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Degradation of sulfonamide antibiotics in the rhizosphere of two dominant plants in Huixian karst wetland, Guangxi, China

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ABSTRACT

In this work, *Phragmites australis* and *Vallisneria natans* were selected as the research objects and were cultured for 10 d under 0.10 μg L^{-1} sulfadiazine (SD) stress in a simulated surface flow wetland reactor. SD degradation was conducted at pH = 7 and 25 °C for 96 h. Each plant group conformed to the first-order kinetic model of degradation, and the degradation rate increased with time, reaching the maximum at 96 h. At 96 h, the degradation rate of *P. australis* communities was higher than that of *V. natans*. SD metabolites showed that the degradation pathways in the plant rhizosphere were mainly hydroxylation, aminolation, and S–N bond cleavage. In the analysis of rhizosphere bacterial community structure, the bacterial phyla that could degrade antibiotics accounted for a large proportion. Compared with before degradation, the dominant phylum and genus did not change after degradation (96 h), but their abundance changed to varying degrees, and new genera appeared in the *P. australis* group. This research provides a reference for the degradation of antibiotics in karst areas and new information on the mechanism of SA degradation in the plant rhizosphere.

Key words: bacterial community, degradation, karst wetland, rhizosphere secretion, sulfonamides antibiotics

HIGHLIGHTS

- Under sulfonamide (SD) stress, the contents of organic acid esters in rhizosphere exudates of *P. australis* and *V. natans* increased.
- New genera were produced in rhizosphere soil of *P. australis* group during SD degradation.
- Antibiotics in karst wetlands may exist in the form of complexes and be adsorbed in soil or sediments.
- After SD degradation, the diversity of bacteria decreased, but the total number of bacteria increased.

1. INTRODUCTION

Sulfonamide antibiotics (SAs) are synthetic antibacterial agents derived from sulfanilic acid. They are often added to veterinary feed or drugs in the form of additives to promote animal growth and disease prevention (Man *et al.* 2019). SAs are only partially metabolized in organisms, and 50–90% of the SAs in feces or urine will be discharged into the environment with their original structures intact, thus accumulating in various environmental media, such as surface water, groundwater, and soil (Zhang *et al.* 2014; Cui *et al.* 2020). Using plants to purify SA-polluted water has become a feasible technology (He *et al.* 2016). Phytoremediation is affected by rhizosphere microorganisms, rhizosphere soil contains a large number of SA-degrading bacteria that can use SAs as a designated carbon source for metabolic activities (Chen *et al.* 2016). Compared with oxidation processes, adsorption on activated carbon, and membrane filtration, phytoremediation has the advantages of high efficiency and low cost (Hu *et al.* 2019).

Karst aquifers are important sources of drinking water in many areas of the world. Because of the high hydraulic conductivity and short residence time of karst systems, antibiotics can be transported in the karst pipeline (Hillebrand *et al.* 2015), as a result, karst ecosystems are easily polluted by antibiotics. At present, antibiotics have been detected in the groundwater of karst areas in countries including Switzerland, USA, Germany, and France (Dodgen *et al.* 2017). The degradation of antibiotics in the environment is affected by environmental factors including temperature, pH, and ionic strength (Menció &

Mas-Pla 2019). The hydrochemical characteristics of karst areas are quite different from those of non-karst areas. The long-term corrosion of carbonate rocks easily forms the special environment of 'high calcium (Ca^{2+}), alkali, high dissolved inorganic carbon (HCO_3^-)' found in karst areas (Li *et al.* 2015; Zhang *et al.* 2018). Therefore, the degradation of antibiotics in karst areas may be different from that in non-karst areas. With frequent human production activities (domestic sewage, medical wastewater, aquaculture and livestock farming, etc.), antibiotics, as a trace pollutant in the environment, are continuously discharged into natural water bodies such as wetlands, altering the dynamic balance of antibiotics in the original environment. A study by Conkle *et al.* (2008) showed that at initial concentrations of 4.090 and 0.068 ug L^{-1} for sulfamethoxazole and sulfadiazine, respectively, the non-karst forest wetlands were less effective in removing the two sulfonamides and less effective in treating and diluting the antibiotics.

As the largest karst wetland in China, Huixian wetland plays important ecological roles in water conservation, climate regulation, water purification, biodiversity conservation, and flood storage in Li River. As part of its involvement in urban water recycling, the Huixian wetland is to some extent able to convert engineered reclaimed water into ecologically reclaimed water, a process that is particularly important in controlling the conversion of new pollutants such as antibiotics. It was reported that SAS pollution was detected in surface water, shallow groundwater, water near farms, and pond water of Huixian wetland (Qin *et al.* 2019). Therefore, the management of SAs in Huixian wetland has gradually attracted the attention of Chinese and international scholars. There are many types of vegetation in Huixian wetland. The plant coverage can reach 80–95%, and the dominant plants are *Cladium chinense Nees*, *Typha orientalis Presl*, *Vallisneria natans*, *Phragmites australis*, and *Canna indica* (Tu *et al.* 2019). *P. australis* is an emergent plant with strong resistance, fast growth, high yield, and strong adsorption, and is the preferred plant for purifying water quality (Lambertini *et al.* 2020; Wang *et al.* 2021a). *V. natans* is a perennial submerged plant that can effectively remove nitrogen, phosphorus, and heavy metals in polluted water (Yan *et al.* 2011). The investigation of dominant plant species can be used to assess biodiversity changes and ecosystem functions in wetland systems. It has been reported that the removal rate of SAs in plant systems was significantly higher than that in non-plant systems, and it was found that the degradation of SAs by plants was related to plant accumulation and stress resistance ability, and the degradation of antibiotics in plants was mainly related to rhizosphere microorganisms (Chen *et al.* 2016, 2021). It has also been reported that root exudates can play a very significant role in promoting microbial degradation of antibiotics (Zhi *et al.* 2019), and different kinds of substances in root secretions will increase rhizosphere microbial biomass and activity, and then affect how effectively wetland systems purify pollutants (Du *et al.* 2020).

However, few studies have revealed the degradation efficiency and mechanism of SAs in the rhizosphere of wetland plants in karst areas, and little attention has been paid to changes in the rhizosphere microbial community structure before and after antibiotic degradation. Therefore, this work selected Huixian wetland dominant plants (*P. australis* and *V. natans*) as the research object. In the simulated surface flow wetland reactor, the root exudates were extracted after 10 d of incubation under sulfadiazine (SD) stress. The root exudates were mixed with the rhizosphere soil to explore the degradation mechanism of SD by root exudates, and the composition and changes of bacterial communities in the rhizosphere soil before and after degradation were analyzed by high-throughput sequencing technology to reveal the mechanism of SD degradation in the rhizosphere so as to provide a theoretical basis for antibiotic treatment in Huixian wetland.

2. METHODS

2.1. Sample collection and pretreatment

The two dominant wetland plants (*P. australis* and *V. natans*) used in the experiment were all obtained from the Qixing Wharf Basin of Huixian wetland (25°01′30″–25°11′15″N, 110°08′15″–110°18′00″E) (Figure 1). The sampling time was March 2020, and plants with high density and vigorous growth were selected in an effort to ensure the selection of plants with similar growth characteristics, such as the plant height, number of roots, and leaves. After the collection of plant samples, to ensure that the root system was not damaged, tap water was first used to gently rinse the roots, and the soil, residual leaves, snail shells, and gravel were rinsed away. After washing, the roots and stems were soaked in ultrapure water for 5 min to reduce other pollutants. After soaking, the roots and stems were washed with ultrapure water more than three times. After air-drying, the rhizosphere soil is ground down to 10 and 60 mesh, one is used for degradation experiment (10 mesh), and the other is used to detect the initial physical and chemical properties (60 mesh).

