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Maize Rotation Combined with *Streptomyces rochei* D74 to Eliminate *Orobanche cumana* Seed Bank in the Farmland

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Abstract: *Orobanche cumana* wallr. is the sunflower root parasitic weed with special life stage in which seed germination and parasitism take place in the soil. In practice, applying microbial agents and trapping crop rotation are utilized separately, or just one of them is selected to control *O. cumana*. The development of the sunflower industry is severely constrained on the farmland, where there is high density of *O. cumana*'s seed banks. In this study, two biological control methods were combined to solve the problem of *O. cumana* parasitism. The bioassay experiment showed that the high concentration fermentation filtrates of *Streptomyces rochei* D74 could effectively inhibit the germination and growth of the germ tube of *O. cumana* seeds. As the concentration was increased to 3.1 mg/mL, *O. cumana* was almost unable to sprout. A two-year pot experiment revealed that the use of D74 agents and sunflower–maize–sunflower rotation together promoted sunflower growth, as shown by the biomass accumulation, plant height, and denser root systems. The combined method resulted in a significant decrease in the number of *O. cumana* parasitism, compared to one method alone. Additionally, it affected the bacterial community composition of sunflower rhizosphere, mostly leading to an increase in *Streptomyces* and *Brevibacterium* and a decrease in *Arthrobacter*. This experiment, combined with multiple biological control, means significantly reducing the parasitism of *O. cumana*, which provides an effective foundation for practical application.

Keywords: *Orobanche cumana*; *Streptomyces rochei*; maize rotation; sunflower; rhizosphere microbial

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1. Introduction

Orobanche cumana Wallr is a fully parasitic weed that lacks chlorophyll and has no photosynthetic capacity [1]. Its seeds can detect strigolactone (SLs), a germination signal substance secreted by the root of the host plant, and initiate the germinating process [2,3]. The end of the germ tube of the germinating seeds can form haustorium, a special nodular protruding tissue that can be adsorbed on the host root surface, penetrate the root epidermis, grow to the xylem and phloem, and form a continuous xylem between the host and the *Orobanche* [4]. *Orobanche* feeds itself by absorbing carbohydrates, water, and mineral elements from the host [5], and then grows to the surface, blooms, and produces tons of tiny seeds eventually [6,7]. *O. cumana* has specific host requirements and can only parasitize sunflowers. *Orobanche* has affected many countries. In China, the area affected by *Orobanche* reached 20,000 hectares, with yield losses ranging from 20% to 50% [8]. In France, the economic losses caused by *Orobanche* are incalculable, and the Ministry of Agriculture claimed that, if no effective measures are taken, yield loss could reach 90% (https://doi.org/10.1079/pwkb.species.37741 (accessed on 1 November 2022)). The serious

parasitism of *O. cumana* reduces sunflower yield and quality, affecting farmers' income and stifling agricultural development. Manual uprooting, herbicide spraying, soil fumigation, irrigation, trapping crop rotation, and allelopathy are currently the most commonly used methods [9–12].

Maize was used as a trap crop in this study because its root exudates can stimulate the germination of *Orobanche* seeds in the surrounding soil, but cannot establish an effective connection with the crop, and it will eventually die due to nutrient deficiency [13]. Planting trap crops repeatedly, making the active parasitic weed seeds germinate in vain without producing new seed replenishment. Additionally, this has been shown to effectively reduce soil seed bank content [14]. In recent years, more and more studies have been conducted on the influence of microorganisms on *Orobanche* parasitism [15,16]. *Pseudomonas fluorescens* and *Myrothecium verrucaria* isolated from rhizosphere soils reduced the germination rate of *O. foetida* and *O. ramosa*, respectively [17,18]. Studies have shown that *Streptomyces enissocaesilis* can inhibit the germination of *Orobanche* seeds by 47% [19]. *Streptomyces rochei* D74, an actinomycete with germination inhibitory potential discovered by Chen in the early stages [19], was used in this experiment. *S. rochei* D74 has been shown in studies to promote the growth of a variety of crops, including *Amorphophallus konjac*, *Aconitum carmichaelii*, tomato, pepper, sunflower, and jujube [20–22].

Because of the nature of *Orobanche* seed germination and parasitism occurring under the ground, it has already caused crop damage and displayed infection symptoms before reaching the surface. More studies have used one method alone to prevent the parasitism of *Orobanche* in the past, and there was a lack of research on combining multiple methods to reduce parasitism. Therefore, in order to achieve the purpose of long-term and effective control of parasitism, this study combined biological control and crop rotation, for the first time, to study the effects of *Streptomyces* and maize rotation on parasitism.

2. Materials and Methods

2.1. Preparation of S. rochei D74 Fermentation Filtrates

S. roche D74 was incubated into Gauze's synthetic medium [23] and was shaken at 28 °C and 100 rpm for 14 days. Afterwards, the fermentation filtrates of D74 above were centrifuged at 4 °C and 10,000 rpm to separate supernatant and precipitate. Filter the supernatant with a 0.45 μm microporous membrane to remove excess impurities. The collected precipitate was dried in a drying oven at 35 °C and weighed afterwards. The concentration of D74 fermentation filtrates was 3.1 mg/mL, which was calculated from the volume of the supernatant and the mass of the dried precipitate. The obtained D74 fermentation filtrates was diluted in a gradient of 10–10^5 times of the original filtrates. The diluted fermentation filtrates concentration was in the range of 3.1×10^{-1} mg/mL–3.1×10^{-5} mg/mL. Finally, we dispensed the diluted solution and kept it in −80 °C refrigerator.

2.2. Petri Dish Bioassay Experiments

The pretreatment of the *O. cumana*'s seed: sterilize the seeds' surfaces by dipping them in 75% ethanol for 3 min, cleaning them from impurities, and then drying them naturally. Lay a round layer of plain filter paper with a diameter of about 9 cm flat on the bottom of the Petri dish. Place the 5 mm diameter round glass fiber filter paper neatly on the round plain filter paper in order. Then, soak the filter paper sheet with sterilized water and then spread the seeds evenly on it (about 40–60 *O. cumana*'s seeds on each piece of filter paper). Put the Petri dishes into the artificial climate incubator at 28 °C for 5 days.

Prepare some new Petri dishes. Place the sterilized glass fiber filter paper with a diameter of 5 mm to the bottom of the Petri dish in an arc shape. Pipette 25 μL of the prepared D74 fermentation filtrates at different concentrations into Petri dishes, while the blank control group was added with an equal amount of sterilized water. Each treatment was replicated 5 times. A pre-cultured seed piece of *O. cumana* was placed on the top layer of each filter paper sheet, and 25 μL of the artificial hormone GR24 at 1 ppm was added in turn. For the control group, sterile water was used instead of GR24 to verify whether the

seeds could germinate in the absence of hormonal stimulation. Place a moistened triangular filter paper sheet in the center of each Petri dish to avoid the death of seeds due to water loss. The dishes were then sealed with Parafilm and incubated at 28 °C for 7–10 days in the dark. After the culture was completed, the seeds were examined microscopically, and the number of germinations and the number of seeds of which the lengths of germ tubes were between 0–1 mm (short) and 1–3 mm (long) were counted [19]. The germination rate of sunflower seeds and the percentage of seeds of which the lengths of germ tubes were between different ranges were calculated according to the following formula (1):

$$\text{Percentage of 0–1 mm} = \frac{(\text{Number of 0–1 mm seeds})}{(\text{Total number of germinated}) \times 100\%} \quad (1)$$

2.3. Co-Culture Experiments

Pretreatment of sunflower seeds: prepare 100-hole cavity trays. Then, mix them according to the ratio of substrate:vermiculite = 2:1 and put them into the cavity trays. Sterilize the seeds in 75% ethanol for 3 min, and then wash off impurities on the surface with sterilized water and press them into the cavity tray (2 seeds in each hole). Water with 20 mL water and incubate in an artificial climate chamber (28 °C; light incubation: dark incubation = 12 h: 12 h) for 5–8 days. When sunflower plants grow to about 5 cm in height, take them out with roots. Rinse off excess soil and impurities from the roots with sterile water, and the seedlings for testing are ready. The pretreatment method of seed is the same as in Section 2.2. Simply sprinkle the seeds of *O. cumana* on a 9 cm glass fiber filter paper, incubate under the same conditions for 5 days, and they are ready for use.

