), and it was added to the water at the same concentrations, with three replicates per concentration; soil samples without the addition of florporauxifen-benzyl were used as a blank control. The florporauxifen-benzyl recovery rate was determined as a ratio of the measured and added concentrations. The coefficient of variation was defined as the ratio of the standard deviation and average recovery rate.

2.2.3. Drawing of herbicide standard and digestion curve and calculation of residual content

The florporauxifen-benzyl standard was prepared by diluting a stock solution (50 mg/L) to concentrations of 0.05, 0.1, 0.5, 1, 1.5, and 2 mg/L. According to the relationship between the standard concentration and the peak area measured by UPLC, the standard curve and regression equation were established for the calculation of the florporauxifen-benzyl concentration.

The florporauxifen-benzyl residual curves were prepared. The degradation kinetics Equation (1) and the correlation coefficient R^2 were obtained by adding an index trend line. The half-life of florporauxifen-benzyl in the soybean field was calculated according to Formula (2). Note: C_0 is the initial concentration of fomesafen; C_t is the residual concentration at time t ; $t_{1/2}$ is the half-life, the time required for the concentration to decrease by half; and k is the dissolution rate constant.

$$C_t = C_0 e^{-kt} \quad (1)$$

$$t_{1/2} = (\ln 2)/k \quad (2)$$

2.3. Exploration of degradation mode of florporauxifen-benzyl

To explore the hydrolysis dynamics of florporauxifen-benzyl in different water bodies, samples were collected from four water bodies: ground water (Yongsheng Village in Harbin), river water (Songhua River in Harbin), distilled water and pure water. Each water body was repeated three times. Add florporauxifen-benzyl to the water sample to make the concentration of florporauxifen-benzyl in the water body 0.5 mg/L. The water body was placed in a constant incubator at 25 °C and stored away from light. Samples were taken every 48 h to determine the residue of florporauxifen-benzyl, and the residue trend line of florporauxifen-benzyl in different water bodies was drawn (Cao et al., 2013).

To explore the residual dynamics of florporauxifen-benzyl under different light sources, UV, LED and sunlight were selected. The acetonitrile solution of florporauxifen-benzyl (0.5 mg/L) was placed in a quartz test tube and sealed. The illumination group was irradiated under an ultraviolet lamp (30 W), LED lamp (18 W) and sunlight. The dark group was wrapped with aluminium foil for opaque treatment, and each group made three repetitions. The ultraviolet lamp irradiation group was sampled at 0 min, 10 min, 20 min, 30 min, 40 min, 50 min and 60 min. The LED lamp and sunlight irradiation group were sampled at Day 0, Day 1, Day 2, Day 3, Day 4, Day 5 and Day 6. After 0.22 μM organic filter membrane filtration, the samples were stored in a –20 °C refrigerator for detection (Cao et al., 2013).

To explore the degradation mode of florporauxifen-benzyl in soil and water, a rice pot experiment was carried out to analyse the residue dynamics of florporauxifen-benzyl. The control groups were sterilized soil and water samples, and the treatment groups were nonsterilized soil and water samples. Different application quantities of florporauxifen-benzyl were added to each pot, 0.1 mg/L, 0.5 mg/L and 1.0 mg/L. The soil samples and water samples were collected at Day 1, Day 5, Day 15 and Day 30. After pretreatment, the florporauxifen-benzyl residue was determined by UPLC and plotted (Wang et al., 2017).

2.4. Determination of physical and chemical properties of rice pot soil

In this study, six physical and chemical properties of the soil in each group were measured. The total nitrogen content of the soil was determined by sulfuric acid digestion and the Kjeldahl method. The content of total phosphorus in the soil was determined by sodium hydroxide melting and the molybdenum antimony sulfate colorimetry method. The content of total potassium in the soil was determined by the flame photometer method. The content of soil organic matter was determined by potassium dichromate capacity and the external heating method. The pH value and air-dried water content of the soil were determined by using a soil pH and moisture tester (Sun et al., 2019).

2.5. High throughput sequencing of microbial diversity in rice pot soil after florporauxifen-benzyl application

The above potted soil samples were frozen in liquid nitrogen and stored at –80 °C for high-throughput sequencing analysis. After extracting the total DNA of the sample, 16 S rDNA-specific primers (F: AGRGTTTGATYNTGGCTAG, R: TASGGHTACCTTGTASGACTT) with barcodes were synthesized according to the full-length primer sequence and amplified by PCR, and the products were purified, quantified and homogenized to form a sequencing library (SMRT Bell). The constructed library was subjected to library quality inspection first, and the qualified library was sequenced with PacBio Sequel II (Callahan et al., 2016; Bokulich et al., 2013).

2.6. Data analysis

Florporauxifen-benzyl residual dynamics were constructed with Excel and were analysed using the Duncan repeated comparison of SPSS 17.0 for Windows (SPSS Inc., USA). $P < 0.05$ was considered a significant difference. High-throughput sequencing data preprocessing: after exporting PacBio offline data to the CCS file (the CCS sequence was obtained by the smrtlink tool provided by PacBio), there were three main steps as follows: CCS identification: use Lima v1.7.0 software to identify CCS through barcodes to obtain raw CCS sequence data; CCS filtering: use Cutadapt 1.9.1 software to identify and remove primer sequences and filter the length to obtain clean CCS sequences without primer sequences; remove chimaeras: use UCHIME v4.2 software to identify and remove chimeric sequences to obtain effective CCS sequences. Information analysis content: feature (OTUs, ASVS), diversity analysis, difference analysis, function prediction analysis and correlation analysis (F.G. Wang et al., 2020; L. Wang et al., 2020; M. Wang et al., 2020; L.Y. Wang et al., 2020; Chen et al., 2014).

3. Results

3.1. Exploration of degradation mode and law of florporauxifen-benzyl

The conditions for the detection of florporauxifen-benzyl by liquid chromatography were explored. The standard curve equation of the florporauxifen-benzyl standard determined by UPLC was $y = 91248x + 1329$ ($R^2 = 0.9993$). The average recoveries of florporauxifen-benzyl were 85.70%–101.90% in soil and 87.71%–99.81% in water, and the coefficient of variation was 2.47–4.86%. The extraction method and detection conditions met the requirements of pesticide residue analysis. To explore the main methods of natural degradation of florporauxifen-benzyl, a hydrolysis test of florporauxifen-benzyl was carried out in four water bodies. The results showed that there was no significant difference in the florporauxifen-benzyl content between sterilized and nonsterilized water bodies. There was no significant change in the residual concentration of florporauxifen-benzyl in pure water and distilled water within 18 days under dark conditions. The residual concentration of florporauxifen-benzyl in ground water and river water showed a downwards trend, and their degradation rates reached 27.84–33.67% and 39.47–49.04%, respectively, in 18 days, but their trend line showed poor correlation with the negative exponential function (Fig. S1).

To explore the main methods of natural degradation of florporauxifen-benzyl, three light sources were used for photolysis in this experiment. The results showed that there was no significant change in the concentration of florporauxifen-benzyl after 60 min of ultraviolet irradiation and 6 days of LED light and sunlight, respectively, compared with the dark groups. It was speculated that photolysis may not be the main method of natural degradation of florporauxifen-benzyl (Fig. S2).

To explore the main methods of natural degradation of florporauxifen-benzyl, the soil from the rice field was treated with sterilization and nonsterilization, and a rice pot experiment was carried out in this experiment. The results showed that the total residual

concentrations of F1, F5 and F10 florporauxifen-benzyl in sterilized soil and water did not change significantly within 30 days ($p > 0.05$). However, the total residual concentration of florporauxifen-benzyl in nonsterilized soil and water showed a significant downwards trend from Day 5–30 ($p < 0.05$). Compared with sterilized soil, 30 days after application, the total degradation rates of florporauxifen-benzyl in nonsterilized soil and water were 53.94%, 66.61% and 64.15%, respectively. The florporauxifen-benzyl content in water and soil also decreased significantly. In sterilized and nonsterilized soil, the distribution proportion of the three application quantities of florporauxifen-benzyl in water was large, and the distribution proportion in soil was small. The distribution proportions of florporauxifen-benzyl of F1, F5 and F10 were 80–88.38 %, 76.32–79.64 %, and 66.58–71.27 % in water and 11.62–19.63 %, 20.36–27.19 %, and 28.73–34.42 % in soil, respectively. On the 30th day of the application of F1, F5 and F10, the ratio of florporauxifen-benzyl content in soil and water in the nonsterilized group was significantly higher than that in the sterilized group, which was caused by the significant decrease in florporauxifen-benzyl content in the nonsterilized group (Fig. 1). The degradation trend of florporauxifen-benzyl in water and soil conforms to a negative exponential function. The half-lives of F1, F5 and F10 in water are 25.67, 21.00 and 22.36 days, respectively; in soil are 15.07, 15.75 and 17.77 days, respectively; and in the water and soil mixture are 23.10, 19.80 and 20.39 days, respectively (Table S1).

3.2. Effects of florporauxifen-benzyl application on physical and chemical properties of pot soil

The soil physical and chemical properties of each treatment group over time are shown. The total potassium content of each group showed no significant change with time, and the other physical and chemical indicators of each group showed significant changes with time, as shown below. Compared with the first day, the total phosphorus content in the F0 group increased significantly on Day 5 and Day 30, and the water content on Day 5, Day 15, and Day 30 decreased significantly. Compared with Day 1, the pH of the F1 group increased significantly on Days 15 and 30. Compared with Day 1, the total nitrogen content in the F5 group decreased significantly on Day 15, the total phosphorus content increased significantly on Day 30, the organic matter content decreased significantly on Day 5, Day 15, and Day 30, and the water content increased significantly. Compared with Day 1, the total nitrogen content in the F10 group increased significantly on Day 15 and Day 30 (Table S2).

The soil physical and chemical properties of different treatment groups in different time periods are shown. On the first day, there was no significant difference in each soil physical and chemical index among the F0, F1, F5 and F10 groups, and there was a significant change in the physical and chemical indices among the other groups on other days, as shown below. On Day 5, compared with F0 and F1, the total phosphorus content in the F5 and F10 groups decreased significantly, and the pH increased significantly. On Day 15, compared with F0 and F1, the organic matter content in the F5 and F10 groups decreased significantly.