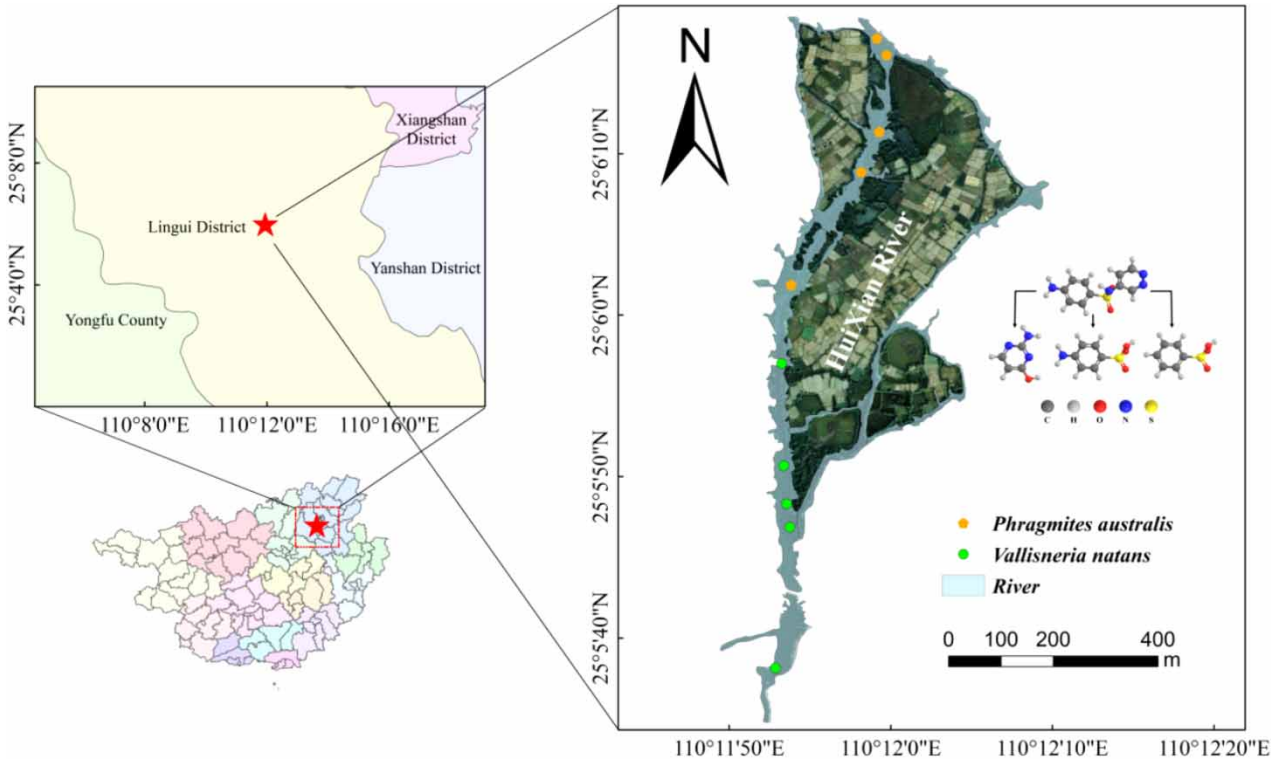

Figure 1 | Information of sampling sites and molecular structure of the sulfadiazine degradation pathway.

2.2. Design of the experiment

The total length of Qixing Quay in Huixian karst wetland is 1,600 m, and there are large areas of *Eichhornia crassipes* growing in the latter half of the water area of about 300–400 m. The river width is 5.2–51.4 m and mainly ranges from 20 to 30 m, and the water depth is about 0.5–3.0 m. To simulate the actual situation of Qixing Quay, the Huixian karst wetland simulation system was designed (Figure 2). The whole system was outdoors with rain shelter measures. The overall length and width of the system is approximately 100 times smaller than that of the Huixian wetland simulation system. The wetland device specifications were 3.10 m × 1.2 m × 0.4 m, the total length was 12 m, the width of each corridor was 0.3 m, the height was 0.4 m, and the width of the outlet was 0.1 m. The water was directly fed by a peristaltic pump with a hydraulic load of 0.05 $m^3 \cdot (m^2\ d)^{-1}$ and a water depth of 0.3 m. Different plant groups were separated by baffles. The bottom of the plant group was equipped with a cylindrical fixed device, and many holes conducive to the passage of water were welded at the bottom. The device was used to carry plants for hydroponics. There were eight channels in the river channel of the simulation system, and a partition was set between the blank group and the experimental group. Ten plants per species were hydroponically supplied with Hoagland nutrient solution (Supplementary Table S1) and ultrapure water, and tin foil was used to shield the root area from light. The water inflow mode of the system was continuous water inflow, and the hydraulic retention time was 6 d. The plants in the system underwent an adaptation period of 1 month. If the plant dies during the 1-month adaptation period, then a plant of the same level of growth must be selected for reseeding. Stress culture was conducted after the plants had adapted to the growth conditions.

In the stress culture, according to the water consumption of the simulation system, referring to the results of Xia *et al.*'s (Xia *et al.* 2021) study and combining the detection limits of the actual instrument, SD was added only to the experimental group so that the aqueous solution contained 0.10 μg L^{-1} SD, which was used as a stressor. After 10 d of cultivation, the plants in each group were taken out, and the root exudates of plants were extracted and analyzed by the root soaking method (Zhalnina *et al.* 2018).

The root exudates used were the original aqueous solutions collected from the two wetland plants after a 10-d cultivation under SD stress with a concentration of 0.10 μg L^{-1}. The collected original aqueous solution was filtered with 0.45 μm filter membranes to prepare a culture medium containing 0.10 μg L^{-1} SD. The rhizosphere soil of each plant was collected by the