A 9 cm diameter piece of sterilized glass fiber filter paper was laid flat on the bottom of the Petri dish in a clean bench. Then, we added 2 mL of D74 fermentation filtrates at a concentration of 3.1 mg/mL, while the blank control group (CK) was added with an equal amount of sterilized water. After soaking for 1.5 h, the filter paper sheets were removed to dry in Petri dishes. We placed the dried filter paper sheet in the middle of the PE bag with tweezers, and then overlaid a glass fiber filter paper sheet that has been evenly sprinkled with pre-cultivated *O. cumana* seeds. We inserted the cleaned sunflower seedling plants through the gap above the cut PE bag to make the roots of the sunflower plants lay evenly and flatly on the filter paper sheet and make full and close contact with the *O. cumana* seeds. The prepared Hoagland nutrient solution [24] was then added in the amount of 15 mL/bag and incubated in an artificial climate chamber (28 °C; light incubation: dark incubation = 12 h: 12 h) for 30 days [16]. During this period, the PE bags were supplemented with 5 mL of Hoagland nutrient solution daily, and we examined the germination and parasitism of *O. cumana* seeds by microscopy after 30 days.

2.4. Design of Pot Experiments

The design of experimental treatment group: the experimental sample site of this study is located at the Institute of Soil and Water Conservation, Northwest A&F University. The maize variety used was Zheng Dan 958, and the sunflower variety was 363. To investigate the effects of maize rotation and addition of D74 agents on the growth of sunflower and the emergence of *O. cumana*, we set up a crossover test with three groups of factors. With (L) and without (N) the addition of *O. cumana* seeds; with (J) and without (N) the addition of D74 agents; with (Y) and without (N) the rotation of maize. A total of 10 treatment groups were set up in this study, with 7 pot replicates for each treatment. Added *O. cumana* groups: CKL (blank control soil); LXJY (crop rotation + D74); LXJN (addition of D74); LXNY (maize rotation); LXNN (sunflower planting only). Non-addition of *O. cumana* group: CKN (blank control soil); NXJY (rotation + D74); NXJN (addition of D74); NXNY (rotation of maize); NXNN (sunflower planting only). With three sowings, the pot planting trial was divided into three crop rotation stages, namely sunflower–maize–sunflower. To ensure consistent planting conditions, the work was performed in the same pot (20 cm in diameter, 25 cm in height) for each crop rotation stage. Stage 1, plant sunflower with 4 seeds per pot in

April 2019, according to treatment group. When the sunflower seedlings had grown for about 15 days, we kept two plants in each pot with the least difference in growth. The planters in different treatment groups were moved every two weeks to ensure that the environmental conditions, such as light and water, were consistent between treatments, and they were watered as needed throughout the period. After 2 months of plant growth, we harvested the aboveground and underground parts of the sunflower and measured their physiological parameters. We harvested and recorded the number of *O. cumana* above the ground, as well as the total number of parasites. Collect the rhizosphere soil for future research. Stage 2, following sunflower harvest, fertilizer, and D74 were reapplied to the original soil in June 2019, in accordance with the treatment group settings. After 1 week of resting, the maize hybrid Zhengdan 958 was planted according to the treatment group setting for crop rotation. Each pot was planted with 10 maize seeds and picked to two plants per pot according to growth after the maize seeds sprouted out of the soil. The aboveground portion of maize was harvested in August 2019 after 45 days of planting to evaluate its physiological parameters, while the underground root portion was left in the soil to facilitate its continued production of root secretions for subsequent research on the effect of maize root secretions on *O. cumana*'s seeds. Stage 3, in April 2020, we opened the pots, removed the underground portion of the maize root system, and re-mixed the soil with fertilizer and D74. We resowed sunflower seeds in the original soil in May 2020, 10 seeds per pot. According to the growth situation, we kept 2 plants in each pot to continue to grow. We harvested the sunflower when it was fully parasitized. In June 2020, fresh samples of above- and underground sunflower plants were harvested, and their physiological parameters were measured, as well as the number of unearthed *O. cumana* and total parasites. Fresh samples of rhizosphere soils were collected and promptly transported back to the laboratory at low temperatures before being divided into two parts. One part of the rhizosphere soil was stored at 4 °C and isolated culturable microorganisms by serial dilution plating. We compared the number and species of culturable microorganisms in the soil of different treatments by counting. We screened and identified the dominant strains. Another part of sunflower rhizosphere soil was stored at −80 °C for extracting total soil DNA extraction. Using 16S rRNA gene amplicon sequencing, we analyzed the structure of the soil microorganism community.

Treatment of sunflower seeds: the full-grained sunflower seeds were selected and divided into two parts based on the needs of the treatment groups. We took out a part of sunflower seeds, weighed D74 at 4% of the total mass, and mixed them with D74 using sodium carboxymethyl cellulose (CMC-Na) at a concentration of 0.6% as a binder, so that the D74 was fully coated with the shell of sunflower seeds. Another portion of sunflower seeds were coated with only an equivalent concentration of 0.6% CMC-Na as a control treatment. Maize seeds were soaked in D74 fermentation filtrates for 10 h and then coated with 0.6% CMC-Na at 15% of their total weight.

Treatment of soil: the test soil was a small mound collected from the cultivated land around Yangling. The soil was obtained from farmland and passed through 5 mm sieve to remove debris, and each pot was filled with 8 kg of soil mixture. In each pot, 25 g of organic fertilizer, 3.44 g of urea, and 1.2 g of calcium superphosphate were included. In the treatment group, D74 and *O. cumana* seeds were evenly mixed with the above soil at 1.5 g/kg and 3.4 mg/kg, respectively. The packed soil was left to stand for 1 week, and then the plants were planted.

2.5. Measurement of Plant Physiological Parameters

We measured the plant height, stem diameter, and root length and weighed the dry stem and root of sunflowers at maturity. *O. cumana* was collected and counted at the same time and then divided into the part aboveground and the part underground.