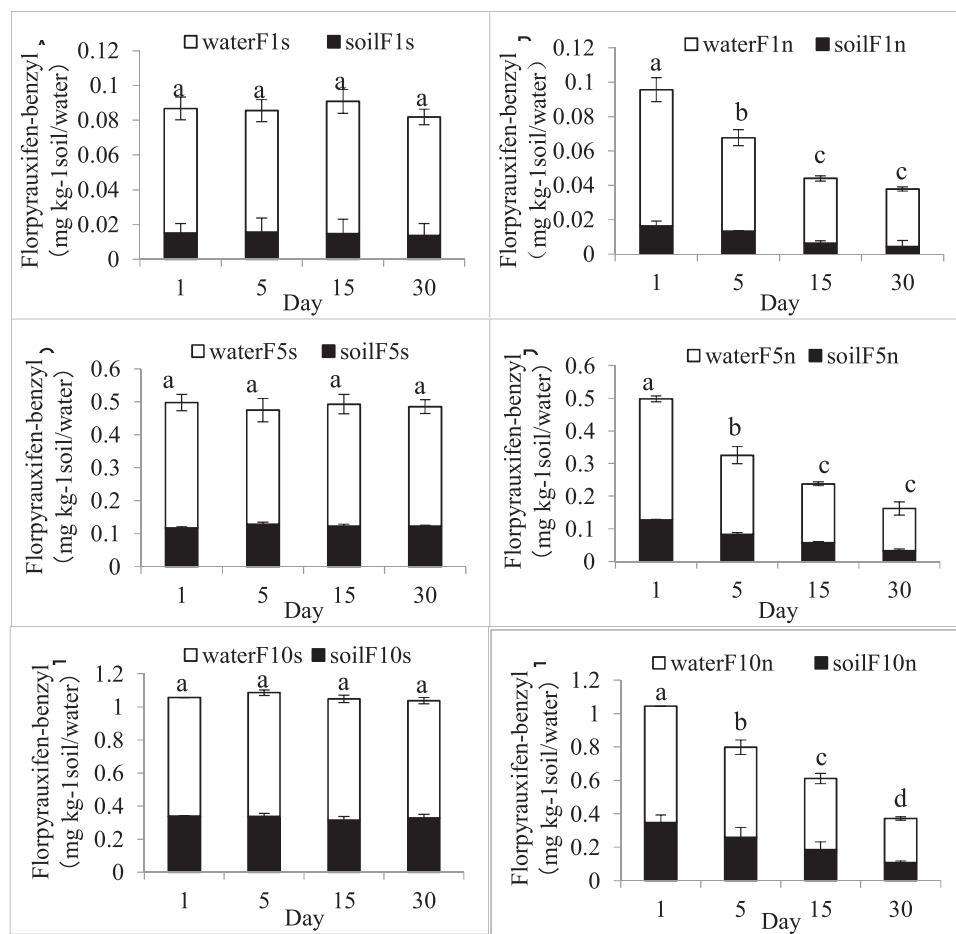


Fig. 1. Dynamics of florporauxifen-benzyl residue in water and soil of rice pot culture. (A) F1 s, (B) F1n, (C) F5s, (D) F5n, (E) F10s, (F) F10n. ■: soil, □: water. “s” and “n” represent sterilization and nonsterilization, respectively. Each value represents the mean \pm SD ($n = 3$). where a, b, and c indicate that there is no significant difference ($p > 0.05$) with the same letter, and there is a significant difference ($p < 0.05$) with different letters.

On Day 30, compared with F0, the total nitrogen content in the F1 group decreased significantly, the organic matter content in the F5 group decreased significantly, and the water content increased significantly. The total potassium content in the F10 group decreased significantly, and the pH increased significantly (Table 1).

3.3. Effects of flupyrauxifen-benzyl on bacterial diversity in rice pot soil

3.3.1. Analysis of soil bacterial composition

The ten phyla, genera and species with the highest abundance are listed in Table S3. *Proteobacteria* accounted for the largest proportion and was the largest phylum in bacteria. *Sphingomonas* and uncultured bacterium_c_subgroup_6 were the most abundant genera and species, respectively (Fig. S3).

Among the top ten genera, the genera in which the bacterial abundance in the treatment group decreased significantly with time were *Sphingomonas*, *Lysobacter*, *Arenimonas*, *Terrimonas*, and *Flavisolibacter*. The genera abundance in the treatment group increased significantly with time were *uncultured_bacterium_f_Gemmamimonadaceae*, *uncultured_bacterium_c_subgroup_6*, and *Ellin6067*. However, the abundance of *uncultured_bacterium_f_Chitinophagaceae* and uncultured bacterium_o_Saccharimonadales in the treatment group did not change significantly with time ($p > 0.05$). Compared with the control group, the abundance of *Sphingomonas* increased significantly in the F1, F5, and F10 groups on Day 1. The abundance of *Sphingomonas* and *Lysobacter* increased significantly in the F1, F5, and F10 groups on Days 5 and 15. The abundance of *Flavisolibacter* increased significantly in the F10 treatment group on Day 15. The abundance of *Sphingomonas* increased significantly in the F1 and F5 treatment groups, *Lysobacter* increased significantly in the F5 and F10 treatment groups, and *Flavisolibacter* increased significantly in the F10 treatment group on Day 30 ($p < 0.05$). However, the abundance of uncultured bacterium_c_subgroup_6 decreased significantly on Days 5 and 15, and *Terrimonas* decreased significantly on Day 5 in the F1, F5 and F10 treatment groups (Fig. 2 and Fig. S4).

3.3.2. Analysis of soil bacterial alpha diversity

Alpha diversity reflects the species richness and diversity of a single sample. Shannon and Simpson indices are used to measure species diversity and are affected by species abundance and community evenness in the sample community. The Chao1 and ACE indices measure species abundance, that is, the number of species. The diversity index of the F5 and F10 groups fluctuated greatly in the early stage of application and gradually recovered in the later stage. On Day 5, compared with the F1

group, the Shannon ($p < 0.05$) index of the F5 group decreased significantly, and the Shannon ($p < 0.01$) and ACE ($p < 0.05$) indices of the F10 group decreased significantly. On Day 15, compared with the F0 group, the Simpson index of the F10 group decreased significantly ($p < 0.01$). On Day 30, compared with F1, the Chao1 ($p < 0.05$) index of F5 increased significantly (Fig. 3 and Fig. S5).

3.3.3. Analysis of soil bacterial beta diversity

By analysing the composition of OTUs (97 % similarity) of different samples, the difference and distance of samples were reflected. PCA uses variance decomposition to reflect the difference of repeated groups of data on the three-dimensional coordinate map, and the coordinate axis takes three eigenvalues that can reflect the variance to the greatest extent. The closer the two samples are, the more similar the composition of the two samples is. There were significant differences with the extension of processing time (Day 1, Day 5, Day 15 and Day 30) in the different herbicide groups (F0, F1, F5 and F10). The time-varying trends of the F1, F5 and F10 groups were similar, but their changing trends were different compared with the F0 group. However, there was no significant difference between the different dose groups on Day 1. Compared with the F0 group, the F10 group was significantly different; compared with the F1 group, the F5 and F10 groups were significantly different on Day 5. Compared with the F0 and F1 groups separately, the F5 and F10 groups were significantly different on Day 15. There was no significant difference between the different dose groups on Day 30 (Fig. S6 and Fig. S7).

R^2 obtained by PERMANOVA indicates the degree of interpretation of sample differences by different groups, that is, the ratio of group variance to total variance. A greater R^2 indicates a higher degree of interpretation of differences by group and a greater difference between groups. When the p value is less than 0.05, it indicates that the reliability of the test is high. In the Day 1, Day 5, Day 15 and Day 30 groups, there were significant differences in binary Jaccard distance among the F0, F1, F5 and F10 groups, and the differences between groups were greater than those within groups. The reliability of the Day 5 group test was high ($p < 0.05$). In terms of subordination level, in the F0, F1, F5 and F10 groups, there were significant differences in binary Jaccard distance among the Day 1, Day 5, Day 15 and Day 30 groups, and the difference between groups was greater than that within groups. The reliability of the test was high ($p < 0.05$) (Fig. 4 and Fig. S8).

The box chart of beta diversity difference analysis between groups can intuitively reflect the median, dispersion, maximum, minimum and abnormal values of sample similarity in the group to analyse whether the beta diversity difference between groups is significant. β NTI < -2

Table 1

Comparison of soil physical and chemical properties of different treatment groups.

Groups	TN g/kg	TP mg/kg	TK g/kg	OM g/kg	MC %	pH
F0D1	8.31 ± 0.34a	320.62 ± 45.97a	15.50 ± 0.38a	153.99 ± 11.55a	1.92 ± 0.34a	6.00 ± 0.00a
F1D1	8.36 ± 0.21a	431.97 ± 20.10a	15.21 ± 0.27a	159.05 ± 5.19a	1.44 ± 0.23ab	6.17 ± 0.29a
F5D1	8.20 ± 0.16a	352.83 ± 35.16a	15.07 ± 0.31a	167.59 ± 3.41a	1.43 ± 0.33ab	6.17 ± 0.58a
F10D1	7.60 ± 0.16a	326.11 ± 99.08a	15.00 ± 0.59a	161.13 ± 7.92a	1.45 ± 0.36ab	6.67 ± 0.29a
F0D5	8.17 ± 0.42ab	474.42 ± 9.03a	15.62 ± 0.23a	168.17 ± 10.51a	1.31 ± 0.20ab	6.00 ± 0.00a
F1D5	8.62 ± 0.28a	451.76 ± 44.37a	15.19 ± 0.41a	159.54 ± 8.49a	1.56 ± 0.30ab	6.00 ± 0.00a
F5D5	7.87 ± 0.34b	362.10 ± 29.21b	15.66 ± 0.94a	156.30 ± 4.54a	1.73 ± 0.07a	6.33 ± 0.29b
F10D5	7.85 ± 0.06b	370.95 ± 53.24b	15.25 ± 0.24a	160.48 ± 4.58a	1.25 ± 0.33b	6.50 ± 0.00b
F0D15	8.73 ± 0.09a	412.61 ± 64.86a	15.58 ± 0.28ab	169.66 ± 6.72a	1.32 ± 0.29a	6.00 ± 0.00a
F1D15	7.63 ± 1.29a	442.25 ± 113.58a	15.33 ± 0.48ab	163.20 ± 1.98ab	1.32 ± 0.21a	6.50 ± 0.00a
F5D15	7.37 ± 0.26a	368.42 ± 9.39a	15.96 ± 0.12b	154.89 ± 8.12b	2.01 ± 0.11a	6.50 ± 0.50a
F10D15	8.08 ± 0.35a	380.76 ± 25.68a	15.09 ± 0.60a	157.36 ± 3.21b	1.65 ± 0.72a	6.50 ± 0.00a
F0D30	8.53 ± 0.22a	438.75 ± 60.81a	15.48 ± 0.02ab	159.03 ± 4.68a	1.26 ± 0.18a	6.17 ± 0.29a
F1D30	7.04 ± 0.88b	434.13 ± 53.99a	15.02 ± 0.36 BCE	158.50 ± 2.42a	1.80 ± 0.11ab	6.50 ± 0.00ab
F5D30	7.71 ± 0.27ab	410.99 ± 9.37a	15.75 ± 0.27a	148.76 ± 3.56b	2.13 ± 0.21b	6.50 ± 0.00ab
F10D30	8.18 ± 0.31a	408.46 ± 20.60a	14.54 ± 0.36c	160.43 ± 6.59a	1.18 ± 0.75a	6.83 ± 0.29b

Note: TN: total nitrogen; TP: total phosphorus; TK: total potassium; OM: organic matter; MC: air dried moisture content; pH: pH value. Each value represents mean \pm SD ($n = 3$). There was significant difference between groups with different letters in the same column ($p < 0.05$), and there was no significant difference between groups with the same letters ($p > 0.05$).

