agronomy

MDPI

Article

Research on a Biofilter for a Typical Application Scenario in China: Treatment of Pesticide Residue Wastewater in Orchards

Jin Zeng [1,2,†], Quanchun Yuan [1,†], Wenzhi Xu [1], Hailong Li [1], Menghui Li [3], Xiaohui Lei [1], Wei Wang [1], Qiang Lin [1], Xue Li [1], Rui Xu [2,*] and Xiaolan Lyu [1,*]

[1] Institute of Agricultural Facilities and Equipment, Jiangsu Academy of Agricultural Sciences/Key Laboratory of Modern Horticultural Equipment, Ministry of Agriculture and Rural Affairs, Nanjing 210014, China; j-zeng@jaas.ac.cn (J.Z.); yuanquanchun@jaas.ac.cn (Q.Y.); xuwenzhi678@163.com (W.X.); l747626393@163.com (H.L.); leixiaohui@jaas.ac.cn (X.L.); jaaswangwei@163.com (W.W.); 19930010@jaas.an.cn (Q.L.); neigelee99@163.com (X.L.)

[2] Yunnan Provincial Rural Energy Engineering Key Laboratory, Yunnan Normal University, Kunming 650500, China

[3] Nanjing Environment Group Co., Ltd., Nanjing 210028, China; limohcr@163.com

* Correspondence: ecowatch_xr@163.com (R.X.); lxlanny@126.com (X.L.)

† These authors contributed equally to this work.

Abstract: To reduce pesticide pollution and promote sustainable agricultural development in China, we designed a pilot-scale biofilter system to treat residual imidacloprid wastewater in an orchard. The biofilter system demonstrated a high rate of removal of imidacloprid from the biodegradation wastewater, with removal rates from the outlet exceeding 99% at different concentrations of pesticides. Among environmental factors, imidacloprid concentration at the inlet and biomixture significantly affected the activity of imidacloprid-degrading bacteria. The dominant microbial communities during the stable operation of the biofilter system included *Firmicutes*, *Actinobacteria*, *Proteobacteria*, and *Bacteroidetes* at the phylum level and *Bacillus*, *Methylobacter*, and *unclassified_f__Microbacteriaceae* at the genus level. In future initiatives to improve biofilter performance and applicability, increasing attention should be paid to the dominant microbial communities, the number of biofilter units, and important environmental factors. Orchard workers in China should improve the existing treatment of residual pesticide wastewater to mitigate agricultural non-point source pollution.

Keywords: biofilter; straw; imidacloprid; biodegradation; orchard

Citation: Zeng, J.; Yuan, Q.; Xu, W.; Li, H.; Li, M.; Lei, X.; Wang, W.; Lin, Q.; Li, X.; Xu, R.; et al. Research on a Biofilter for a Typical Application Scenario in China: Treatment of Pesticide Residue Wastewater in Orchards. *Agronomy* **2024**, *14*, 934. https://doi.org/10.3390/agronomy14050934

Academic Editor: Wanting Ling

Received: 7 March 2024
Revised: 13 April 2024
Accepted: 24 April 2024
Published: 30 April 2024

1. Introduction

Pesticides play an important role in preventing and controlling pests and diseases, as well as promoting stable crop yields and increasing incomes. Approximately 2.6 million tons of pesticides are used worldwide each year [1], and China accounts for approximately 10–15% of this usage. However, the utilization rate of pesticides in China is low (approximately 40%). The planting area of orchards in China is approximately 18 million hectares, and pesticides are applied eight times a year. Imidacloprid, a member of the neonicotinoid class of insecticides, is widely used to control leaf-dropping pests in fruit orchards and is one of the most used pesticides in fruit production. Imidacloprid is easily released into the environment and can persist for long periods, thereby accumulating in the food chain and contaminating groundwater and surface water [2].

To improve the effectiveness and efficiency of pesticide application in orchards and reduce pesticide use, scholars worldwide have developed pesticide application equipment such as air-assisted sprayers, plant protection drones, and multifunctional robots. However, after applying pesticides, this equipment often produces small amounts of high-concentration pesticide residue in addition to low-concentration pesticide residue within the large volume of water that is used to flush the interior components, such as the box

and pipeline [3]. Developed countries such as those in Europe and the United States are vigorously promoting the use of biofilter systems to treat pesticide residues in wastewater [4–8]. However, most Chinese farmers currently lack awareness regarding pesticide wastewater treatment and do not have access to effective treatment measures. Generally, pesticide wastewater residues are discharged directly into farmland soil or nearby bodies of water, which can lead to environmental pollution and pose safety risks [9] that endanger human health. Therefore, it is imperative to utilize precise and effective measures to reduce pesticide pollution in orchards and develop green technologies that can address the aforementioned issues and promote the green, healthy, and sustainable development of the orchard industry.

Microorganisms play a crucial role in pesticide degradation in ecosystems. Extensive research has shown that microbial degradation of pesticides is the primary pathway for ensuring the complete elimination of pesticide residues [10,11]. Understanding the types, quantities, and activities of microorganisms is essential for understanding pesticide metabolism [8]. During biodegradation, microorganisms can completely break down the most toxic organic compounds into CO_2 and H_2O [4]. Although the remediation period can be long, microbial degradation has become an important area of research for facilitating pesticide residue degradation owing to its economic, environmentally friendly, safe, and efficient nature, with no secondary pollution. Microbial degradation is particularly suitable for soils and water bodies contaminated with low to moderate levels of pesticide residues.