2.6. Sunflower Rhizosphere Soil Collection, Isolation and Identification of Bacteria

Silwet L-77 was added at 0.02% of the volume of phosphate buffer (pH 7.0), and it was mixed as the rhizosphere soil eluted. In the laboratory, we kneaded the fresh sunflower root samples with sterile gloves at low temperature to allow the soil in the root zone to fall naturally. We put the plant roots in a 100 mL sterile centrifuge tube and added an appropriate amount of phosphate buffer. We vortexed for 15 s at the maximum speed to disperse the rhizosphere soil. We removed impurities such as plant debris and large sediments with forceps, then transferred the roots to a new 100 mL sterile centrifuge tube, added appropriate amount of phosphate buffer, centrifuged at 3200 rpm for 15 min, and collected the sediment in a 10 mL centrifuged tube, then centrifuged at 8000 rpm for 10 min and removed the supernatant. One part of the precipitate was stored at 4 °C for isolation and identification of soil rhizosphere bacteria, and one part of the precipitate was stored in a refrigerator at −80 °C for extraction of total DNA [25]. Isolation and identification of sunflower rhizosphere bacteria were carried out on TSB and R2A media to analyze the differences in the composition, and the detailed methods are described in [16].

2.7. Extraction and Sequencing of Total DNA from Rhizosphere Soil

Microbial DNA from soil was extracted using FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA), and the extraction procedure was based on the operation manual. Sequencing libraries were constructed by TruSeq® DNA PCR-Free Sample Preparation Kit (Illumina, Inc., San Diego, CA), and the libraries were quality assessed by Qubit 2.0 fluorometer. Finally, libraries were sequenced on the NovaSeq PE250 platform (Thermo Fisher Scientific, Waltham, MA, USA) to generate 250 bp sequences of paired-end reads. Sequences with ≥97% similarity in clean reads were clustered into identical OTUs (operational taxonomic units) for species annotation.

2.8. Statistical Analyses

SPSS IBM Statistics software (Version 21) [26] was used for two-way ANOVA test at 0.05 level. The plotting was performed using Origin software (Version 2018) [27]. Amplicon analysis was performed through the Novomagic Cloud Platform (https://magic.novogene.com). PCoA plots were obtained using the ImageGP web server (http://www.ehbio.com) [28,29].

3. Results

3.1. Effects of D74 Fermentation Filtrates on the Germination of O. cumana Seeds

The results of the effect of D74 fermentation filtrates on the germination of *O. cumana* seeds are shown in Figure 1a. The germination rate of *O. cumana* seeds in the control group (GR24) without the addition of D74 fermentation filtrates was 83.31%, and the germination rate of *O. cumana* seeds in the treated group with the addition of D74 fermentation filtrates was lower than control group ($p < 0.05$). The germination rate of *O. cumana* seeds was 0% when D74 fermentation filtrates on concentration of 3.1 mg/mL was added, indicating that D74 fermentation filtrates had a significant inhibition effect on the germination of *O. cumana* seeds ($p < 0.05$), and the inhibition rate could reach 100%. When D74 fermentation filtrates was diluted 10–10^5 times, the germination rate of *O. cumana* seeds was reduced by 19.82%, 17.31%, 17.51%, 12.26%, and 8.00%, respectively, compared with the control. The results indicated that the high concentration of D74 fermentation filtrates had a better effect on the germination inhibition of *O. cumana* seeds.

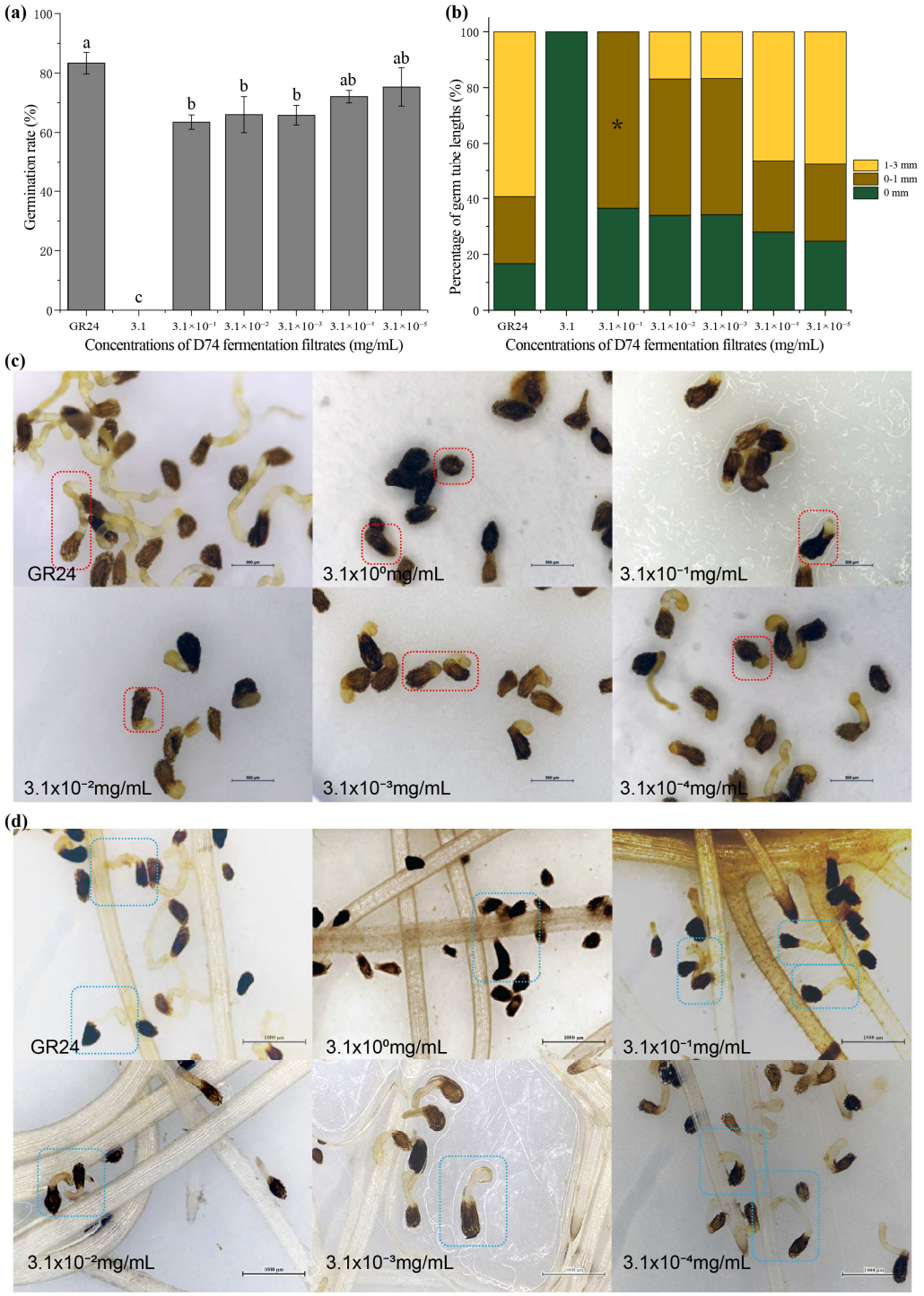

Figure 1. Effects of different concentrations of D74 fermentation filtrates on the germination rate (**a**,**c**), germ tube length (**b**), and parasitism (**d**) of *O. cumana* seeds. Different letters indicate significant differences between the groups (Tukey's test, $p < 0.05$), bar: 1000 µm. * Critical level of significance of constructs at 5%.