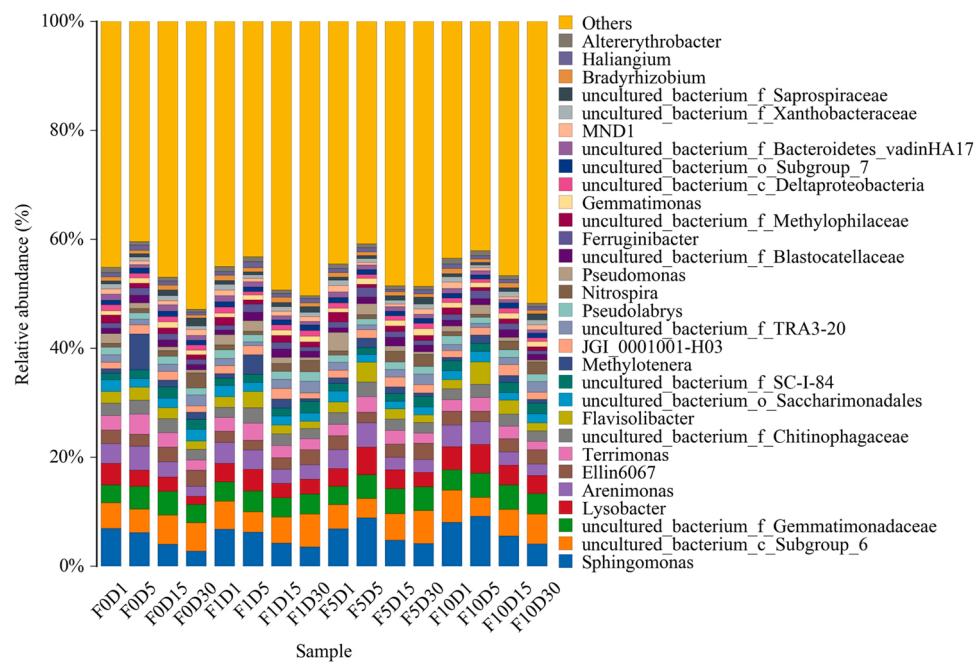


Fig. 2. Histogram of soil bacterial distribution at the genus level. Colour and patch length represent species and the proportion of species in relative abundance. The abscissa and ordinate represent the sample names and relative abundance percentage, respectively.

or $> + 2$ indicates $\beta\text{MNTD}_{\text{obs}}$ deviation from mean $\beta\text{MNTD}_{\text{null}}$ (the expected level of phylogenetic turnover dominated by random ecological process) is more than two standard deviations, indicating that it is significantly less than or greater than the expected phylogenetic turnover. The deviation of $|\beta\text{NTI}| < 2$ indicates that the stochastic process is dominant, and the deviation of $|\beta\text{NTI}| > 2$ indicates that the deterministic process is dominant. In the F0 group, the beta diversity index of Day 15 and Day 30 increased and was located in the deterministic region compared with the incomplete determinacy of Day 1 and Day 5. In the F1, F5 and F10 groups, compared with Day 1, the beta diversity index of Day 5 decreased and was located in the incomplete determinacy region, while Day 15 and Day 30 increased and were located in the determinacy region. On Day 15 and Day 30 in the F0, F1, F5 and F10 groups, $|\beta\text{NTI}| > 2$, which indicates that the deterministic process of the beta diversity index over time is dominant. The beta diversity index of F0, F1, F5 and F10 in the Day 1 and Day 5 groups was mostly located in the balance region between deterministic and stochastic processes. The beta diversity index of the F10 group in the D5 group was higher than that of the F0 group. The beta diversity index of F10 in the D15 group was lower than that in the F0 group, the beta diversity index of F1, F5 and F10 in the Day30 group was lower than that in the F0 group, and F10 was higher than F1, and they were all located in the area of incomplete determinacy (Fig. 5 and Fig. S9).

3.3.4. Significance analysis of soil bacterial difference between groups

The results of LEfSe analysis usually include an LDA value distribution histogram and evolutionary branch diagram (Fig. 6 and Fig. 7). The core flora in the soil of Group F0 changed from *Actinobacteria* to *Terrimonas*, uncultured bacterium *Terrimonas*, *Arenimonas*, *Nitrosomonadaceae*, and then to *Deltaproteobacteria* on Day 1, Day 5 and Day 30, respectively. The core flora in the soil of Group F5 was transformed from *Lysobacter ginsengisoli* and *Sphingomonas sediminicola* to *Planctomycetes* on Day 5 and Day 30, respectively. On Day 5, the core flora in the F0 group soil are *Terrimonas*, uncultured bacterium *Terrimonas*, *Arenimonas* and *Nitrosomonadaceae*; the core flora in the F1 group soil are *Bacteroidetes*, *Bacteroidia*, *Chitinophagales*, *Chitinophagaceae* and *Flavisolibacter*; the core flora in the F5 group soil are *Lysobacter ginsengisoli* and *Sphingomonas sediminicola*; the core flora in the F10 group soil are

Sphingomonadaceae, *Sphingomonadales*, *Sphingomonas*, *Xanthomonadales*, *Xanthomonadaceae*, *Lysobacter*, and *Sphingomonas daechungensis*. On Day 30, the core flora in the F0 group soil was *Deltaproteobacteria*; the core flora in the F5 group soil was *Planctomycetes* (Fig. 6).

The core flora in the F1 group from phylum to species on Day 5 are *Bacteroidetes* (Phylum), *Bacteroidia* (Class), *Chitinophagales* (Order), *Chitinophagaceae* (Family) and *Flavisolibacter* (Genus), which are located on the same branch. The core flora in the F10 and F5 groups from door to species on Day 5 are *Sphingomonadales* (order), *Sphingomonadaceae* (family), *Sphingomonas* (genus), *Sphingomonas daechungensis* (species), and *Sphingomonas sediminicola* (species), and they are located in the same branch. Another core flora in the F10 and F5 groups on Day 5 are *Xanthomonadales* (order), *Xanthomonadaceae* (family), *Lysobacter* (genus), and *Lysobacter ginsengisoli* (species), which are located on the same branch. These core flora in these groups are not located on the same branch: *Actinobacteria* (Phylum) in F0D1, *Terrimonas*, uncultured bacterium *Terrimonas*, *Arenimonas* and *Nitrosomonadaceae* in F0D5, *Deltaproteobacteria* (Class) in F0D30, and *Planctomycetes* (Class) in F5D30 (Fig. 7).

3.3.5. Prediction and analysis of soil bacterial functional genes

The histogram of the COG metabolic pathway shows the relative abundance of 24 metabolic functions of 29 phyla in the soil samples (Fig. S10). The COG function classification statistics chart shows the functional differences after 30 days between the soil with different concentrations of herbicides and the soil without herbicides. On Day 30, compared with F0, the functions with a significant increase in F1 were lipid transport and metabolism, translation, ribosomal structure and biogenesis, function unknown, posttranslational modification, protein turnover, chaperones, energy production and conversion, secondary metabolite biosynthesis, transport and catabolism, and replication, recombination and repair. The functions with a significant decrease in F1 were cell motility, carbohydrate transport and metabolism, signal transduction mechanisms, amino acid transport and metabolism, cell wall/membrane/envelope biogenesis, inorganic ion transport and metabolism, transcription, and cytoskeleton. Compared with F0, the functions with a significant increase in F5 were nucleotide transport and metabolism, carbohydrate transport and metabolism, translation,