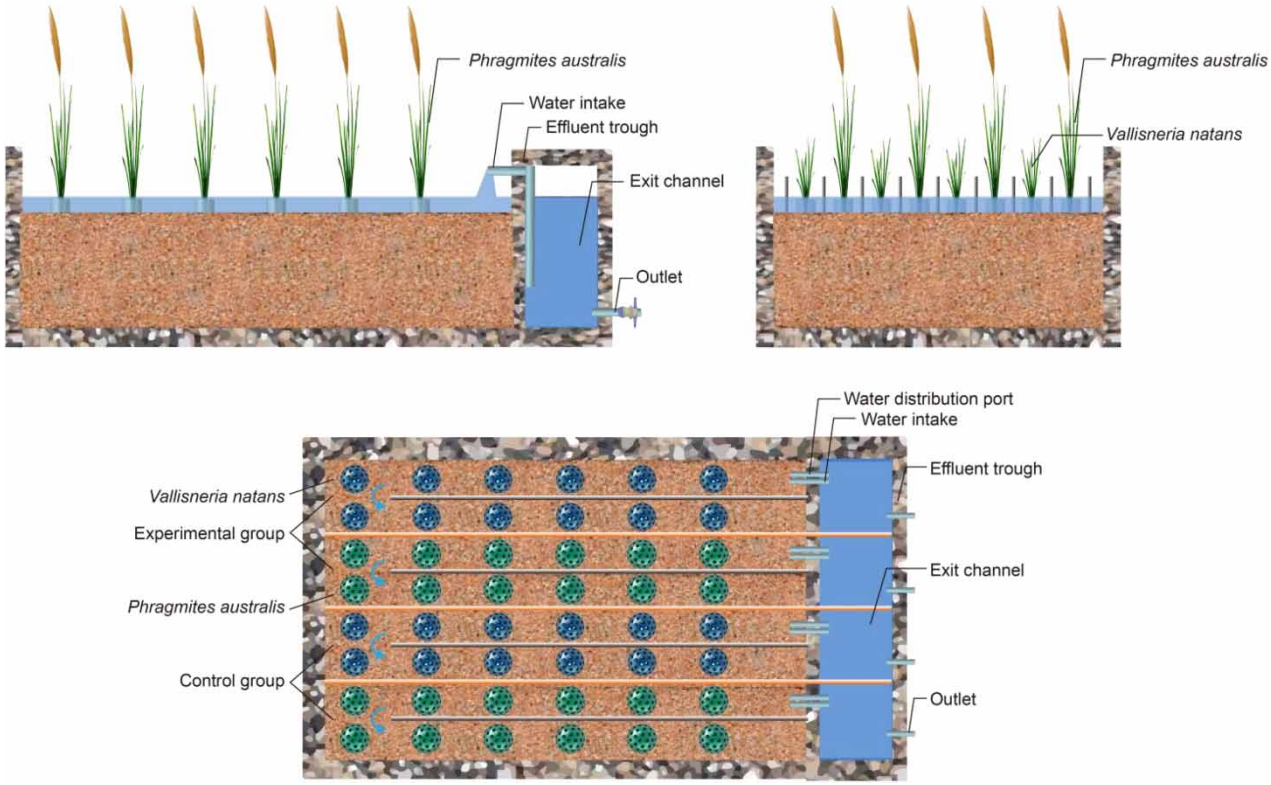

Figure 2 | Simulation of surface flow wetland reactors.

root shaking method (Tu *et al.* 2019). One hundred grams of rhizosphere soil collected from each plant was weighed and placed in a conical flask, and 300 mL of the above antibiotic-containing culture medium was added. The soil and root exudates were fully mixed. After determining that the rhizosphere soil and root exudates of plants corresponded, they were recorded as *P. australis* experimental group (P), *P. australis* control group (CP), *V. natans* experimental group (V), and *V. natans* control group (CV). In the SD degradation experiment, the oscillation time was set at $t = 0/2/8/16/30/48/96$ h as seven time points, and the concentration of SD in the aqueous solution was measured at each time point to obtain the SD degradation rate in different time periods. In the degradation experiment, *P. australis* and *V. natans* were termed as PD and VD, respectively. The rhizosphere soil of 0 and 96 h samples collected to determine microbial community structure, and the aqueous solutions of 0 and 96 h samples were used to detect SD degradation products. The 0 h *P. australis* and *V. natans* groups were labeled as P_0 and V_0, respectively, and the 96 h *P. australis* and *V. natans* groups were P_{96} and V_{96}, respectively.

2.3. Determination of root exudate components

The solution collected by the root soaking method was analyzed by gas chromatography–mass spectrometry (GC-MS) after treatment. The root washing solution was extracted with 300 mL CH_2Cl_2 three times and the CH_2Cl_2 extraction solution was concentrated to dry by rotary evaporation at 35 °C. Five milliliters of CH_2Cl_2 filtered by a 0.45-μm membrane was then added, the solution was dried with anhydrous Na_2SO_4, and finally, 0.5 mL of treated CH_2Cl_2 was taken for GC-MS analysis. The instrument used for determination was a GC-MS-type gas chromatography–mass spectrometer (Claus 600T Mass Spectrometer and Claus 680 Gas Chromatograph), Elite-5MS (30 m × 0.25 mm × 0.25 μm) chromatographic column.

2.4. Determination of SD content and degradation products

SD was detected by solid phase extraction-high-performance liquid chromatography. The instruments used included the following: a Japan AQUAT race ASPE799 solid phase extraction instrument, Agilent 1260 high-performance liquid chromatography, a 24-bit nitrogen blowing instrument, an HLB extraction column, and an ultrasonic cleaner.

The detection samples of SD degradation products were tested Guangxi Guilin RID Testing Co., Ltd. A triple quadrupole liquid chromatography–mass spectrometry (HPLC-MS) system was used with a chromatographic column: an Eclipse Plus C18 RRHD 2.1 × 50 mm, 1.8 μm. Sample treatment was conducted as follows: the sample solution was moved to a centrifuge tube, centrifuged at 4,000 r min^{-1} for 5 min, and the supernatant was collected for filtration. One hundred milliliters of the sample solution were taken in a conical flask with a measuring tube. The sample solution was concentrated to 100 times by solid phase extraction and detected by HPLC-MS/MS.

2.5. Bacterial DNA extraction and PCR amplification from rhizosphere soil

The E. Z. N. ATM Mag-Bind Soil DNA Kit (Omega Bio-Tek Company, Guangzhou International Business Incubator) was used for DNA extraction, and then the Qubit 3.0 DNA detection kit was used for the accurate quantification of genomic DNA to determine the amount of DNA added to the PCR reaction. The PCR primers were fused with 16S V3–V4 primers on the sequencing platform. The PCR reaction conditions were 94 °C, 3 min → (94 °C, 30 s → 45 °C, 20 s → 65 °C, 30 s) 5 → (94 °C, 20 s → 55 °C, 20 s → 72 °C, 30 s) 20 →72 °C, 5 min → 10 °C. DNA extraction, PCR amplification and purification were performed by the Sangon Biotech Co., Ltd (Shanghai).

2.6. Statistical analysis

UPARSE software was used for operational taxonomic unit (OTU) sequence clustering of valid sample data. The Mothur method and SILVA's SSU rRNA database were used to perform species annotation analysis on the representative OTU sequences and obtain the community composition of the sample at each taxonomic level. The alpha diversity index was calculated using Qiime 1.9.1. A species abundance column diagram, degradation rate analysis diagram, degradation kinetics diagram, and dilution curve were analyzed and drawn by Origin 2018. The *p*-value was obtained using Welch's *t*-test. Abundance differences were plotted using STAMP software. Data analysis of the results was performed in SPSS 25.0.

3. RESULTS AND DISCUSSION

3.1. Composition of root exudates

Each measured peak (Supplementary Figure S1(a)–S1(d)) was searched in the atlas library to obtain the corresponding compounds (Supplementary Table S2). A total of 37 compounds were detected in the CP group, including alkanes, aldehydes, esters, alcohols, and amides. A total of 31 compounds were detected in group P, including alkanes and esters. A total of 35 compounds were detected in the CV group, including alkanes, aldehydes, esters, amides, and alcohols. A total of 34 compounds were detected in root exudates of group V, including alkanes and esters.

From the perspective of the most abundant component (Table 1), the most abundant component of the CP group was heptadecane, 2,6,10,15-tetramethyl, with a content of 8.46%, but that of the P group was 9-octadecenoic acid (*Z*)-, methyl ester, with a content of 31.88%. In the CV group, the content of 9-octadecenamide, (*Z*)- was 9.6%, and in the V group, the content of 9-octadecenoic acid (*Z*)-, methyl ester was 22.31%. According to the above results, the most abundant components of the two plant groups changed to esters under SD stress, and the content increased. Under material stress, the secretion of some compounds in plant root exudates was inhibited or changed (Rolfe *et al.* 2019). In this study, dichloromethane was used for extraction, and the obtained experimental results covered relatively complete root exudates.