The germination rate of *O. cumana* seeds increased with increasing dilution of D74 fermentation filtrates. Additionally, more typical haustorial papillae, probably due to the presence of haustorial-inducing substances in the D74 fermentation filtrates, which induced haustorial formation on *O. cumana* tubes. Tubes of germinating seeds were measured (Figure 1b), and 59.33%·of which in the control group ranging from 1–3 mm. It was significantly higher than the 23.98% with 0–1 mm of tubes length. After the fermentation

filtrates was diluted 10 times, the seeds of *O. cumana* started to germinate, and the length of the shoot tubes were all concentrated in the 0–1 mm range (63.49%), which was significantly higher than the control group ($p < 0.05$). When the fermentation filtrate was diluted 10^2–10^5 times, the percentages of *O. cumana* with shoot tube lengths in the 1–3 mm range were 16.9%, 16.78%, 46.29%, and 47.25%, respectively. As observed from the germination phenotype of *O. cumana*, the seeds of *O. cumana* treated with D74 fermentation filtrates were brown, expanded, and spherical at the end of shoot tube, showing a non-healthy state (Figure 1c).

The results of the sunflower–*O. cumana* co-culture experiment (Figure 1d) were consistent with the results of the germination experiment, and high concentrations of D74 fermentation filtrates had a significant inhibitory effect on the germination of *O. cumana* seeds. With the increase of the dilution of D74 fermentation filtrates, the inhibitory effect on the germination of *O. cumana* seeds gradually diminished and began to parasitize.

3.2. Effects of Maize Rotation and S. rochei D74 Agents on Sunflower Growth

We harvested sunflowers at maturity and observed their growth. The sunflowers in the group with *O. cumana* seeds (L) had weaker growth, while the sunflowers in the group without *O. cumana* seeds (N) had normal growth. The short plant, thinner stalks, and shriveled leaves indicated that *O. cumana* can severely restrict the growth of the host sunflower (Figure S1). In the first year, we took samples primarily to study the effect of D74 alone on sunflower growth. After adding *O. cumana*, the dry matter accumulation, plant height, and root length of the sunflower above and below the ground were significantly lower than those without *O. cumana*. (Figure 2a, Table 1). The dry weights of plants (LXJY, LXJN, NXJY, NXJY) after the addition of D74 were higher than those in the control groups (LXNY, LXNN, NXNY, NXNN), regardless of the presence or absence of *O. cumana*, indicating that D74 can promote sunflower biomass accumulation to varying degrees.

Table 1. The growth index of sunflower in two consecutive years under different treatments.

Sampling Time	Group	Plant Height/cm	Stalk Diameter /mm	Root Length/cm
First year	LXJY	62.00 ± 1.59 b	9.13 ± 0.17 bc	13.71 ± 0.32 bc
	LXJN	60.50 ± 1.4 b	9.28 ± 0.19 bc	10.64 ± 0.92 c
	LXNY	60.75 ± 1.28 b	8.99 ± 0.21 d	12.29 ± 0.21 c
	LXNN	61.00 ± 2.25 b	8.75 ± 0.12 d	11.06 ± 1.05 c
	NXJY	126.76 ± 5.65 a	9.63 ± 0.36 bc	17.38 ± 2.38 b
	NXJN	140.81 ± 5.23 a	10.57 ± 0.64 a	22.31 ± 2.68 a
	NXNY	135.34 ± 7.65 a	9.58 ± 0.21 bc	23.24 ± 1.97 a
	NXNN	137.10 ± 7.91 a	10.05 ± 0.21 ab	24.50 ± 1.93 a
Second year	LXJY	103.06 ± 0.10 c	8.23 ± 0.22 ab	14.70 ± 0.51 c
	LXJN	79.84 ± 0.60 e	7.85 ± 0.18 b	10.27 ± 0.36 e
	LXNY	92.67 ± 2.38 d	8.28 ± 0.19 ab	13.14 ± 0.56 cd
	LXNN	87.26 ± 1.53 d	7.05 ± 0.17 c	10.87 ± 0.68 de
	NXJY	116.29 ± 2.14 a	7.81 ± 0.08 b	28.90 ± 1.09 a
	NXJN	108.94 ± 1.58 b	7.79 ± 0.24 b	21.71 ± 1.33 b
	NXNY	114.16 ± 2.55 ab	8.42 ± 0.18 a	23.79 ± 1.01 b
	NXNN	117.97 ± 2.86 a	8.03 ± 0.08 ab	22.36 ± 1.11 b

Different letters indicate significant differences at $p < 0.05$, as determined by ANOVA, followed by Tukey's test. Groups with *O. cumana* added: LXJY (maize rotation + D74); LXJN (D74 only); LXNY (maize rotation only); LXNN (sunflower planting only). Groups without *O. cumana* added: CKN (blank control); NXJY (maize rotation + D74); NXJN (D74 only); NXNY (maize rotation only); NXNN (sunflower planting only).