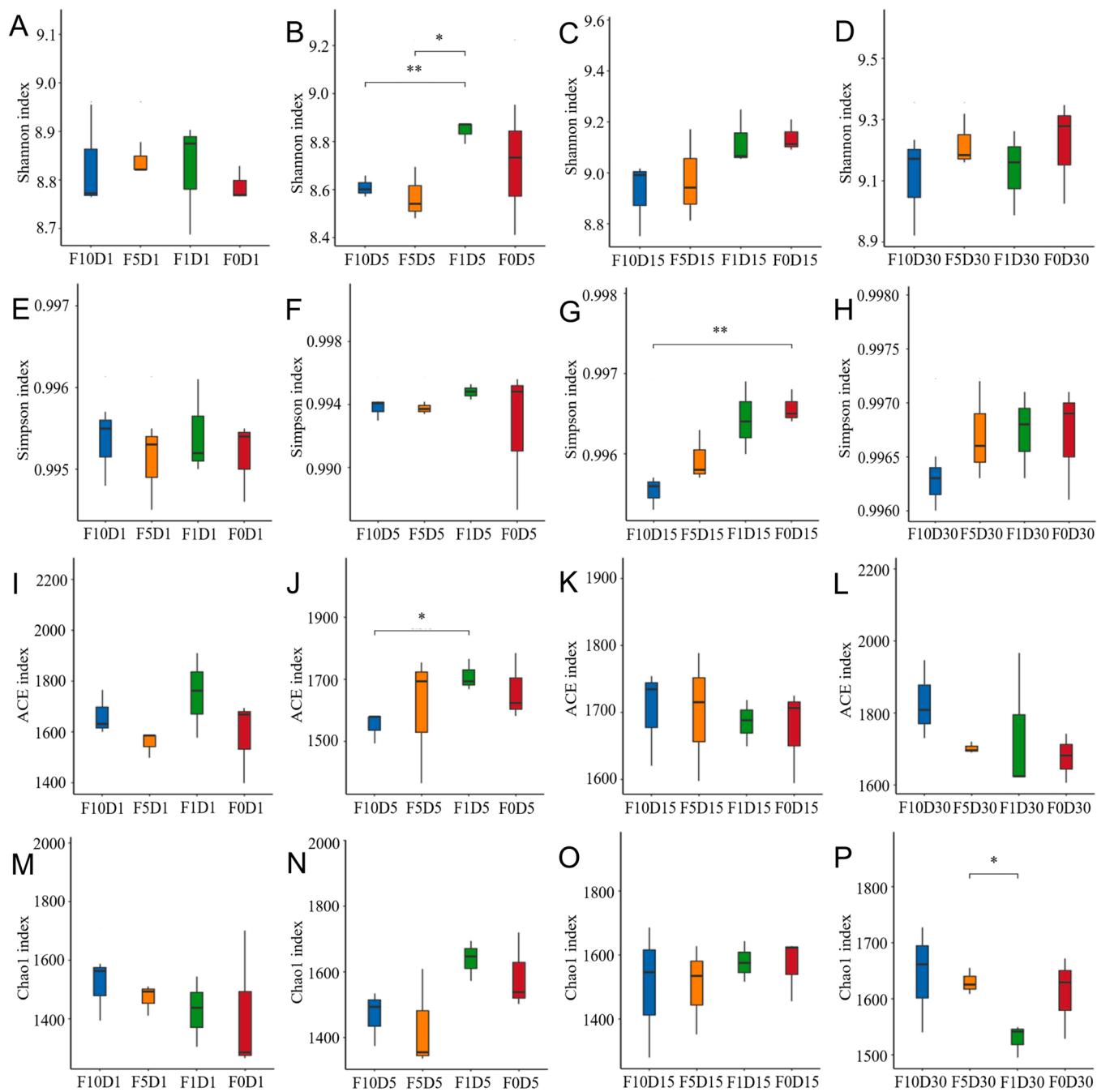


Fig. 3. Analysis of differences between groups of alpha diversity index at OTU level. (A, B, C, D) Shannon; (E, F, G, H) Simpson; (I, J, K, L) Ace; (M, N, O, P) Chao. The abscissa and ordinate are the group names and alpha diversity index, respectively. The difference in the alpha diversity index between different treatments was evaluated by Student's T test. * represents $p < 0.05$, and ** represents $p < 0.01$.

ribosomal structure and biogenesis, replication, recombination and repair, and coenzyme transport and metabolism. The functions with a significant decrease in F5 were function unknown, signal transduction mechanisms, cell motility, secondary metabolite biosynthesis, transport and catabolism, inorganic ion transport and metabolism, energy production and conversion, and cytoskeleton. Compared with F0, the functions with a significant increase in F5 were intracellular trafficking, secretion, vesicular transport, cell motility, translation, ribosomal structure and biogenesis, replication, recombination and repair. The functions with a significant decrease in F10 were amino acid transport and metabolism, carbohydrate transport and metabolism, function unknown, general function prediction only, and cytoskeleton. Compared with F0, the functions with significant increases in F1, F5 and F10 were

translation, ribosomal structure and biogenesis, and replication, recombination and repair. The function with a significant decrease in F1, F5 and F10 was cytoskeletal (Fig. 8).

The family-level bacterial species in soil treated with different application quantities are shown in each function. The aerobic bacteria in the soil samples were mainly *Chitinophagaceae*, *Comamonadaceae*, *Pirellulaceae*, *Sphingomonadaceae*, *Xanthomonadaceae* and others. The relative abundance of aerobic bacteria in the F10 group was lower than that in the F0, F1 and F5 groups. Anaerobic bacteria in the soil samples were mainly *Bacteroidales*, *Syntrophaceae*, *Fimbiimonadaceae*, and others. The facultative anaerobic bacteria in the soil samples were mainly *Bradyrhizobiaceae*, *Comamonadaceae*, *Coxiellaceae*, *Hypomicrobiaceae*, *Rhodospirillaceae* and others. gram-positive bacteria in the soil samples

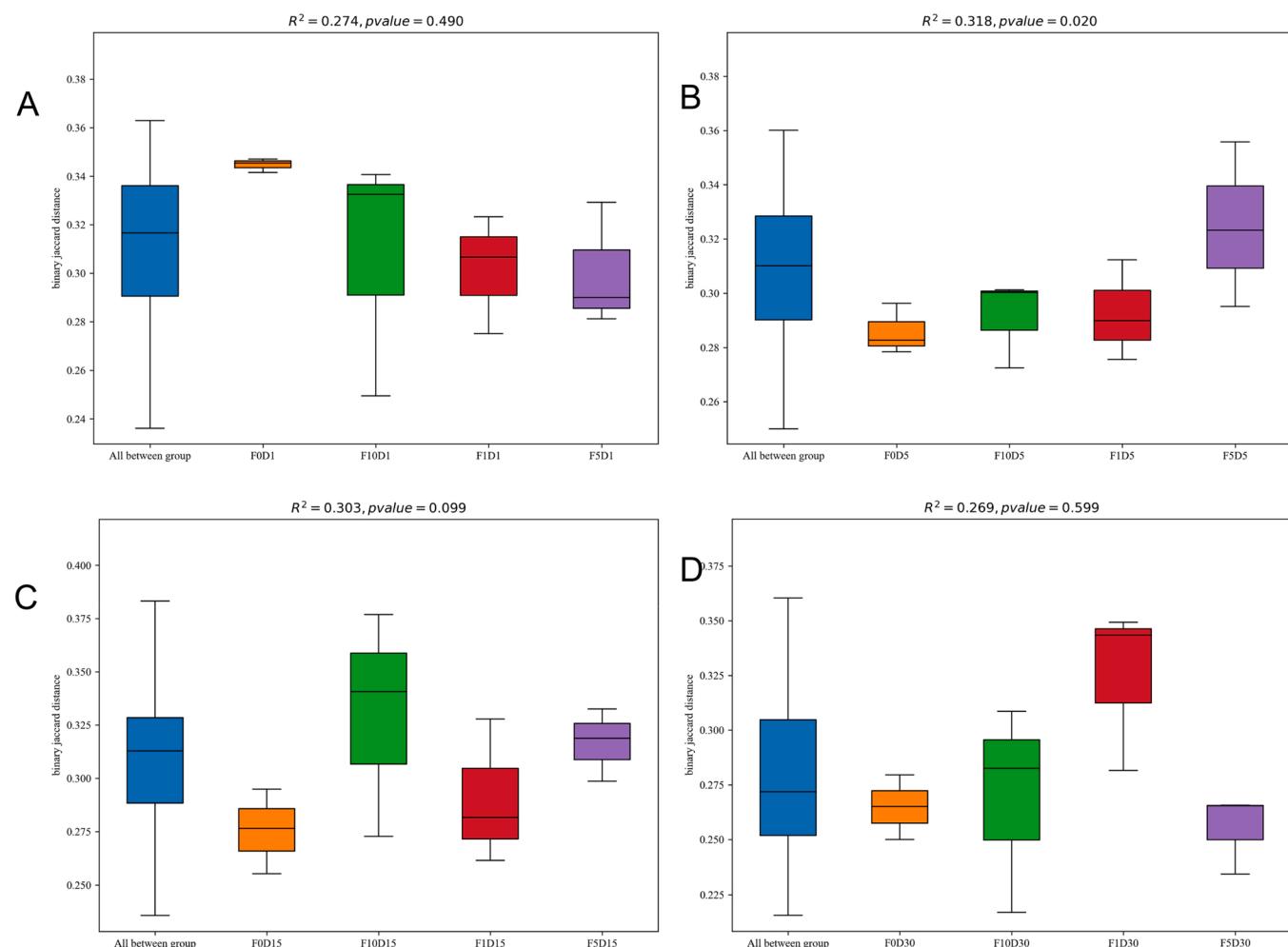


Fig. 4. PERMANOVA intergroup analysis box diagram. (A) Day 1; (B) Day 5; (C) Day 15; (D) Day 30. The ordinate represents the beta distance. The abscissa "All between group" represents the beta distance between samples in all groups, and the box diagram behind is the beta distance data between samples in different groups. The first box chart represents the intergroup differences between groups. The higher the median line is, the greater the intergroup differences. The lower the median line and the shorter the tentacle in the other box line diagrams, the smaller the difference within the group and the better the repeatability of the samples within the group.

were mainly *Acidimicrobiales*, *Gaiellaceae*, *Iamiaceae*, *Micrococcaceae*, *Nocardioidaceae*, *Fimbriimonadaceae* and others. The relative abundances of facultative anaerobic, gram-positive and anaerobic bacteria in the F1, F5 and F10 groups were lower than those in the F0 group, among which the facultative anaerobic and gram-positive bacteria in the F10 group were the lowest and the anaerobic bacteria in the F5 group were the lowest. The gram-negative bacteria in the soil samples were mainly *Chitinophagaceae*, *Comamonadaceae*, *Hypomicrobiaceae*, *Nitrospiraceae*, *Pirellulaceae*, *Sphingomonadaceae*, *Xanthomonadaceae* and others. Form biofilm bacteria in the soil samples were mainly *Comamonadaceae*, *Hypomicrobiaceae*, *Nitrospiraceae*, *Pirellulaceae*, *Sphingomonadaceae*, *Xanthomonadaceae*, and others. The relative abundance of gram-negative biofilms and biofilm forms in the F1, F5 and F10 groups were higher than those in the F0 group, in which gram-negative biofilms in the F10 group were the highest and biofilm forms in the F5 group were the highest. Stress Tolerant, Contains Mobile Elements, Potentially Pathogenic in soil samples are mainly *Comamonadaceae*, *Xanthomonadaceae*, and others. The relative abundance of stress tolerance, containing mobile elements and potentially pathogenicity in the F5 and F10 groups were higher than those in the F0 and F1 groups, of which the F10 group was the highest (Fig. 9 and Fig. S11).