Studies used different nitrogen and phosphorus concentrations to culture plants, and they concluded that the amount of compounds in the root exudates of the low nutrient treatment was higher than that of the high nutrient treatment (Wu *et al.* 2012). However, the detection of compounds in this study was higher than that in the above studies. Some scholars have found that the root exudates of rape seedlings mainly consist of compounds including hydrocarbons, alcohols, esters,

Table 1 | Changes in the most abundant components in rhizosphere secretions before and after degradation

Plant group	Substance name	Category	Content (%)
CP	Heptadecane, 2,6,10,15-tetramethyl	Alkanes	8.46
P	9-Octadecenoic acid (*Z*)-, methyl ester	Esters	31.88
CV	9-Octadecenamide (*Z*)-	Amides	9.6
V	9-Octadecenoic acid (*Z*)-, methyl ester	Esters	22.31

and acids (Escolà Casas & Matamoros 2021). This finding is similar to the results in this study, but the specific characteristics of compounds are not consistent. After SD stress at a concentration of 0.10 μg L^{-1}, the species of compounds in the rhizosphere exudates of the two plants decreased, which may have been due to the fact that wetland plants would actively adapt to the environment by self-regulating the composition and quantity of secretions by roots under external stress (Duan *et al.* 2020).

3.2. Degradation analysis of SD

As shown in Figure 3, the degradation efficiency of antibiotics in all of the plant groups increased with time, and was the highest at 96 h and the lowest at 2 h, the degradation efficiency of VD was 15.53% in 2 h, and the degradation efficiency of PD reached 12.40% in 2 h. From 2 to 30 h, the degradation efficiency of VD was always higher than that of PD. From 30 to 96 h, the degradation rate of PD gradually became higher than that of VD, the degradation rate of VD was 1.15, 0.98, and 0.57 μg L^{-1} h^{-1} at 30, 48, and 96 h, and the degradation rate of PD reached 0.74, 0.65, and 0.55 μg L^{-1} h^{-1} at the same time. At 96 h, the degradation efficiency of the *P. australis* group was 97.20% (Supplementary Table S4), and that of the *V. natans* group was 85.60% (Supplementary Table S4).

The experimental results showed that the removal rate of antibiotics with these species was higher than that of other constructed wetlands or non-karst wetland plants (Yan *et al.* 2019). Owing to the long-term erosion of carbonate rocks, the Huixian wetland ecosystem contains a large amount of Ca^{2+} (Li *et al.* 2017), and the form of calcium that occurs in the soil used in this study is mainly exchangeable, with an ECa/TCa value of 52.82–69.48% (Supplementary Table S3). The high content of exchangeable calcium indicates that calcium in the soil is active in migration and bioavailability, and Ca^{2+} can form a complex with antibiotics and then be adsorbed by the soil matrix (Liang *et al.* 2018). However, SAs are a typical amphoteric compound. When the soil is alkaline, antibiotics are difficult to adsorb, and when the pH is close to neutral, the removal effect of antibiotics is the best (Kurade *et al.* 2019). Huixian wetland soil is rich in calcium and alkaline, and its water quality is mostly weak alkaline. In this study, the rhizosphere soil pH was 7.16–7.22 (Supplementary Table S3), which indicated that SD was difficult to adsorb by the soil matrix in the karst wetland, and probably existed in the form of complexes. In addition, the removal mechanism of SD in Huixian wetland may have been mainly dependent on the interaction between plants and rhizosphere microorganisms.

The data were fitted by the first-order equation and the second-order equation of degradation kinetics (Figure 4(a) and 4(b)). The residual concentration of SD decreased gradually with time, indicating that the degradation effect increased with time, which was consistent with the expression of the degradation rate. Figure 4(a) and 4(b) and Supplementary Table S4 also show that the remaining concentration gradually slows down after 48 h and tends to flatten at 96 h. In wetland environments, the plant rhizosphere is often flooded. The soil carbon mineralization rate under anaerobic conditions is much lower than that

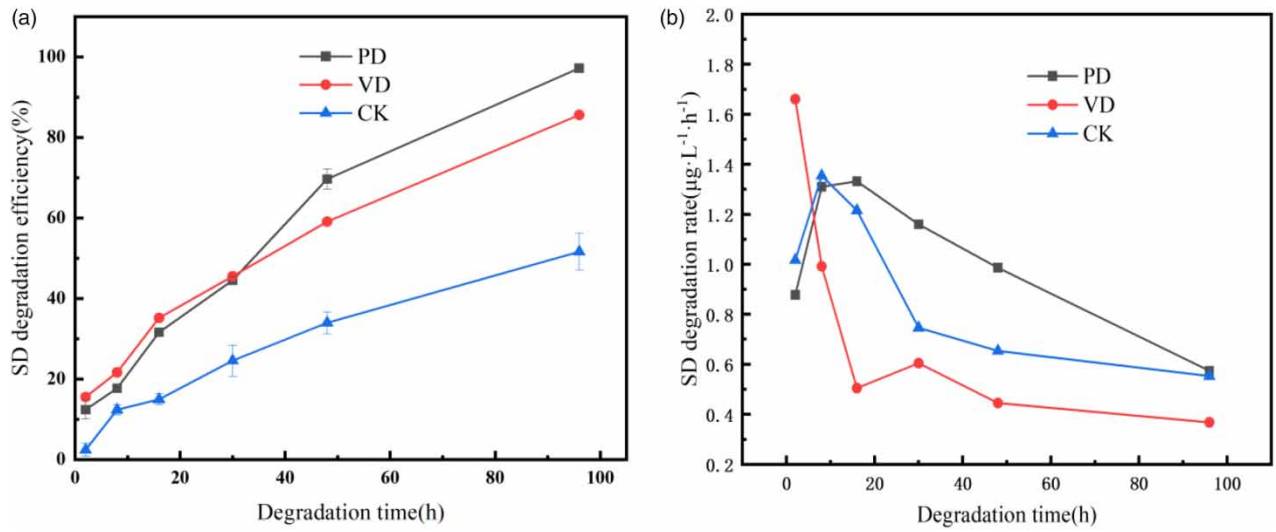

Figure 3 | Variation of sulfadiazine (SD) degradation efficiency (a) and degradation rate with time (b).