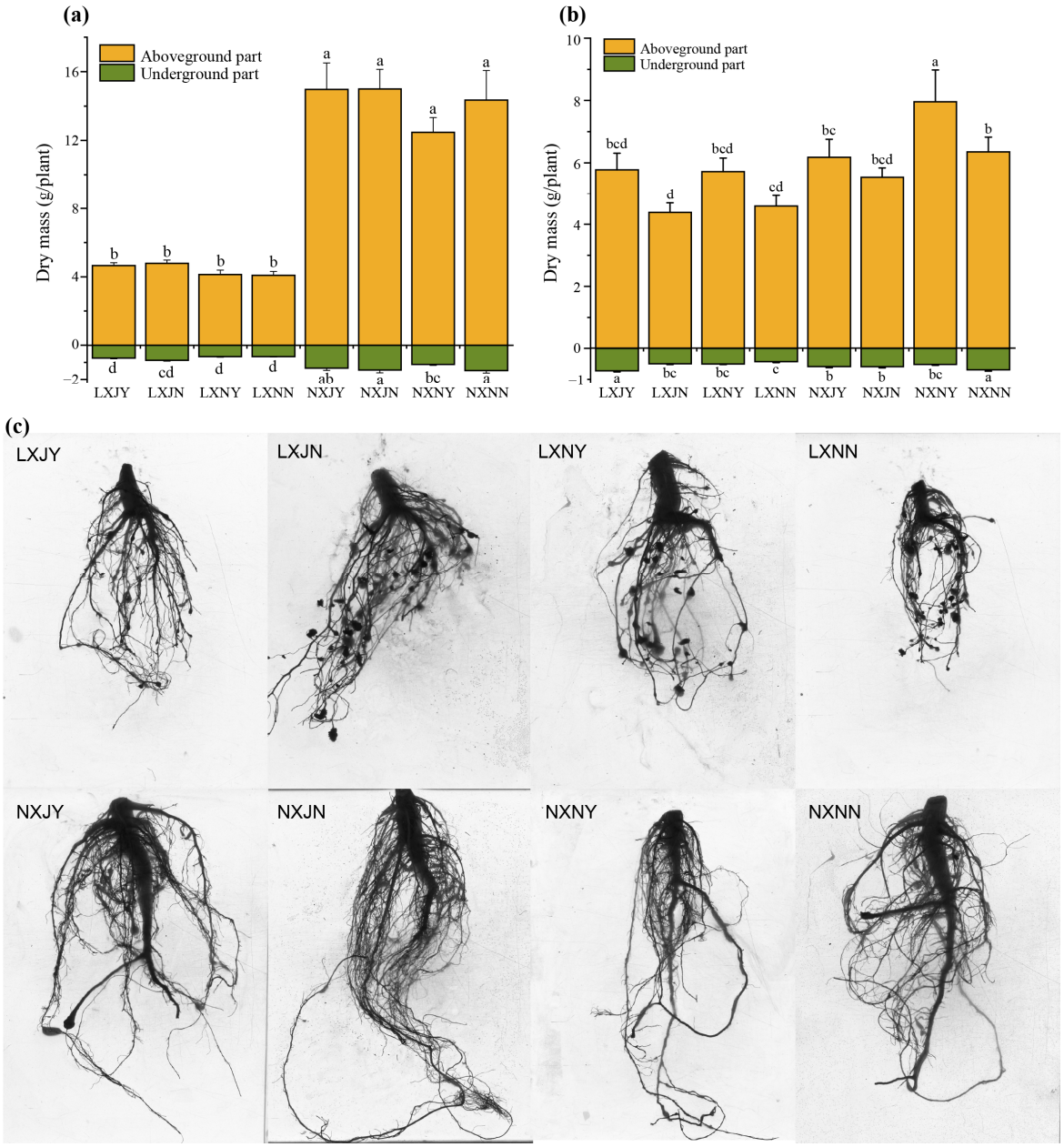

Figure 2. The D74 agent and maize rotation promote sunflower growth. Aboveground and underground dry weights of sunflowers at maturity in the first (**a**) and second (**b**) year. Scanning image (**c**) of sunflower roots at mature stage in second year. Different letters indicate significant differences between the groups (Tukey's test, $p < 0.05$). Groups with *O. cumana* added: LXJY (maize rotation + D74); LXJN (D74 only); LXNY (maize rotation only); LXNN (sunflower planting only). Groups without *O. cumana* added: CKN (blank control); NXJY (maize rotation + D74); NXJN (D74 only); NXNY (maize rotation only); NXNN (sunflower planting only).

The second year, we took samples to study the effects of D74 and maize rotation on the growth of sunflowers. When *O. cumana* was present, dry matter accumulation was higher in the maize rotation group (LXJY, LXNY) than in the non-maize rotation group (LXJN, LXNN), and it was highest in the group that had both maize rotation and the addition of D74 (LXJY) (Figure 2b). The sunflower biomass (including plant height, stem diameter, and root length) was highest in the LXJY group, with plant height increasing by 29.08%, 11.21%, and 18.11% ($p < 0.05$), when compared to the other three groups (LXJN, LXNY, and LXNN). Additionally, the root length increased by 43.14%, 11.87%, and 35.23%,

respectively (Figure 2c, Table 1, $p < 0.05$), while the difference between stem diameter and other treatment groups was minor. When only the effect of maize rotation was considered, the plant height, stem diameter, and root length of LXJN and LXNN increased by 29.08% and 6.2%, 4.84% and 17.45%, and 43.14% and 20.88%, respectively, compared to the non-maize rotation groups (LXJY and LXNY, Table 1). In the absence of *O. cumana*, the root length of sunflower in the maize rotation and D74 treatment group (NXJY) increased by 33.12%, 21.48%, and 29.25% ($p < 0.05$), compared to the other treatment groups (NXJN, NXNY, NXNN). The length of the main root and the number of fibrous roots and lateral roots increased significantly with the addition of D74 groups (NXJY and NXJN), compared to the control group (NXNY and NXNN). The roots, particularly in the NXJY group, were well-developed and formed a root network. This aids in the absorption of nutrients and moisture from the soil.

Based on the results of two years, the plant height, stem diameter, dry weight of above-ground and underground parts, and root length of sunflower added *O. cumana* seeds were significantly lower than those without *O. cumana* seeds ($p < 0.05$), indicating that *O. cumana* can cause a severe depletion of sunflower plant biomass. However, under the parasitic conditions of *O. cumana*, the combination of maize rotation and D74 has a significant effect on sunflower growth, which is higher than that of D74 or maize rotation alone.

3.3. Effect of Maize Rotation and S. rochei D74 Agents on O. cumana Parasitism

In the first year, sunflowers were planted to study the effect of D74 alone on their growth (Figure 3a). The emergence rate of *O. cumana* in the group with the addition of D74 (LXJY) was lower than that of LXNY and LXNN in the group without the addition of D74. However, using D74 alone reduced the *O. cumana* unearthed, as well as parasitism, but the effect was not statistically significant. The next year, sunflowers were planted to study the effects of D74 and maize rotation alone or in combination on its growth (Figure 3b). The number of *O. cumana* unearthed and parasites in the maize rotation group (LXNY) were significantly inhibited. Compared with the control group, LXNN was significantly reduced by 65.22% and 64.36% ($p < 0.05$). In comparison to LXJN, the group of maize rotation combined with D74 application (LXJY) had the best inhibition effect, with the 78.26% inhibition rate on the unearthed number of *O. cumana*, and the inhibition rate against the total number of parasites was 48.51%.

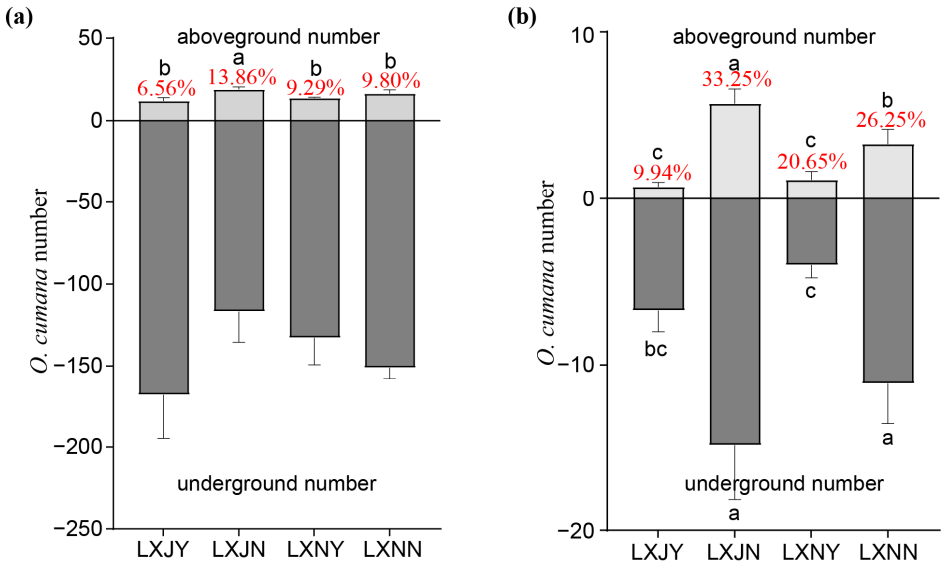

Figure 3. Effects of D74 agents and maize rotation on *O. cumana* parasitism in the first (**a**) and second (**b**) year. The red numbers represent the unearthed rate, different letters indicate significant differences between the groups (uncorrected Fisher's LSD, $p < 0.05$).

In conclusion, the unearthed number and total amount of parasitism of *O. cumana* in the different treatment groups at harvest in the second year were significantly lower than in the first year. This indicates that the continuous planting of crops for induction can effectively reduce the number of *O. cumana* seed banks in the soil. *O. cumana* parasitism can be reduced by either maize rotation alone or D74 application alone. When the two measures were used in combination, the inhibitory effect on *O. cumana* was the best ($p < 0.05$).