3.3.6. Correlation analysis of genus and environmental factor

The environmental factors positively correlated with the florpyrauxifen-benzyl residue were the moisture content and total potassium content of the air-dried soil, and the correlation was MC>TK. The environmental factors negatively correlated with the florpyrauxifen-benzyl residue were the number of days of treatment, the content of organic matter and total nitrogen, and the correlation was Days>OM>TN. pH and total phosphorus content were the environmental factors that had little correlation with the florpyrauxifen-benzyl residue. The order of correlation of the sample group with a positive correlation with the florpyrauxifen-benzyl residue is as follows: F1D5 > F5D5 > F10D5 > F10D1 > F1D1 > F5D15 > F10D15 > F0D5. The rank of correlation of the sample group negatively correlated with the florpyrauxifen-benzyl residue is as follows: F1D15 < F5D30 < F0D15 < F10D30 < F0D30 < F1D30. The order of the magnitude of the correlation between the positive correlation with the residue of florpyrauxifen-benzyl and the genus is as follows: *Lysobac-ter*>*Methylotenera*>*Flavisolibacter*>*Sphingomonas*>*Terrimonas*>*Arenimonas*; the abundance of these genera decreases with the decrease in herbicide concentration. The order of magnitude of the genus correlation negatively related to the florpyrauxifen-benzyl residue is as follows: *Nirospira*<*Pseudolabrys*<*Ellin6067*; the abundance of these genera

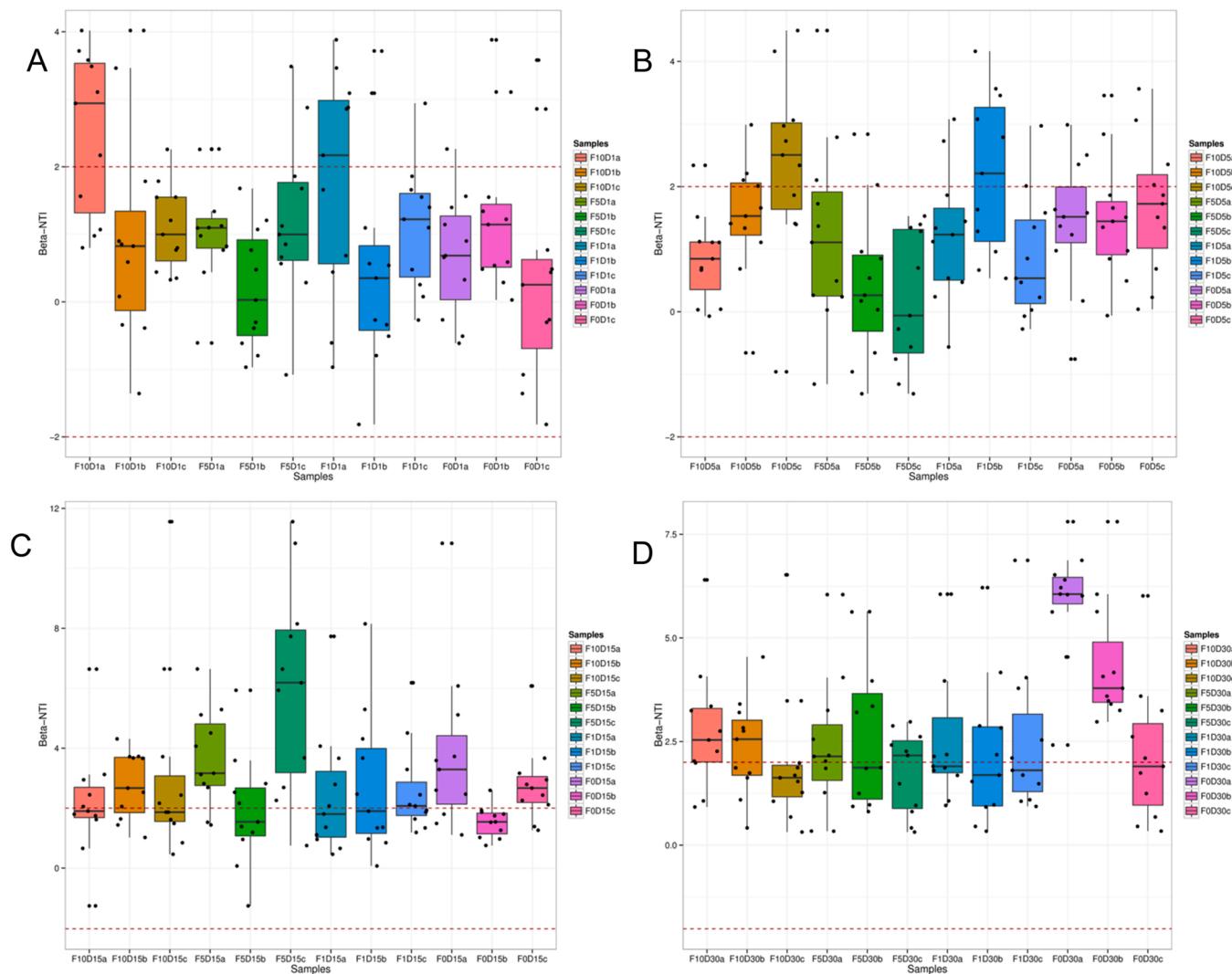


Fig. 5. Beta distance box diagram. (A) Day 1; (B) Day 5; (C) Day 15; (D) Day 30. The abscissa represents the group, the ordinate represents the distance, and the boxes of different colours represent each group. The upper and lower end lines of the box represent the upper and lower interquartile range. The horizontal line in the box represents the median, the edges of the upper and lower tentacles extending outside the box correspond to the maximum and minimum values, and the points outside the tentacle edge represent outliers. The red horizontal dotted lines represent β NTI = +2 and -2 at upper and lower significance thresholds.

increases with the decrease in the florpyrauxifen-benzyl concentration.

The environmental factor positively related to the treatment days was pH; that is, the pH increased with increasing treatment days. The order of correlation of environmental factors negatively related to the number of days of treatment is as follows: MC < OM < TN < TP < Residue < TK; these environmental factors decrease with the increase in treatment days. The rank of correlation of sample groups with positive correlation with treatment days is as follows: Day30 > Day15. The rank of correlation of sample groups negatively correlated with the number of days of treatment is as follows: Day 1 > Day 5. The environmental attribute values of the sample groups with different doses and the same days were similar. The rank of the animal correlation positively related to the treatment time was as follows: *Nitrospira* > *Pseudolabrys* > *Ellin6067*; its abundance increased with the extension of treatment time. The rank of genus correlation negatively related to treatment time was as follows: *Flavisolibacter* < *Methylotenera* < *Lysobacter* < *Sphingomonas* < *Terrimonas* < *Arenimonas*; the abundance of these genera decreased with the prolongation of florpyrauxifen-benzyl treatment time (Fig. 10). The species correlation network diagram was used to obtain the coexistence relationship of species and the interaction and important model information of species

in the same environment (Fig. S12).

4. Discussion

4.1. Degradation mode and law of florpyrauxifen-benzyl

Due to the small solubility of florpyrauxifen-benzyl in water, an organic solvent with weak polarity was directly used to extract it in this study, which is different from that reported in the current literature. In the literature (N. Zhang et al., 2021; J.Y. Zhang et al., 2021), pure water was used to soak the soil, and an organic solvent with strong polarity was used to extract it, but ultrasound and purification technology were used, and the recovery and standard deviation met the requirements. In this study, ultrahigh-performance liquid chromatography was used, and the detection conditions were optimized. At present, the literature reports use high-performance liquid chromatography technology (Teng et al., 2021), and the detection conditions are different with different instruments. This study provides a reference for the determination of florpyrauxifen-benzyl residues in the future.

According to the literature, the migration of most herbicides in soil mainly occurs through the downwards movement of osmotic water. Additionally, herbicides undergo photolysis, hydrolysis and

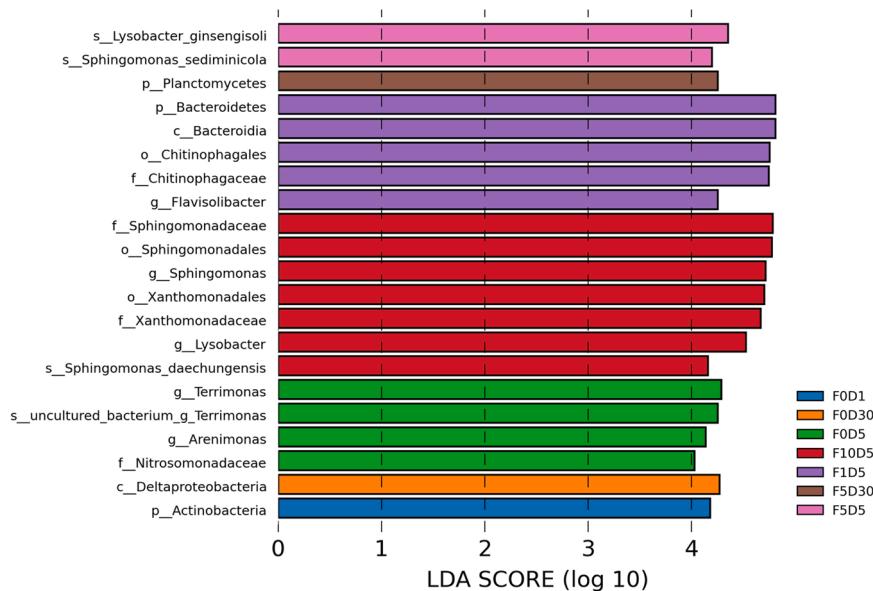


Fig. 6. Histogram of LDA value distribution. The figure shows the significantly different species whose LDA score is greater than the set value (the default setting is 4.0), i.e., biomarkers with significant differences. The colour of the histogram represents their respective groups, and the length of the histogram represents the impact degree of different species (LDA score), i.e., the impact degree of significantly different species among different groups.