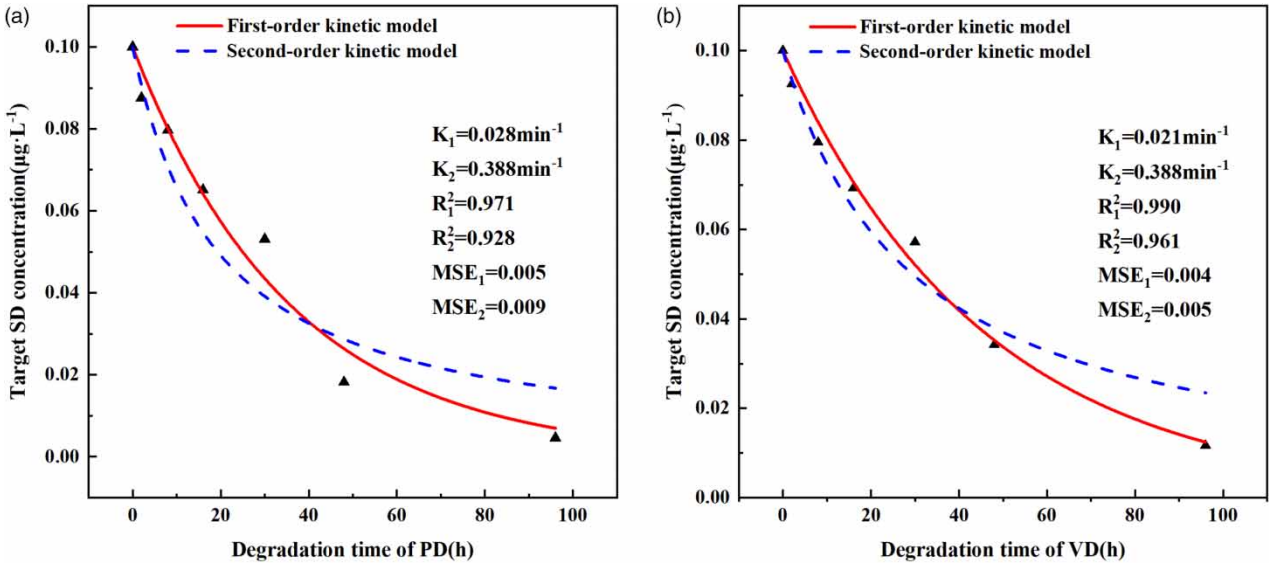

Figure 4 | SD degradation kinetics of *P. australis* (PD) (a) and *V. natans* (VD) (b); K_1 and K_2 are the first- and second-order kinetic degradation rate constants, respectively, R_1^2 and R_2^2 are the first- and second-order kinetic fitting coefficients, respectively, and MSE_1 and MSE_2 are the mean square deviation of the target concentration expressed by the first- and second-order kinetics, respectively.

under aerobic conditions, resulting in high organic carbon content in wetlands (Dos Santos Teixeira *et al.* 2021). Supplementary Table S3 also shows that the wetlands had abundant carbon sources (21.86–22.28 g kg^{-1}). SD can be used as a microbial carbon source, but a rich carbon source in soil will reduce microbial demand for SD. With the decline of a biodegradable carbon source in the environment, microorganisms will use SD as a carbon source, and the biodegradation of SD will be enhanced. However, with the continuous consumption of SD, microbial activity will gradually decrease.

The R_1^2 values of PD and VD were higher than R_2^2 and MSE_1 was lower than MSE_2, indicating that the fitting degree and correlation of the first-order equation of degradation kinetics were higher than those of the second-order equation, and the first-order kinetic model was more reasonable to express the degradation of SD in this study. This result was consistent with the results of research on the photodegradation, Fenton-oxidation degradation, and chlorination degradation of antibiotics (Dirany *et al.* 2010). Different plants had different pollutant removal capacities, and the selection of plant species was a key part of pollutant removal (Rezania *et al.* 2015). Therefore, the cultivation of *P. australis* was of great significance for the prevention and control of SD in Huixian wetland.

3.3. Analysis of SD degradation products

Microorganisms can degrade the phenyl portion of SD molecules, but the pyrimidine ring of SD is stable and produces an equal molar amount of 2-aminopyrimidine. When 2-aminopyrimidine is formed, it will be transformed into 4-hydroxy-2-aminopyrimidine by bacterial metabolism (Deng *et al.* 2016). The intermediates of SD also include sulfonamide aniline acid, p-aminobenzenesulfonic acid, 2-hydroxypyrimidine, aniline, and pyrimidine-2-sulfonamide acid. The degradation pathways of the intermediates may be one of three parallel pathways: first, the S–N bond, N–C bond, or C–S bond is broken, and then hydroxylation, formylation, and acetylation occur (Mohring *et al.* 2009). In this experiment, the two plant groups obtained the same three metabolites (Supplementary Figure S2(a) and S2(b)): *m/z* 171.2 (p-aminobenzenesulfonic acid), *m/z* 114.1 (4-hydroxy-2-aminopyridine), and *m/z* 224.2 (phenyl sulfoxide).

Therefore, we can deduce the possible degradation pathway of SD (Figure 5). I indicates that the product p-aminobenzenesulfonic acid was obtained by hydrolysis of the S–N bond and hydroxylation; II indicates that the product 4-hydroxy-2-aminopyridine was obtained by hydrolysis of the S–N bond, deamination of the C atom on the connecting chain, and then amination and hydroxylation; III shows the hydrolysis, hydroxylation, and deamination of the S–N bond to obtain phenyl sulfoxide. The results are consistent with previous studies; that is, SAs can be degraded by chlorine substitution, S–N bond cleavage, S–C bond cleavage, hydroxylation/oxidation, and desulfonation, and they can generate a series of degradation products (Wang & Helbling 2016).

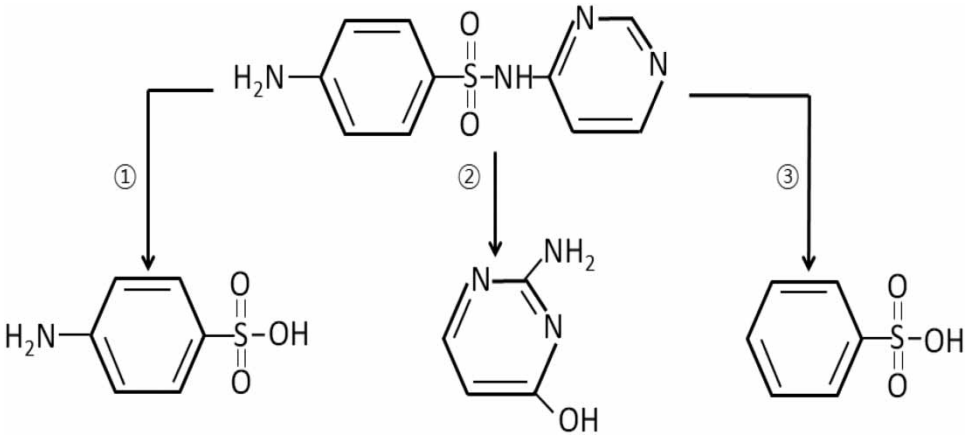

Figure 5 | Possible degradation pathways of sulfadiazine (SD).

3.4. Effect of SD degradation on rhizosphere microorganism phyla and genus abundance

3.4.1. The relative division of microorganisms at the phylum level

In this research, 37 phyla, 87 classes, 137 orders, 238 families, and 448 genera were detected, indicating that the bacteria were widely distributed, and the bacterial community had high diversity. Thirteen categories with the highest abundances were selected to generate histograms (Figure 6), including *Proteobacteria* (34.49–43.21%), *Chloroflexi* (15.75–26.11%), *Acidobacteria* (8.92–11.02%), *Planctomycetes* (3.58–6.77%), *Firmicutes* (1.79–2.31%), *Actinobacteria* (2.01–2.85%), *Verrucomicrobia* (1.11–3.12%), *Bacteroidetes* (1.41–2.41%), *Aminicenante* (0.46–1.06%), *Nitrospirae* (0.19–0.66%), and *Cyanobacteria-Chloroplast* (0.14–0.54%) (Supplementary Table S5), which were similar to those reported in other rhizosphere bacterial communities (Hua *et al.* 2018; Tian *et al.* 2020; Yang *et al.* 2021). Tu *et al.* (2019) also investigated the distribution characteristics of rhizosphere bacterial communities in Huixian wetland, but the bacterial abundance in this study was lower than