3.4. Effect of Maize Rotation and S. rochei D74 Agents on Rhizosphere Microbiota

3.4.1. Comparison between Culturable Microbial Dominant Strains in Sunflower Rhizosphere Soil

We obtained similar results of culturable bacterial species and relative abundance in sunflower rhizosphere soil from different treatment groups by using TSB and R2A media. The culturable bacteria we obtained were all distributed in three phyla: Actinobacteria, Firmicutes, and Proteobacteria. On TSB medium, 14 genera of culturable dominant bacteria were isolated. *Streptomyces*, *Paenibacillus*, *Microbacterium*, *Brevibacterium*, and *Ensifer* were the top 5 genera, accounting for 44.46%, 14.01%, 12.65%, 7.03%, and 4.35%, respectively (Figure 4a, Table S1 and Figure S2). On R2A medium, 21 genera of culturable dominant bacteria were isolated. *Streptomyces*, *Microbacterium*, *Ensifer*, *Cellulosimicrobium,* and *Brevibacterium* were the top 5 genera, accounting for 48.51%, 10.64%, 8.77%, 4.23%, and 3.26%, respectively (Figure 4b and Figure S3 and Table S2).

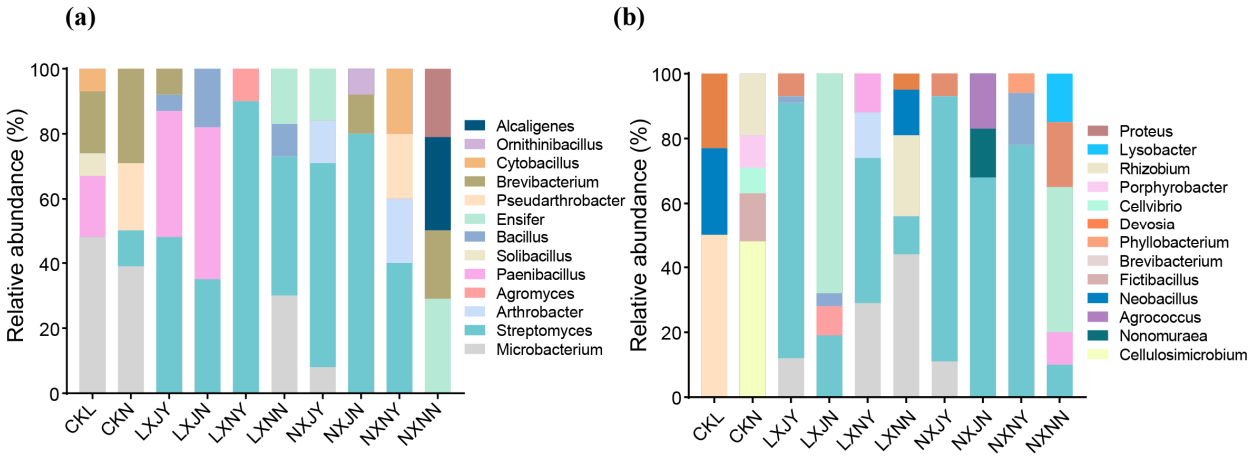

Figure 4. Relative abundance of bacterial genus levels in TSB (**a**) and R2A (**b**) media. CKL (blank control with *O. cumana* added), CKN (blank control without *O. cumana* added).

The number of culturable microorganisms in sunflower rhizosphere soil can be affected by the addition of D74 and maize rotation. When *O. cumana* present, the highest number of bacteria was isolated from the treatment group that was applied with D74 and maize rotation (LXJY) by TSB and R2A medium (TSB: 1.46×10^7 CFU/g; R2A: 2.05×10^7 CFU/g), while 1.28×10^7 CFU/g and 9.71×10^6 CFU/g were obtained from LXNY (*O. cumana* with maize rotation). Regardless of the presence or absence of *O. cumana*, the number of bacteria isolated after D74 application, maize rotation, or co-treatment with both was higher than the corresponding control group. *Streptomyces* numbers increased significantly in the sunflower rhizosphere soil in the D74 application group, possibly due to the D74 multiplied on the sunflower roots (Tables S1 and S2). In the unplanted control group (CKL), *Solibacillus* (TSB) and *Pseudomonas* (R2A) were isolated from the two media, respectively. In conclusion, different treatments have different effects on the types and numbers of culturable microorganisms in the sunflower rhizosphere soil. The isolation results in the R2A medium were similar to those in the TSB medium, and the results showed that the total number and proportion of *Streptomyces* were the largest in the culturable bacteria in the rhizosphere of sunflowers after D74 treatment.

3.4.2. Effects of *S. rochei* D74 Agents and Maize Rotation on Sunflower Rhizosphere Bacterial Community

The top five phyla of sunflower rhizosphere bacterial community in different treatment groups were Proteobacteria, Acidobacteria, Actinobacteria, Gemmatimonadetes, and Bacteroidetes, with relative abundance of 27.71%, 18.77%, 12.78%, 9.38%, and 8.12%, respectively (Figure 5a,b and Figure S4). Compared with the CKL group, the relative abundance of Bacteroidetes increased by 7.59%, 12.51%, 11.37%, and 7.77%; Actinobacteria increased by 6.48%, 6.32%, 5.98%, and 6.83%; Acidobacteria decreased by 3.84%, 2.69%, 4.69%, and 3.11%; and Gemmatimonadetes decreased by 3.13%, 5.66%, 3.49%, and 3.62%, respectively, in the treatment groups (LXJY, LXJN, LXNY, and LXNN groups) that added *O. cumana*. Compared with the CKN group, the relative abundance of Bacteroidetes increased by 6.54%, 13.82%, 5.6%, and 29.39%; Actinobacteria increased by 10%, 6.72%, 6.08%, and 2.77%; Acidobacteria decreased by 5.47%, 5.75%, 2.43%, and 5.81%; Gemmatimonadetes decreased by 1.57%, 5.45%, 0.85%, and 5.84% in the no *O. cumana* treatment group (NXJY, NXJN, NXNY, and NXNN), respectively. The results showed that, regardless of the presence or absence of *O. cumana*, compared with the control group (CKL and CKN), the maize rotation or the addition of D74 agent significantly increased the relative abundance of Bacteroidetes and Actinobacteria and decreased the relative abundance of Acidobacteria and Gemmatimonadetes.