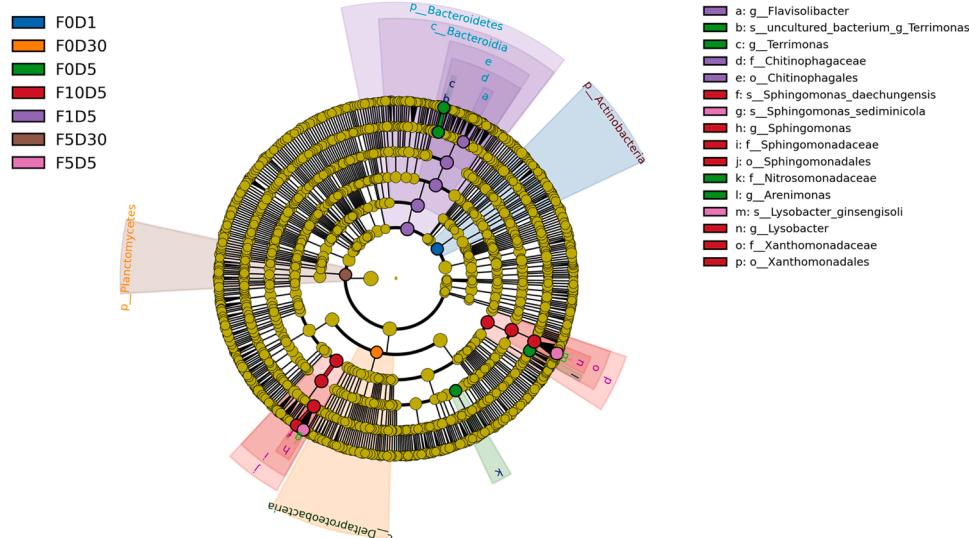


Fig. 7. Evolutionary branching cladogram. Circle in the figure: the circle radiating from inside to outside in the figure represents the classification level from phylum to species. Each small circle at different classification levels represents a classification at that level, and the diameter of the small circle is directly proportional to the relative abundance. Colour: the species with no significant difference are uniformly yellow, and the species with significant difference are coloured with the group. The node colour indicates the microbial groups that play an important role in the same colour group. The species names corresponding to biomarkers that cannot be displayed in the figure are displayed on the right, and the letter numbers correspond to those in the figure.

biotransformation in the environment. The degradation pathways of herbicides in aquatic environments are mainly hydrolysis and photolysis, and microbial degradation is the main mechanism of herbicide dissipation in soil (J.Y. Zhang et al., 2021; N. Zhang et al., 2021). This may also be caused by the combined action of hydrolysis, photolysis and microbial degradation in the natural environment. The residual concentration in groundwater and river water shows a downwards trend, possibly because some minerals and salt ions in groundwater and river water promote the hydrolysis of florporauxifen-benzyl to a certain extent. However, hydrolysis is less common than natural degradation.

To eliminate the influence of hydrolysis, acetonitrile was selected as the solvent in this experiment. It was found that the concentration of the herbicide did not change significantly. It was speculated that photolysis was not the main degradation mode of florporauxifen-benzyl in the natural environment. It was found that the distribution proportion of florporauxifen-benzyl in water was negatively correlated with the initial concentration. The distribution proportion of florporauxifen-benzyl in

the soil is positively correlated with the initial concentration. It is speculated that this is due to the strong adsorption capacity of soil for florporauxifen-benzyl. Additionally, the total residual concentration of florporauxifen-benzyl in sterilized soil and water did not change significantly but decreased significantly in nonsterilized soil and water, and the microbial degradation of florporauxifen-benzyl was higher than hydrolysis. It is speculated that the main degradation mode of florporauxifen-benzyl in soil and water is microbial degradation. This is similar to the degradation law of most herbicides reported in the literature (Magnoli et al., 2020).

It was reported that the half-lives of florporauxifen-benzyl in paddy water and the soil matrix were 0.3 and 1.2 days, respectively. The degradation rate of florporauxifen-benzyl in paddy water was 90% after 5 days of application and 90% after 21 days of application in soil (Tu et al., 2021). However, the half-life of florporauxifen-benzyl in water and soil was longer, and the degradation rate in this study was lower than that reported, which may be related to the recommended dose

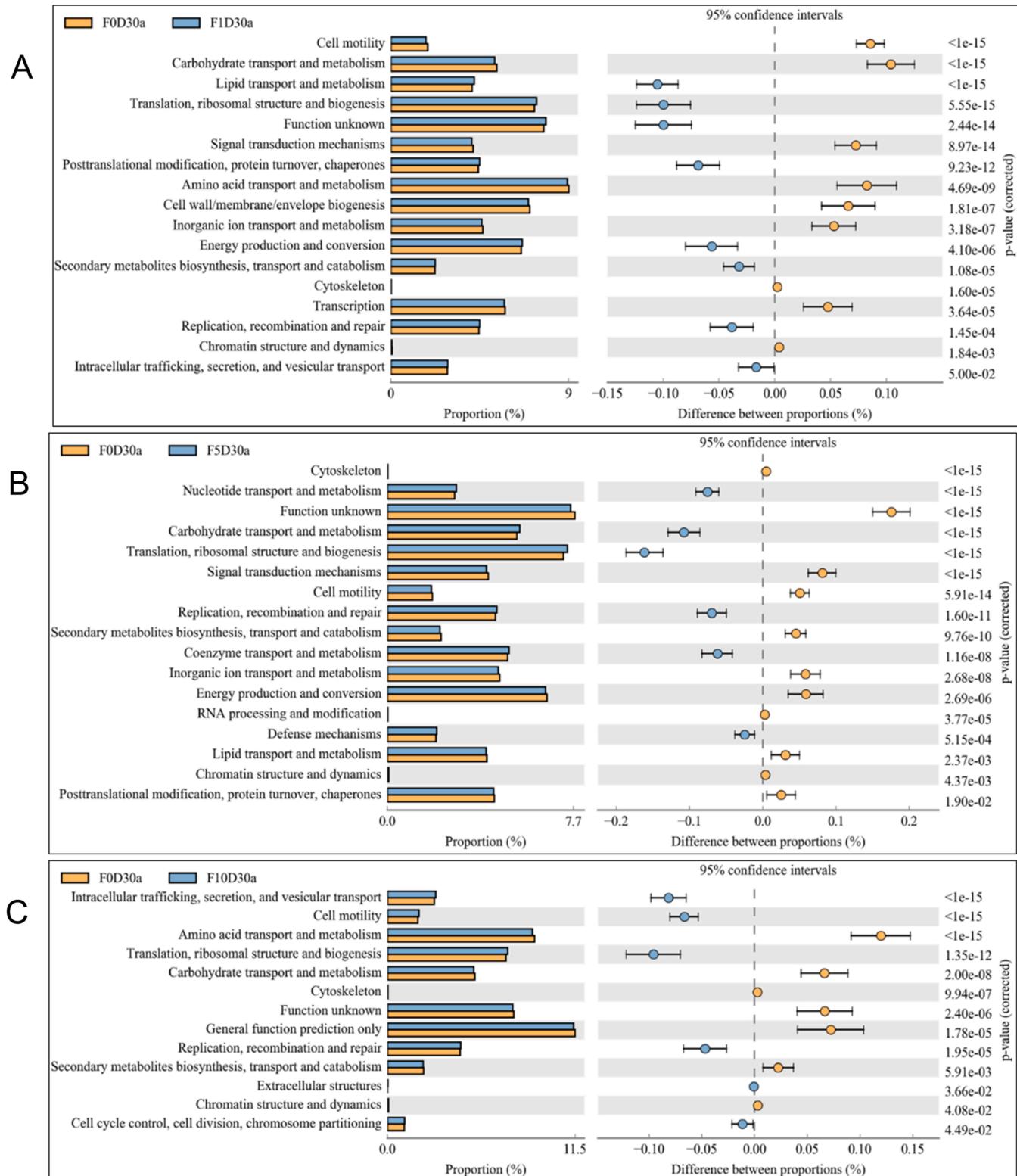


Fig. 8. COG function classification statistics. (A) F0 and F1; (B) F0 and F5; (C) F0 and F10. Different colors represent different groups. The left figure shows the abundance proportion of different functions in the two groups of samples, the middle shows the difference proportion of functional abundance in the 95 % confidence interval, and the value on the far right is the p value.

repeated application or may be caused by the geographical location, soil microbial abundance and environmental climate of the test site.

4.2. Effects of florporauxifen-benzyl on bacterial diversity in rice pot soil

4.2.1. Analysis of soil bacterial composition

Compared with F0, the abundance of the two genera in the F10 group increased significantly on Day 15. It is speculated that these two genera

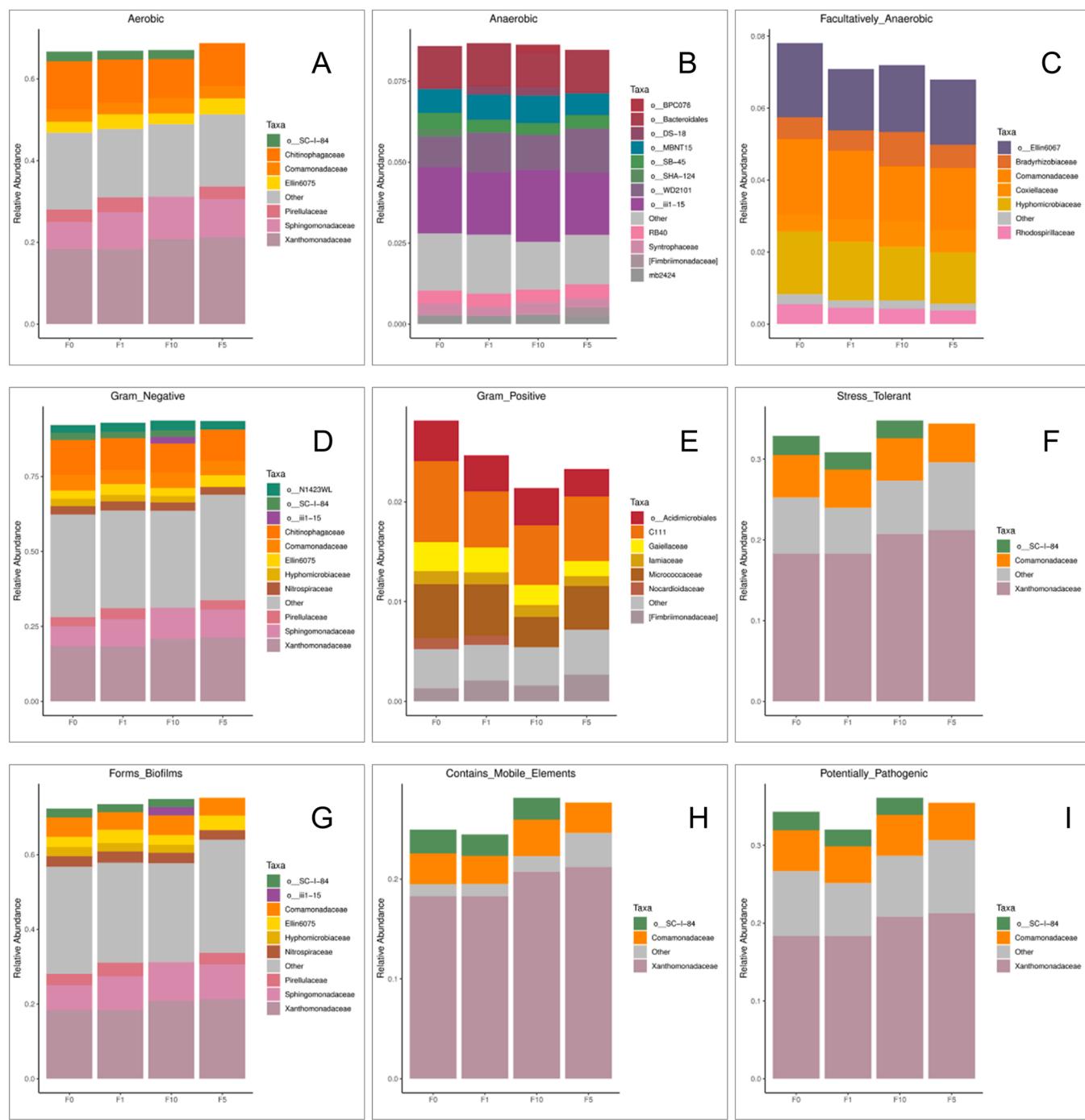


Fig. 9. The variation of BugBase phenotype in the top species of family level abundance inter groups. (A) Aerobic, (B) Anaerobic, (C) Facultatively Anaerobic, (D) Gram Negative, (E) Gram Positive, (F) Stress Tolerant, (G) Forms Biofilms, (H) Contains Mobile Elements, (I) Potentially Pathogenic. The abscissa and ordinate are the group name and the relative abundance percentage of species, respectively.