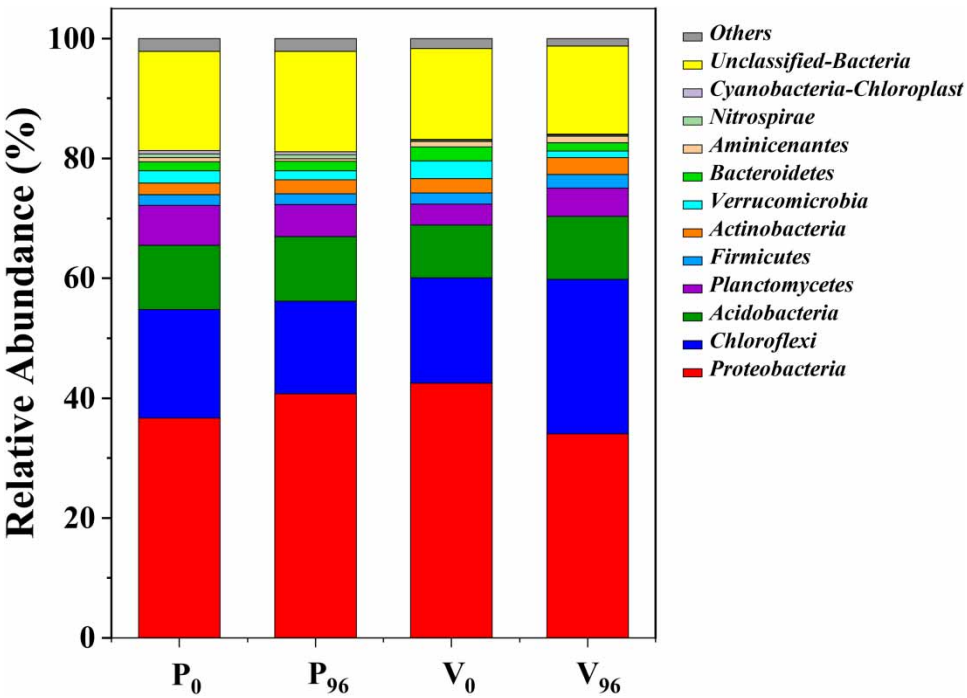

Figure 6 | Comparison of relative abundance at the phylum level.

that in their research, indicating that the presence of SD hindered the growth of certain bacterial communities. Bacteria that degrade antibiotics are mainly composed of *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Actinobacteria*, *Verrucomicrobia*, and *Phytoplankton* (Wu *et al.* 2019). The relative abundances of *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, and *Bacteroidetes* in rhizosphere bacterial communities in this study were high, which was one of the reasons for the high degradation rate in the two plant groups. The dominant phyla did not change before and after degradation, but their relative abundances changed to varying degrees.

3.4.2. The relative division of microorganisms at the phylum level

The first 34 genera with the highest abundances were selected to generate a relative abundance histogram (Figure 7). All of the samples were composed of 34 genera, of which 20 were identified, and the unclassified genera accounted for 42.98–52.17% (Supplementary Table S6). Before and after degradation, the abundance of dominant bacteria in different plant groups changed to varying degrees. It is worth noting that in the *P. australis* group, new genera appeared after 96 h of SD degradation, which was *Methylophilus*, and they belonged to *Methylophilaceae* at the upper level. *Methylophilaceae*, as a kind of *Proteobacteria*, can increase with the concentration of antibiotics in soil-lettuce systems (Shen *et al.* 2021). This is due to *Methylophilaceae*'s potential to degrade exogenous chemicals (Shen *et al.* 2019).

Root exudates can be transported by cells and excreted around the rhizosphere, creating a unique environment for root microorganisms (Hu *et al.* 2018), thus affecting the degradation of SAs. This work found that after SD stress culture, the highest component of root exudates was changed to organic acid esters. In the presence of acid or alkali, organic acid esters can be hydrolyzed to organic acids or alcohols. Many studies have shown that plant roots secrete organic acids, amino acids, sugars, and other substances that contribute to microbial growth (Li *et al.* 2019). Biodegradation has been proved to be the main pathway for SA degradation in plants (Chen & Xie 2018). The contribution of plant roots and pollutants to microbial growth is different. Plant root exudates can increase the activity of microorganisms, and some microorganisms can promote the activity of plants, helping them resist pollutant stress (Cristaldi *et al.* 2017). Therefore, the calcium-rich and alkali-rich characteristics of karst wetland soil will lead to the hydrolysis of some organic acid esters in root exudates

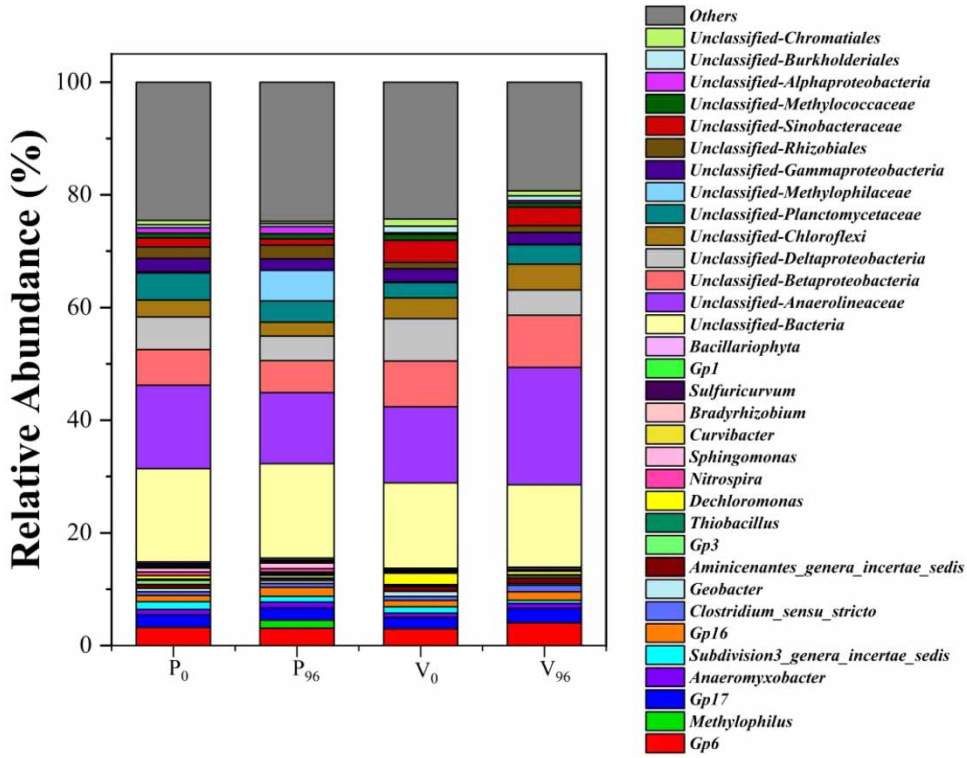

Figure 7 | Comparison of relative abundance at the genus level.

to produce organic acids, which indirectly affects the rhizosphere microbial metabolism, and thus regulates the degradation of antibiotics.

3.5. Difference analysis of bacterial community abundance before and after decomposition

Figure 8 shows the proportion of microbial abundance differences before and after decomposition in the 95% confidence interval. The *p*-value is shown on the rightmost side. This graph only lists the 25 lowest *p*-values. At the phylum level, the abundance of *Synergistetes* increased significantly after degradation.