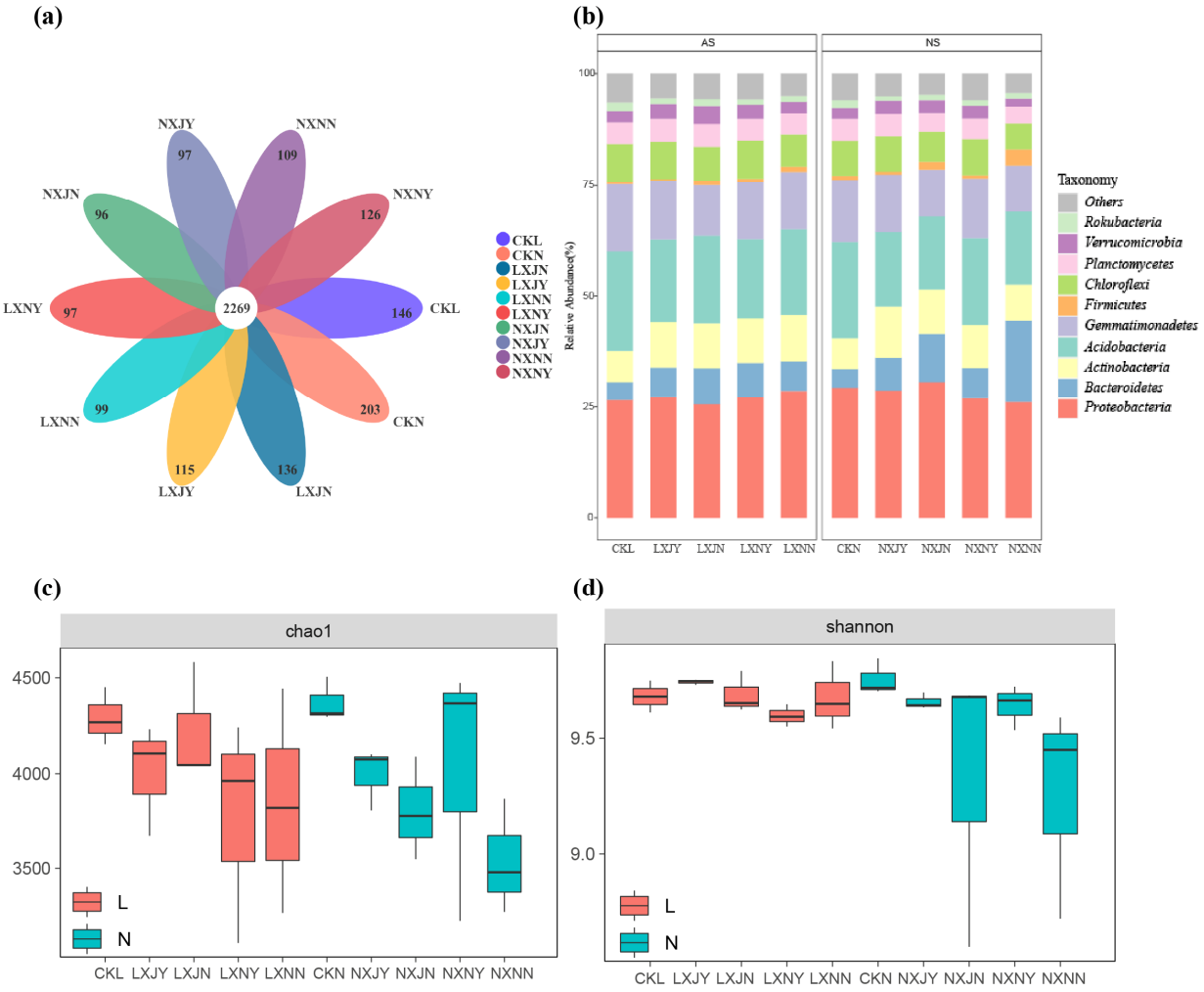

Figure 5. Effects of D74 agents and maize rotation on the α-diversity of sunflower rhizosphere bacterial community. Include OTUs petal map (**a**), relative abundance of Top 10 in phylum level (**b**), AS: added *O. cumana*, NS: unadded *O. cumana*), chao1 index (**c**), and shannon index (**d**).

For the α-diversity of the sunflower rhizosphere bacterial community, the chao1 index and shannon index of the no-plant control group (CKN) were the highest among all treatments, followed by CKL, while the α-diversity index of the sunflower-only group (NXNN) was the lowest (Figure 5c,d and Table S3). The difference of α-diversity index among the groups was small after adding *O. cumana* weeds to the soil, and the chao1 index and Shannon index of the D74 agents added groups (LXJY and LXJN) were higher than those of the corresponding control groups LXNY and LXNN. When no *O. cumana* was present in the soil, the chao1 index and Shannon index of the rotated maize groups (NXJY and NXNY) were higher than those of the non-rotated maize groups (NXJN and NXNN). The α-diversity of soil microbes in either the D74 alone (NXJN) or rotation maize alone (NXNY) groups were significantly higher than that in the control group (NXNN). This indicated that the addition of D74 or maize rotation increased the bacterial community diversity in the rhizosphere of sunflower, and the effect of rotated maize was greater than that of D74. The effect of *O. cumana* seeds on the α-diversity index of microorganisms was small. The results of PCoA for treatment groups (Figure 6a,b) showed that the composition of rhizosphere bacterial community was significantly different ($p < 0.05$), regardless of whether the *O. cumana* was added or not. Based on Bray–Curtis distance, the first two axes explained 20.00% and 26.30% of the total variance. In the *O. cumana*-free soil, the microbial communities were mainly separated along the PCoA1 axis, and the D74 agent and maize rotation had the greatest effect on the community difference, followed by the application of D74 agents alone, and the rotation of maize had the least effect. T-test analysis indicated that maize rotation significantly increased ($p < 0.05$) the relative abundances of *Sphingomonas*, *Altererythrobacter*, *Lechevalieria*, *Aeromicrobium*, and *Acidibacter* in bacterial communities. The D74-added group (LXJY) significantly increased the abundance of *Aquicella* and unidentified Alphaproteobacteria, compared to the LXNY group (Figure 6c,d).

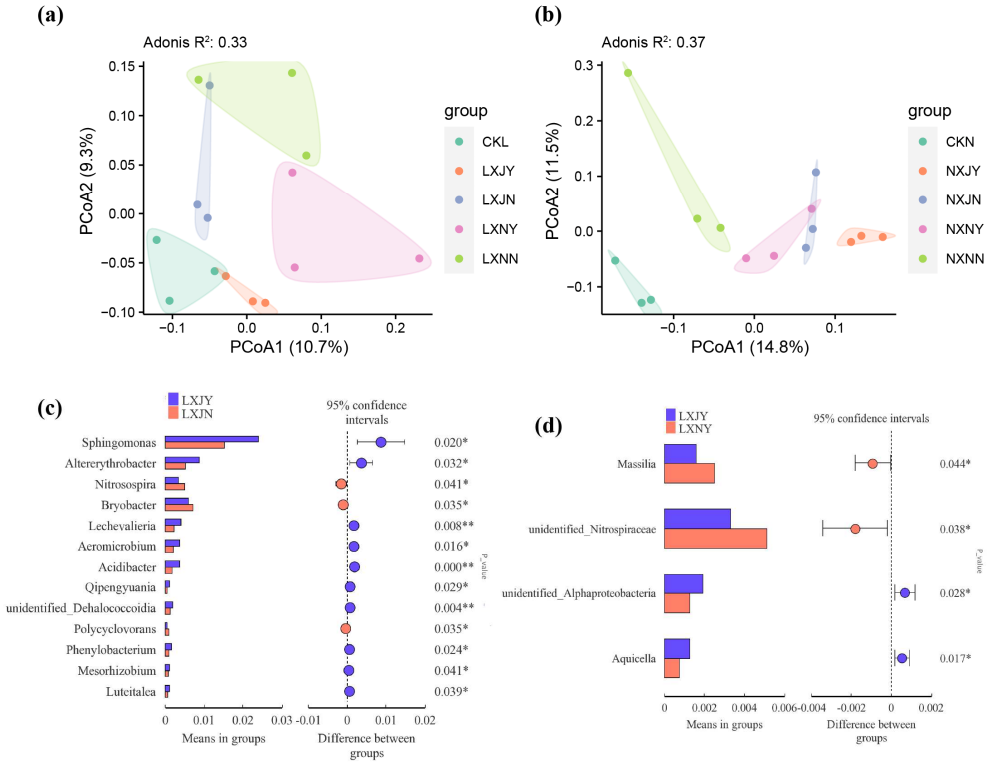

Figure 6. Effects of D74 agents and maize rotation on bacterial community structure. The PCoA based on Bray–Curtis distance with Adonis test ((**a**) added *O. cumana*, (**b**) unadded *O. cumana*, $p < 0.05$). Analysis of species with significant difference in genus level ((**c**) maize rotation, (**d**) added D74 agents, T-test, $p < 0.05$). Critical level of significance of constructs at * 5%, ** 1%.