can grow and reproduce with florporauxifen-benzyl as a carbon and nitrogen source. Although the bacterial community composition of the rice rhizosphere varied with the dose and duration of florporauxifen-benzyl, the two genera *Sphingomonas* and *Lysobacter* showed a significant increasing trend. It has been reported in the literature that the abundance of *Sphingomonas* and *Lysobacter* increased after dry farming, acaricide and allyl isothiocyanate treatment. They have the ability of pollutant degradation, tolerance to heavy metals and symbiosis with plants to reduce pollution (Shi et al., 2021; Gao et al., 2021; B.B. Wang et al., 2021; M.T. Wang et al., 2021; Hu et al., 2021). In addition, *Lysobacter* is a facultative predatory bacterium that can inhibit the

growth of fungi and synthesize antibiotics. It has arsenic resistance, and its abundance increases after being treated with selenium fertilizer, earthworm compost and biochar. It can degrade benzopyrene and bisphenol sulfur; can produce protease, chitinase and lipase; can kill nematodes; and has a high growth rate and carbon assimilation rate (Hungate et al., 2021; Tian et al., 2021; Wang B.B. et al., 2021). *Sphingomonas* can degrade organic pollutants such as acetochlor, organophosphorus, lindane, chlorpyrifos, oestrogen, nonylphenol, atrazine and its three metabolites, glyphosate, diesel, polycyclic aromatic hydrocarbons, fipronil, 2,4-dihydroxybenzophenone, straw, 2,4-D, dibenzofuran and dibenzo-p-dioxin. Its abundance increased after

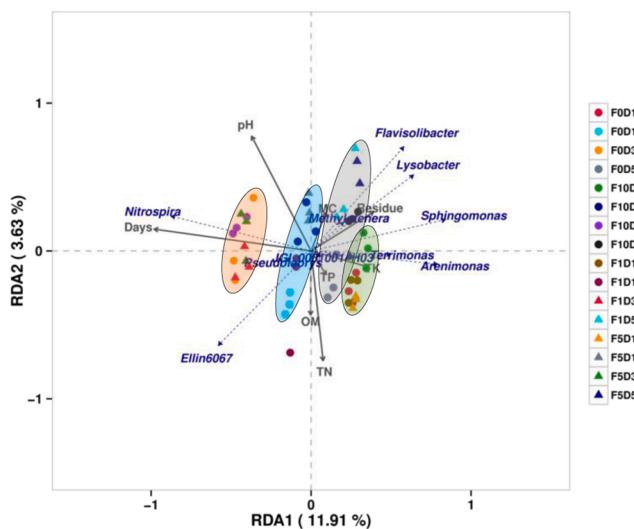


Fig. 10. RDA correlation analysis of bacteria and environmental factors in soil samples. Points of different colors or shapes represent sample groups under different conditions. Blue arrows represent bacteria of different genera. Gray arrows represent environmental factors: TN: total nitrogen; TP: total phosphorus; TK: total potassium; OM: organic matter; MC: air dried moisture content; pH: pH; Residue: residual amount of flupyrauxifen-benzyl; Days: processing days. Green, grey, blue and yellow circles represent sample Day1, Day5, Day15, and Day30, respectively.

arsenic-containing fertilizer, polychlorinated biphenyls in soil, polycyclic aromatic hydrocarbons, pesticide/fertilizer mixture, pesticide treatment only, nicosulfuron + atrazine and magnesium fertilizer application or treatment. It can colonize corn and enhance the absorption of toxic metals. It can eliminate chlorpyrifos oxidative damage, promote the transformation of cadmium, have disease resistance and other plant growth-promoting abilities in colonized rice, and have cold resistance, adaptability to saline alkali, chromate, copper pollution and other environmental tolerances (Feng et al., 2021; Wang H. et al., 2018; Tang et al., 2021; Cheng et al., 2021; Puopolo et al., 2014; F.G. Wang et al., 2020; L.Y. Wang et al., 2020; M. Wang et al., 2020; L. Wang et al., 2020; Wu et al., 2020). The removal rates of polycyclic aromatic hydrocarbons, diesel degradation and fipronil degradation were accompanied by a significant increase in the relative abundance of *Sphingomonas*, which was consistent with the results of this study. Additionally, the relative abundance of bacteria in the two genera increased significantly. This may be because flupyrauxifen-benzyl stress will lead to rice actively recruiting specific symbiotic microorganisms to detoxify pollutants, and rice can survive better under polluted conditions. The decrease in flupyrauxifen-benzyl residue concentration was accompanied by a significant increase in the relative abundance of *Sphingomonas* and *Lysobacter*, which may be due to the growth of bacteria using herbicides as carbon and nitrogen sources.

4.2.2. Analysis of bacterial diversity

Soil microorganisms are the most sensitive biological indicators reflecting soil health. Under pesticide stress, the structure of the soil microbial community often shows a trend from drastic change in the initial stage to recovery in the later stage. PCA showed that there were significant differences between different herbicide dose groups over time. The change trend of the application group was the same, which was different from that of the nonapplication group, indicating that the application of herbicide did have a significant impact on soil microorganisms. On the 5th and 15th days, there was a significant difference between the 5 times and 10 times of the recommended dose group compared with the nonapplication and the application of the recommended dose group, and it returned to the level of the control group on

the 30th day, indicating that the soil microorganism showed a severe impact at the initial stage after the application of the repeated application quantity and naturally returned to the level of the nonapplication of herbicide after one month. PERMANOVA showed that there were significant differences in binary jackard distances between groups at different times. The difference between groups is greater than that within groups, so the reliability of the test is high. This shows that there are significant differences between the two groups over time. At each time point, there was a significant difference in the binary Jaccard distance between groups, and the difference between groups was greater than that within groups. The reliability of the test in the fifth-day group was higher. This showed that there were significant differences between the application group and the nonapplication group and between the groups with different applications. If the herbicide is not applied according to the recommended dose, if it is applied in repeated application quantity, the soil microorganisms in this study may be inhibited in a short time and will not recover until a long time, but their diversity is still lower than that of the group not applied or applied with the recommended dose. Inhibited microorganisms may not be able to use herbicides, and their growth decreases, but some microorganisms can tolerate herbicides and use herbicides as carbon and nitrogen sources for growth and reproduction. With the passage of time, the herbicide content decreases, and the relative abundance of microorganisms increases. It may take longer for microbial diversity to recover to the level of nonapplication or application of the recommended dose group. It has been reported that mesosulfuron-methyl significantly increases the aggregation of microorganisms with pollutant degradation functions in microbial communities (Du et al., 2021). The results of this study are consistent with the change trend of microorganisms reported in the literature.

One of the main objectives of microbial community ecology is to understand the formation process of species abundance with time and space. β NTI analysis showed that the bacterial diversity of the group without herbicide application increased significantly with time and showed a transformation from incomplete determination to certainty. The microbial diversity of the application group decreased significantly with time and showed a transformation from incomplete determination to certainty. On Day 1 and Day 5, each group was in the transition stage of randomness and certainty. After the 15th day of application, the microbial diversity was significantly lower than that of the non-application group and showed a transformation from the complete transition region to the incomplete determination region. There are two types of processes that affect the assembly of species in the community. They are deterministic and stochastic processes. The deterministic process is that in this process, abiotic and biological factors determine the existence/absence and relative abundance of species, which is related to ecological selection. Stochastic processes include random changes in the probability distribution and relative abundance of species (ecological drift), which are not adaptive results determined by the environment (Dini-Andreote et al., 2015). Although the content of herbicide in the soil decreases with time and the corresponding abundance of dominant flora increases, the repeated application of herbicide reduces the soil bacterial diversity and does not involve random processes, indicating that the bacterial change in rice rhizosphere soil was an adaptive result determined by environmental factors.

4.2.3. Significance analysis of difference between groups

The core flora of the nonapplication group and application group changed with time, and the core flora of the nonapplication group and application group with different applications were different at the same time. The soil microorganisms changed from the initial *Terrimonas* to the later *Deltaproteobacteria* and became the core flora. Most bacteria in this class (*Deltaproteobacteria*) live in facultative or obligatory anaerobic life (Kuever et al., 2005). However, in the 5-fold and 10-fold application groups, the core flora in the initial stage was *Lysobacter* and *Sphingomonas*, and in the later stage, the core flora was transformed into

Planctomycetes, which is a small phylum of aquatic bacteria and an important bacterium in sewage treatment. They can use nitrite (NO_2^-) ammonium oxide (NH_4^+) to generate nitrogen to obtain energy in an anoxic environment. This is called anaerobic ammonia oxidation, which is of great significance to the global nitrogen cycle (Ghimire et al., 2021).

The core flora of the application group was located on the same branch and clustered. The initial core flora in the recommended dose group is mainly the parents of *Flavisolibacter*. In the 5-fold and 10-fold recommended application groups, the evolution of the initial core flora was similar, mainly the parents and children of *Sphingomonas* and *Lysobacter*. The core flora of the application group developed in the same direction, and the microbial diversity decreased, which may be the stress response of rice rhizosphere soil microorganisms to adapt to the environment of different herbicides (Pertile et al., 2021).