At the genus level (Figure 9), there were 25 genera with significant differences before and after degradation ($p < 0.05$), among which 6 genera were extremely significant ($p < 0.01$). These genera were *Piscinibacter*, *Unclassified-Xanthomonadaceae*, *Methylotenera*, *Streptococcus*, *Unclassified-Methylophilaceae*, and *Nitrosomonas*. They were all significantly increased compared with those before degradation, indicating that SD degradation played a catalytic role in them.

Root exudates have been shown to affect the composition of rhizosphere microbial communities. For example, salicylic acid can induce systemic resistance in plants and inhibit the growth of pathogens (Badri *et al.* 2013), benzoic acid in

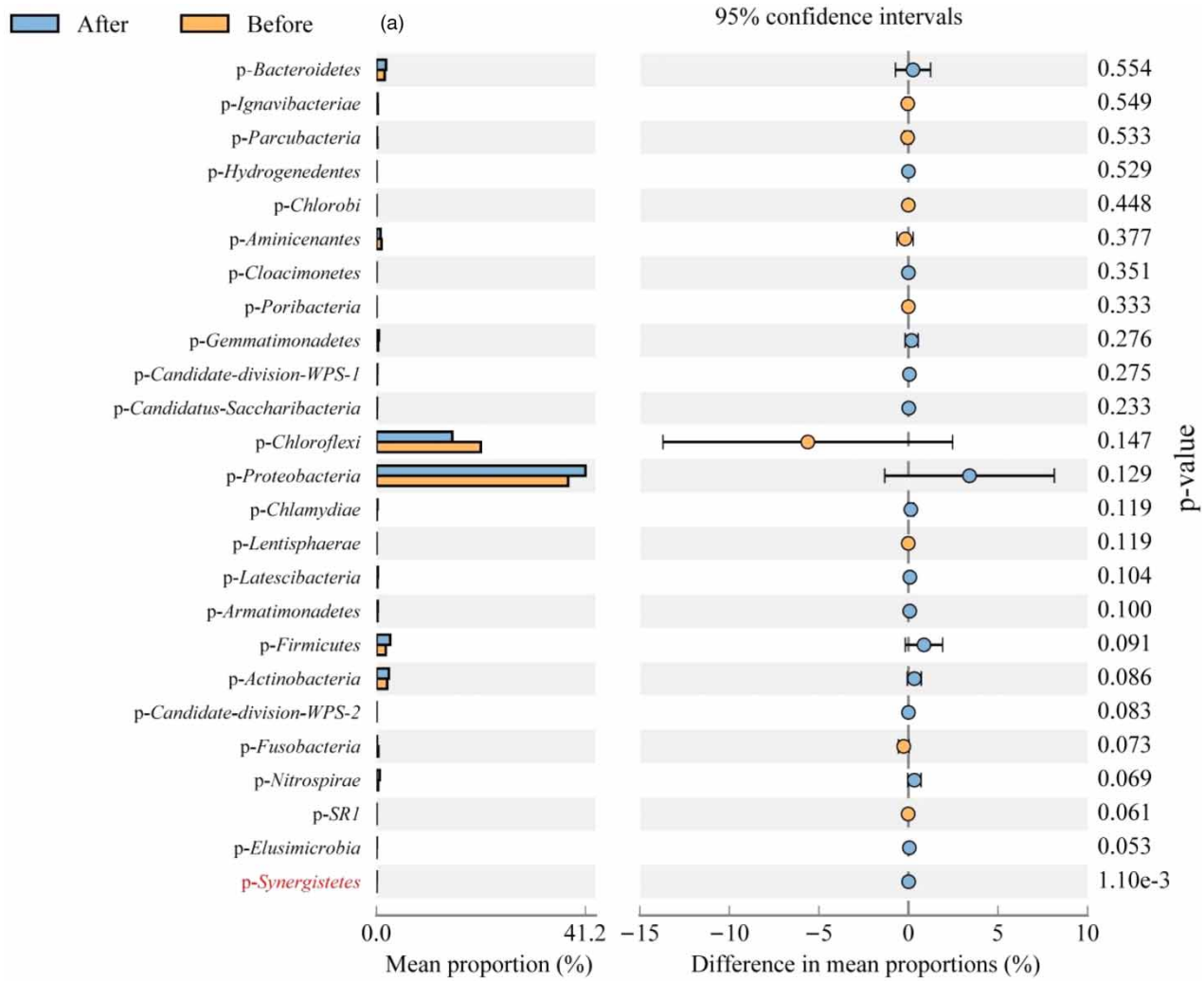

Figure 8 | Analysis chart of abundance differences among plant groups at the phylum level (a); 'Before' indicates the relative abundance of rhizosphere microorganisms when the plant group had degraded SD for 0 h, while 'After' indicates the relative abundance of rhizosphere microorganisms when the plant group had degraded SD for 96 h. Red text represents the species with significant or extremely significant differences before and after degradation (the same below). Please refer to the online version of this paper to see this figure in colour: https://dx.doi.org/10.2166/wrd.2023.062.