4. Discussion

4.1. Effects of D74 Fermentation Filtrates on the Germination of O. cumana Seeds

The early growth stages of parasitic plants, such as seed germination, attachment host, and nodule development, are critical and ideal periods for controlling *O. cumana* parasitism. Therefore, the use of soil microorganisms to interfere with the development of parasitic weeds during the above period is an effective management strategy. Many fungi and bacteria can infect *Orobanche* and prevent its infestation to improve crop growth. In 2007, Zermane discovered natural soils that inhibited *Orobanche* parasitism [17]. Studies have shown that in the rhizosphere of plants, intensive and important interactions occur between parasitic plants, hosts, and microorganisms, such as biochemical reactions and the exchange of signaling molecules [30–32]. The use of microorganisms to control parasites is an environmentally friendly and easy to implement measure. Existing studies have shown that microorganisms not only reduce the number of parasites, but also significantly promote hosts growth. For example, *Fusarium oxysporum* f. sp. *orthoceras* (FOO) inhibited the germination of *O. cumana* and increased sunflower yield [33]. The use of *F. camptoceras* and *F. chlamydosporum* significantly reduced the biomass of *O. cumana* [34,35]. *Bacillus atrophaeus* inhibited *O. aegyptiaca* seeds germination and germ tubes growth, resulting in reduced parasitism number [36]. The *S. rochei* D74 used in this study not only has a good inhibitory effect on a variety of soil-borne diseases of crops, but also has a significant promotion effect on crop growth [20]. The D74 fermentation filtrates significantly inhibited the parasitism ($p < 0.05$), the effect was the best at high concentration, and the length of the seed germ tube was in the range of 0–1mm. In this case, the seeds show enlarged tops and darker colors, resulting in poor growth and reduced germination vigor.

4.2. Effects of Maize Rotation and S. rochei D74 Agents on Sunflower Growth and O. cumana Parasitism

The addition of D74 alone can promote the growth and development of sunflower roots, mainly in promoting the growth of main roots, increasing the number of lateral roots and fibrous roots, and providing more nutrients for the growth of the aerial parts. Additionally, the sunflower stem diameter, stem dry weight, and root dry weight increased after using D74 agents, which promoted the accumulation of sunflower dry matter. Both D74 inoculum or maize rotation could reduce the number of parasites, but the synergistic use of the two (LXJY) had the best inhibitory effect on *O. cumana*. Studies have shown that maize, as a trap crop, and its root exudates can induce *O. cumana* "suicide germination". However, after germination, *O. cumana* died quickly because there was no host to supply nutrients, thus reducing the number of *O. cumana* seed banks in the soil significantly [14,37]. At the same time, D74 inhibited the germination of *O. cumana*, and the parasitic number of *O. cumana* was significantly reduced in the second year. Additionally, after those two are used together, the sunflower biomass is increased due to the reduction of nutrient loss, which is of great significance for improving economic benefits.

4.3. Effects of Maize Rotation and S. rochei D74 Agents on Rhizosphere Microbial Community

The interaction between the root exudates and rhizosphere microorganisms is a very important process. Plant roots affect the species, quantity, and distribution of rhizosphere microorganisms through the secretion of various secondary metabolites and have a selective effect on the rhizosphere microbial community structure [38,39]. Research shows that changes in plant–microbe interactions mediated by root exudates are extremely important for soil fertility, health, and plant growth and development [40–42]. This study investigated the effects of D74 agents and maize rotation on the number and diversity of culturable and non-culturable bacterial communities in the rhizosphere of sunflower. Through the isolation and identification of rhizosphere microorganisms in two mediums, the bacteria isolated on both TSB and R2A media were distributed in three phyla, which were Actinobacteria, Firmicutes, and Proteobacteria. A total of 14 genera and 21 genera were isolated on TSB and R2A medium, respectively. The common genera were *Streptomyces*, *Microbacterium*,

Ensifer, and *Brevibacterium*. The addition of D74 agent significantly increased the number of *Streptomyces* and *Bacillus*. The *S. rochei* has been isolated from tomato, pepper, and other plant tissues. This strain suppressed the growth of the Chinese cabbage seedlings' pathogen *Pythium aphanidermatum* and *Sclerotium rolfsii* [43,44]. In summary, the addition of D74 agents increased the number of beneficial bacteria in the plant rhizosphere soil, thereby increasing soil fertility and promoting plant growth [45,46]. Both addition of D74 and maize rotation can reduce the total number of soil OTUs and the number of unique OTUs, compared with control soil. After analyzing the α-diversity of sunflower rhizosphere bacterial community, the results revealed that the richness and diversity of bacterial community in the addition of D74 increased, to a certain extent, compared with the control group. The bacterial community diversity in only the rotation maize treatment group decreased. In the absence of *O. cumana*, the D74 and maize rotation significantly increased the richness and diversity of bacterial communities, either alone or in combination. The results showed that the addition of D74 and the rotation of maize increased the types of soil microorganisms. The combined approach increased the abundance of Proteobacteria and Actinomycetes and decreased the abundance of Bacteroidetes, compared to the no-plant control (NXNN).

5. Conclusions

In order to reduce the parasitism of *O. cumana* in sunflower, we used a combination of *S. rochei* D74 and maize rotation for control. The combined prevention has proved to be quite effective. It reduced the parasitism of *O. cumana* well, with a reduction rate of 48.51%, and at the same time, could increase the accumulation of sunflower material and promote the growth of sunflowers. This method breaks the traditional idea of using a single method to control *O. cumana*. For practical purposes, we used microbial agents, instead of chemical control, which improves soil microbial diversity, has low environmental impact, adheres to the concept of green environment, and is conducive to the promotion of agriculture.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12123129/s1, Figure S1: Growth of sunflower with different treatments (mature stage); Figure S2: Effects of different treatments in TSB medium on sunflower rhizosphere soil-cultivated bacteria; Figure S3: Effects of different treatments in R2A medium on sunflower rhizosphere soil-cultivated bacteria; Figure S4: Comparison of differences in OTUs of sunflower rhizosphere bacterial communities with (A) and without (B) *O. cumana*; Table S1: Effects of different treatments in TSB medium on the number of dominant strains in sunflower rhizosphere soil ($\times 10^5$ CFU/g); Table S2: Effects of different treatments in R2A medium on the number of dominant strains in sunflower rhizosphere soil ($\times 10^5$ CFU/g); Table S3: α-diversity index analysis of sunflower rhizosphere bacterial community.

Author Contributions: Conceptualization, Y.M. and Y.L. (Yanbing Lin); methodology, J.X. and Q.X.; investigation, W.Q., Z.D. and Y.X.; data curation, J.X.; writing—original draft preparation, J.X. and W.Q.; writing—review and editing, Y.L. (Yanbing Lin) and Y.L. (Yongxin Liu); visualization, J.X. and T.X. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Raw sequence data of soil microbe is available in the National Center for Biotechnology Information (NCBI) database, with the accession number PRJNA898994.

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