4.2.4. Functional gene prediction analysis

Herbicides can induce bacteria to produce enzymes that can metabolize such carbon and nitrogen sources, therefore promoting the transformation of herbicides, thereby enhancing the functions of translation and ribosomes. Under the action of herbicides, the morphology of bacteria changed due to the change in osmotic pressure inside and outside cells. As high concentrations of herbicides inhibit the division and reproduction of bacteria, the function of the bacterial cytoskeleton is reduced (Pichoff et al., 2007).

Overall, the relative abundance of aerobic bacteria in the 10 times recommended application group was lower than that in the non-application group and the 1-fold and 5-fold recommended application groups. The relative abundances of facultative anaerobic bacteria, gram-positive bacteria and anaerobic bacteria in the application group were lower than those in the nonapplication group. However, the relative abundance of morphological biofilms in the application group was higher than that in the nonapplication group. It is possible that the application of herbicides leads to an increase in the relative abundance of gram-negative bacteria in soil and the formation of bacterial biofilms. Bacterial biofilms are a life phenomenon in which bacteria adapt to the natural environment and are conducive to survival. The membrane can buffer the changes in the microenvironment. Acylated homoserine lactones (AHLs) are substances that transmit information between gram-negative bacilli. It plays a decisive role in the final formation of biofilms (Houdt et al., 2007). After bacteria are adsorbed on the surface, how to gather into the cell community and coordinate their behaviour to form the biofilm structure, that is, the information transmission mechanism between bacteria, is under further study. The relative abundances of stress tolerance, mobile elements and potentially pathogenic bacteria in the 5-fold and 10-fold recommended application groups were higher than those in the nonapplication and application recommended application groups. When the external living environment of bacteria changes, bacteria will produce a stress response in a short time, and most genes of their genome will participate in this process. Bacteria containing resistant plasmids can use herbicides as carbon and nitrogen sources for growth and reproduction. These bacteria that can degrade organic pollutants often have potential pathogenicity to animals or plants (Singh et al., 2016). Therefore, the relative abundance of stress-tolerant, mobile elements and potentially pathogenic bacteria in the soil increased after herbicide application.

The relative abundance of aerobic bacteria, pressure-tolerant bacteria, mobile elements and potentially pathogenic bacteria showed a trend of first increasing and then decreasing. This may be due to the gradual decrease in herbicide residues in the soil with increasing time. Pressure-tolerant bacteria containing resistant plasmids and potential pathogens increase to degrade high concentrations of herbicides and obtain growth and reproduction. With the decrease in herbicide residues, the relative abundance of these bacteria gradually decreases due to the lack of carbon and nitrogen sources. The relative abundance of anaerobes and facultative anaerobes first decreased and then increased. It

is speculated that the herbicide residue is high in the early stage of application, so the relative abundance of anaerobic bacteria and facultative anaerobic bacteria is inhibited. With increasing time, the herbicide residue gradually decreased, and the oxygen in the soil gradually exhausted. Due to the coverage of water, the relative abundance of anaerobic bacteria and facultative anaerobic bacteria gradually increased. The relative abundance of gram-negative bacteria showed an upwards trend, which may be related to the formation of biofilms by gram-negative bacteria in soil and their participation in the degradation and metabolism of herbicides. Although the herbicide showed a gradual downwards trend over time, its residue still inhibited gram-positive bacteria. This is consistent with the results reported in the literature (Liao et al., 2021).

4.2.5. Correlation analysis of genus and environmental factor

The change in relative abundance often does not reflect the growth rate of the population. However, there is a strong and symmetrical interaction between the abundance of these core flora, and the positive or negative correlation may be caused by mutualism or competition between them. The interaction between species is not the only driving factor, and many potential environmental factors will increase the complexity of population dynamics. Therefore, the inference of interspecific relationships can be used as a preliminary reference for soil microbial ecology (Carr et al., 2019).

It has been reported that the genera (*Lysobacter*, *Methylorenica*, *Flavisolibacter*, *Sphingomonas*, *Terrimonas*, and *Arenimonas*) positively correlated with herbicide residues can degrade organic pollutants. *Lysobacter* and *Sphingomonas* can also degrade a variety of organics and adapt to the polluted environment, as mentioned earlier. *Methylorenica* is one of the dominant genera in aquatic ecosystems polluted by petroleum hydrocarbons. *Flavisolibacter* and *Sphingomonas* were positively correlated with the content of aromatic compounds in the soil. *Terrimonas* can degrade anthracene, phenanthrene, and benzopyrazine, participate in low organic matter anaerobic fermentation, and respond quickly to AHL, which is significantly negatively correlated with reaction time. *Arenimonas* is a dominant bacterium with the functions of carbon fixation, nitrogen fixation and phosphorus reconciliation. It is speculated that they will decrease with the decrease in herbicide residues, which is consistent with the positive correlation effect of herbicide residues on them. This may be the reason why these genera have a low positive correlation with herbicide residue and a high negative correlation with treatment time. These genera have a certain ability to utilize organic pollutants. It is speculated that they can use herbicides for growth and reproduction. However, they are aerobic. As the soil is covered with water, the oxygen in the soil gradually decreases with increasing time, and its abundance gradually decreases (Hungate et al., 2021; Tian et al., 2021; M.T. Wang et al., 2021; B.B. Wang et al., 2021; Feng et al., 2021; Wang H. et al., 2018; Tang et al., 2021; Cheng et al., 2021; Puopolo et al., 2014; F.G. Wang et al., 2020; L.Y. Wang et al., 2020; M. Wang et al., 2020; L. Wang et al., 2020; Wu et al., 2020; Idomeh et al., 2021; Jin et al., 2021).

It has been reported that the genera (*Nirospira*, *Pseudolabrys* and *Ellin6067*) negatively correlated with herbicide residues can degrade organic pollutants. The herbicide residue concentration is high in the initial stage, and the abundance of these genera is low. The herbicide concentration is low in the later stage, and the abundance of these genera is high. It is speculated that the bacteria of these genera are inhibited by high concentrations of herbicides in the early stage of application, and low concentrations of herbicides promote their growth and reproduction in the later stage. According to the literature reports, the unknown CAH enzyme of *Pseudolabrys* sp. Root1462 was highly expressed in aerobic cell culture. *Pseudolabrys* was identified as one of the dominant genera after aerobic culture of 1,4-dioxane and Co pollutants. *Nirospira* is a typical nitrate-oxidizing bacterium in wetlands. Bacterial diversity values were significantly lower in the constructed wetland with the highest inlet nutrient concentrations. *Ellin6067* and

other bacteria were mainly connected with the network in the TNT group and had strong interactions with metabolites. This showed that they had certain pollutant tolerance. Their abundance increased with the decrease in herbicide residue, which was consistent with the influence of herbicide residue on them (Jia et al., 2021; Arroyo et al., 2015; Yang X et al., 2021).

With the decrease in herbicide residues, the content of organic matter and total nitrogen in the soil increased. It is speculated that herbicide residues and animal and plant residues release some organic matter through microbial decomposition. According to the literature, the contents of NO_3^- -N and NH_4^+ -N were increased by mesosulfuron-methyl. The abundance of bacteria was responsible for the total N contents (Du et al., 2021). With the decrease in herbicide residues, the air-dried water content and total potassium content in the soil also decreased. The hardness of paddy soil is closely related to its moisture content. The lower the water content is, the higher the hardness. The viscoelastic strain rate of paddy soil decreases with decreasing water content (Liu et al., 2021). Irrigation water intake reduced the exchangeable potassium concentration in the paddy soil near the water inlet. Some potassium may be dissolved in water after being soaked and washed, so the total potassium content in the soil decreases (Klevtsova et al., 2022). Through which ecological cycle can these bacteria naturally digest herbicides in paddy soil, therefore reducing the residue, which is worth further studying the abundance changes of carbon and nitrogen cycle functional genes in the future (Luo et al., 2022; Du et al., 2021).

5. Conclusion

This study found that the main degradation mode of florporauxifen-benzyl in soil and water was microbial degradation. After 30 days of application, compared with the control groups (F0), the abundance of *Sphingomonas*, *Lysobacter*, and *Flavisolibacter* in the treatment groups (F1, F5 and F10) increased significantly, and the abundance of two genera, uncultured bacterium c subgroup 6 and *Terrimonas*, decreased significantly. The species diversity of the F0 and F1 groups showed a change trend of significantly increasing over time. Compared with the F0 and F1 groups, the species diversity of the F5 and F10 groups decreased significantly on Day 5 and Day 15. On Day 30, the recovery even exceeded that of the control group. The relative abundances of facultative anaerobic bacteria, gram-positive bacteria and anaerobic bacteria in the F1, F5 and F10 groups were lower than those in the F0 group, and the relative abundances of gram-negative bacteria and morphological biofilms were higher than those in the F0 group. The relative abundance of aerobic bacteria in Group F10 was lower than that in Groups F0, F1 and F5. The relative stress tolerance, mobile elements and potential pathogenicity abundance in Groups F5 and F10 were higher than those in Groups F0 and F1. The genera generally positively correlated with herbicide residues were *Luteimonas* and the other five genera, and the genera generally negatively correlated with herbicide residues were *Pseudolabrys* and the other two genera. In conclusion, repeated application of florporauxifen-benzyl showed a significant impact on the structure of the soil bacterial community in paddy fields, mainly showing a trend from initial dramatic change to later recovery. The results will guide the safe and rational use of florporauxifen-benzyl and provide a scientific basis for florporauxifen-benzyl dynamic supervision of environmental pollution and protection of black soil in Northeast China.

CRediT authorship contribution statement

Chunguang Liu: Conceptualization, Sample collections, Sample treatment, Methodology, Formal analysis, Validation, Writing – original draft. **Yujun Han:** Conceptualization, Methodology, Formal analysis, Validation, Funding acquisition. **Chunhong Teng:** Writing – review & editing, Resources, Supervision. **Hong Ma:** Writing – review & editing,

Resources, Supervision. **Bo Tao:** Methodology, Validation, Funding acquisition, Writing – review & editing. **Fengshan Yang:** Methodology, Validation, Funding acquisition, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.

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.

Data Availability

The authors do not have permission to share data.

Acknowledgements

This research was supported by Key Technologies Research and Development Program (Grant No. 2016YFD0200507), National Natural Science Foundation of China (Grant No. 32072434), Natural Science Foundation Research Team Project of Heilongjiang Province (Grant No. TD2019C002), and Scholar Backbone Project of Northeast Agricultural University (Grant No. 19XG02).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114390.

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