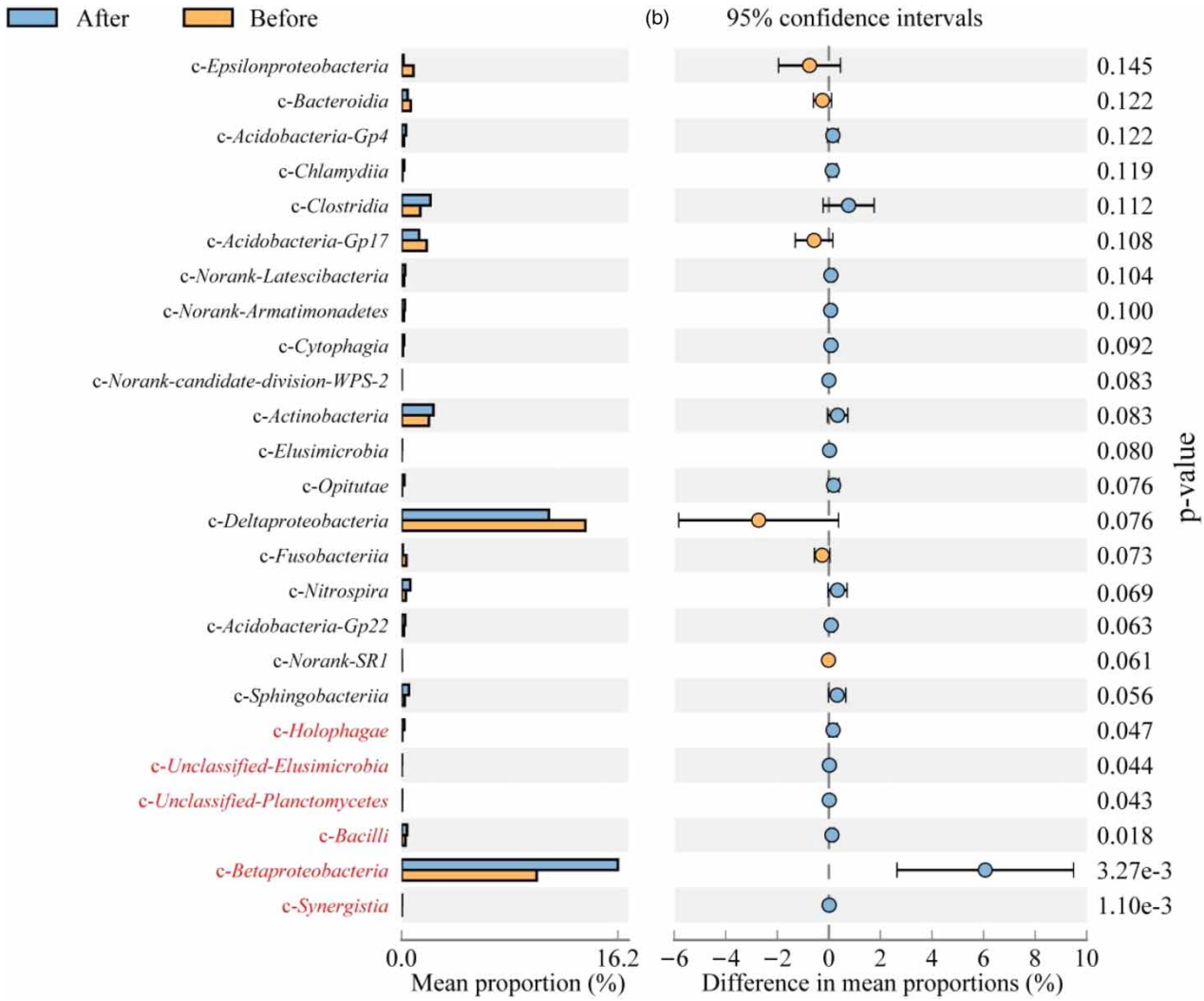

Figure 9 | Analysis chart of abundance differences among plant groups at the genus level (b).

peanut root exudates increases the relative abundance of *Burkholderiaspp* in rhizosphere soil (Liu *et al.* 2017), and ferulic acid in watermelon root exudates can promote the formation and germination of *Fusarium oxysporum* spores (Hao *et al.* 2010). In this experiment, it has been shown that a large amount of organic acid alcohols is produced in the rhizosphere secretions during SD degradation. Organic acid alcohols are hydrolyzed in acid or alkaline environments to produce corresponding acids and alcohols. Some strains exhibit significant absorption of amino acids, organic acids, sugars, and quaternary amines during root growth (Zhalnina *et al.* 2018).

The selective influence of plants on the composition and structure of the inter-rooted microbial community is always dominant, mainly due to the secretions of the plant roots that regulate the structure of the inter-rooted microbial community, which differs in root exudates before and after SD degradation. These root secretions can induce and stimulate the growth of specific bacterial groups and influence the abundance and diversity of inter-rooted microorganisms, which in turn has an impact on degradation efficiency and products. In a study of SD degradation dynamics, it was found that the areas with the highest degradation rates were those with the highest microbial abundance, and that the community structure changed before and after the experiment, probably because the crop roots secreted large amounts of organic matter into the soil, and these root secretions in turn directed the microbial community toward reducing external stresses, promoting SD degradation, causing the inter-rooted microbial community structure to respond.

3.6. Diversity analysis of bacterial communities

The curve was drawn with the alpha diversity index as the vertical axis (Figure 10). All of the curves were not stable; that is, each sample did not reach saturation, indicating that the diversity of rhizosphere bacteria in all of the plant groups in this study was high. When the sequencing quantity is more than 20,000, the curve growth trend slows down until it tends to flatten, indicating that the sample sequencing quantity is reasonable. The diversity and abundance of bacteria changed before and after the degradation of SD. In addition, the OTU number of the *P. australis* group was higher than that of the *V. natans* group.

As shown in Table 2, the number of OTUs in the samples was 2,541–3,353, and the coverage was over 98% (coverage: 98–99%). It can be seen from the table that for the Shannon $P_0 > P_{96} > V_0 > V_{96}$ and for the Simpson $V_{96} > V_0 > P_{96} > P_0$; thus, the bacterial diversity of the rhizosphere in the *P. australis* group was always higher than that in the *V. natans* group before degradation (0 h) and after degradation (96 h). In the above analysis of the degradation rate, that of the *P. australis* group was also greater than that of the *V. natans* group, which was consistent with the expression of diversity analysis. The main bacterial groups involved in SA degradation may be resistant to antibiotics, thus contributing to SA biodegradation (Yang *et al.* 2016).

It can also be found from the table that the rhizosphere bacterial diversity of *P. australis* and *V. natans* decreased after 96 h of SD degradation. Chao1 was $P_{96} > P_0 > V_{96} > V_0$ and Shannoneven was $P_0 > P_{96} > V_0 > V_{96}$, indicating that the degradation of SD increased the total number of bacteria in the rhizosphere of plants, but decreased the uniformity of bacterial distribution. Wang *et al.* (2021b) found that adding root exudates reduced microbial community diversity, but increased community abundance. This may have been due to the presence of a large amount of organic matter in plant root exudates. This

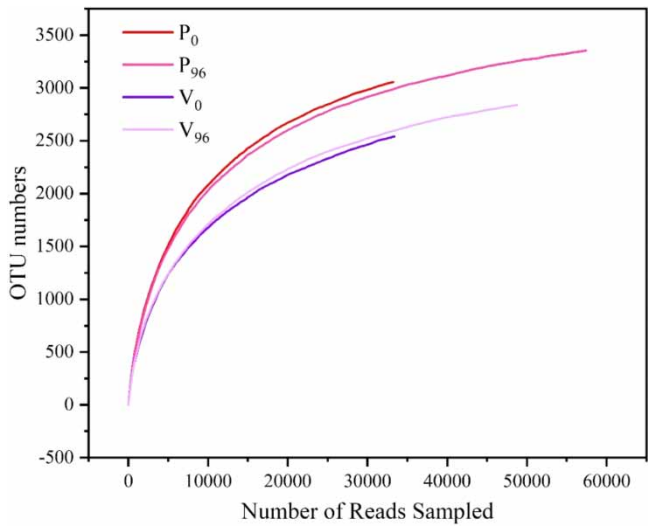

Figure 10 | Dilution curves of plant groups. P_0 was the group of *P. australis* rhizosphere degradation for 0 h, P_{96} was the group of *P. australis* rhizosphere degradation for 96 h, V_0 was the group of *V. natans* rhizosphere degradation for 0 h, and V_{96} was the group of *V. natans* rhizosphere degradation for 0 h.

Table 2 | Alpha diversity index of each plant group

Sample	Shannon	OTUs	Chao	Simpson	Shannoneven	Coverage (%)
V_0	6.39	2,541	3,081.98	0.005	0.82	98
V_{96}	6.32	2,838	3,328.27	0.007	0.80	99
P_0	6.89	3,055	3,509.62	0.004	0.86	98
P_{96}	6.78	3,353	3,678.60	0.006	0.83	99

leads to the evolution of the microbial community toward the reduction of external stress, which promotes the degradation of SD and induces and stimulates the growth of specific bacterial communities, thereby affecting the abundance and diversity of rhizosphere microorganisms (Yuan *et al.* 2017).

4. CONCLUSION

In order to reveal the sulfonamide degradation mechanism of the wetland plant rhizosphere, we compared the composition of rhizosphere exudates of *P. australis* and *V. natans* under 0.10 μg L^{-1} SD stress in this study. The degradation of SD by rhizosphere exudates extracted under stress was also observed. We found that SD stress could increase the content of organic acid esters in plant roots. In the degradation experiment, we found that the degradation effect of the *P. australis* group was stronger than that of the *V. natans* group. During the degradation process, the relative abundance of rhizosphere bacteria will change, the diversity will decrease, and new genera will be introduced. In addition, we conclude that the degradation of SD in plants in karst areas may be controlled indirectly by increasing the content of organic acid esters in roots. Under weak alkaline conditions, organic acid esters break down to produce organic acids that affect the rhizosphere microbial metabolism.

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PUBLISHER'S NOTE

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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