Biobeds are typical biological treatment systems that combine the functions of physical adsorption and microbial degradation [12,13]. Biofilters are traditional biobeds with similar underlying principles that have been appropriately optimized from certain initial designs in practical applications [4,5]. Biofilters are widely used in agricultural production given that the raw materials are easily accessible, investment and operating costs tend to be low, and pesticide removal efficiency is high. However, research on the degradation of pesticide residues in wastewater by biofilters and their application in orchards in China remains limited. The metabolic characteristics and functions of the microbial communities within biofilters are especially unclear. In Belgium, research has primarily focused on adjusting biofilters into small and flexible systems [14], for which the number of biofilter units is determined based on the amount of water to be treated and the concentration of pesticides. Pussemier et al. [15] added activated carbon filter cartridges to the drainage pipes of a biofilter system and found that the concentration of pesticides in the filtrate decreased significantly. Complex automatic electronic computer systems are used in modern biofilters to maintain established usage specifications [16].

The biomixture of a biofilter is crucial to the purification function because the key mechanism for purification in the biofilter is the removal of contaminants via biomixture microorganisms [17]. Typical biomixtures are composed of straw, charcoal, and soil at a volume ratio of 2:1:1 [18]. However, researchers have investigated other biomixture ratios, such as 50% straw + 45% charcoal + 5% pesticide-primed substrate or soil [19], 50% corn cobs + 25% soil + 25% charcoal [4], and 50% soil + 50% millet stubble [20], all of which showed a high pesticide degradation efficacy. Studies have shown that the adsorption and degradation of pesticides are primarily influenced by the composition, balance, duration of use, temperature, and humidity of biomixtures. Yang et al. [8] recommended further research on the variations in pesticide adsorption and degradation efficiency of biomixtures over time. Long-term monitoring can determine the effective lifespan of a biomixture, thereby guiding the compensation or replacement of the substrate when microbial activity is excessively low.

Numerous studies have demonstrated that straw is the primary site of pesticide degradation and microbial activity in biofilters [21]. Straw and other lignocellulosic materials are indispensable in biomixtures [14] because their slow degradation continuously provides carbon, nutrients, and energy to microorganisms. China produces more than one billion tons of crop straw annually; the Ministry of Agriculture and Rural Affairs requires the comprehensive utilization of straw resources, prioritizing agricultural use with a multifaceted

approach. However, improving the comprehensive utilization of straw by exploring sustainable industrial development models and developing efficient utilization mechanisms remains necessary. Owing to its high carbon-to-nitrogen ratio of 60–100:1, straw is an ideal raw material for regulating carbon sources and microbial fermentation. As a raw material, it can be combined with relevant microbial ecosystems to construct high-performance biofilters and explore new methods for resource utilization.

In particular, this study used straw as one of the main raw materials for the biomixture of a biofilter and explored its degradation efficiency on imidacloprid pesticide residues in a typical orchard in China. Additionally, the metabolic characteristics and functions of the microbial communities in the biofilter were analyzed, laying the foundation for detailing the operational mechanisms of the biofilter and the degradation and transformation pathways of pesticide pollutants. Our findings will be useful in efforts to utilize straw resources and mitigate environmental pollution caused by pesticide residue wastewater.

2. Materials and Methods

2.1. Description of the Experimental Site

A biofilter was installed at the Institute of Agricultural Facilities and Equipment, Jiangsu Academy of Agricultural Sciences, Nanjing, Jiangsu Province (32°2′15″ N, 118°52′26″ E). The local climate is a northern subtropical humid climate with four distinct seasons. The biofilter system was installed in September 2022 and tested in 2022 and 2023.

2.2. Preparation of the Biomixture

Considering the typical biomixture formula (straw, peat, and soil in a volume ratio of 2:1:1), the high cost and limited availability of peat, and the recommendation to use agricultural and forestry organic waste straw under a resource-oriented approach [22], the biomixture was prepared by mixing straw, substrate, and soil at a volume ratio of 1:1:1. Rice and wheat straw were obtained from experimental fields of the Jiangsu Academy of Agricultural Sciences, China. After drying, the straw was crushed into powder using a straw crusher. The substrate was a special potting substrate from Shanghai Meizhijia Gardening Co., Ltd. (Shanghai, China), which contained peat, coconut husk, pine phosphorus, perlite, growth regulator and microbial agents. The pH of the substrate was 5.5–8.5, and the organic matter content was between 40% and 85%. The soil was topsoil from a greenhouse at the Institute of Agricultural Facilities and Equipment, Jiangsu Academy of Agricultural Sciences.

2.3. Design of Pilot-Scale Biofilter System

The biobed method has been used in several countries and regions, and most biobeds are simple and efficient. Although the initial design of some biobeds has been adjusted in practice, and some are referred to as a biofilter, biomass bed, phytobac, biobac, or biotable, they remain similar in nature and principle.

Although methods for calculating the relationship between the efficiency of biofilters and their geometric parameters have been proposed, the equipment size in practical applications is mostly determined by empirical methods [16]. Most biofilters that have previously been used are large and operate for extended periods. The biomixture used in these biofilters may undergo decomposition and compaction, leading to a higher pressure decrease and a lower mass transfer efficiency.

Based on the structural design of biofilters used in Belgium [14], a standard 1 × 1 × 1 m cubic PVC box was used as an individual carrier for the biofilter unit. The interior of each unit was filled with a homogeneous biomixture of straw, matrix, and soil. A biofiltration system can be constructed by connecting four biofilter units in series. Physical and operation diagrams of the static chamber are shown in Figures 1 and 2, respectively. The biofilter system consisted of the following main elements: the pool to collect the pesticide residue sewage, the pool to collect the circulating water, the cubic PVC boxes to carry biomixture,

the centrifugal pumps to transfer the liquid to the biofilter and the micro-sprinklers to produce a uniform distribution to the surface of the biomixture.

Figure 1. Physical diagram of the biofilter system.

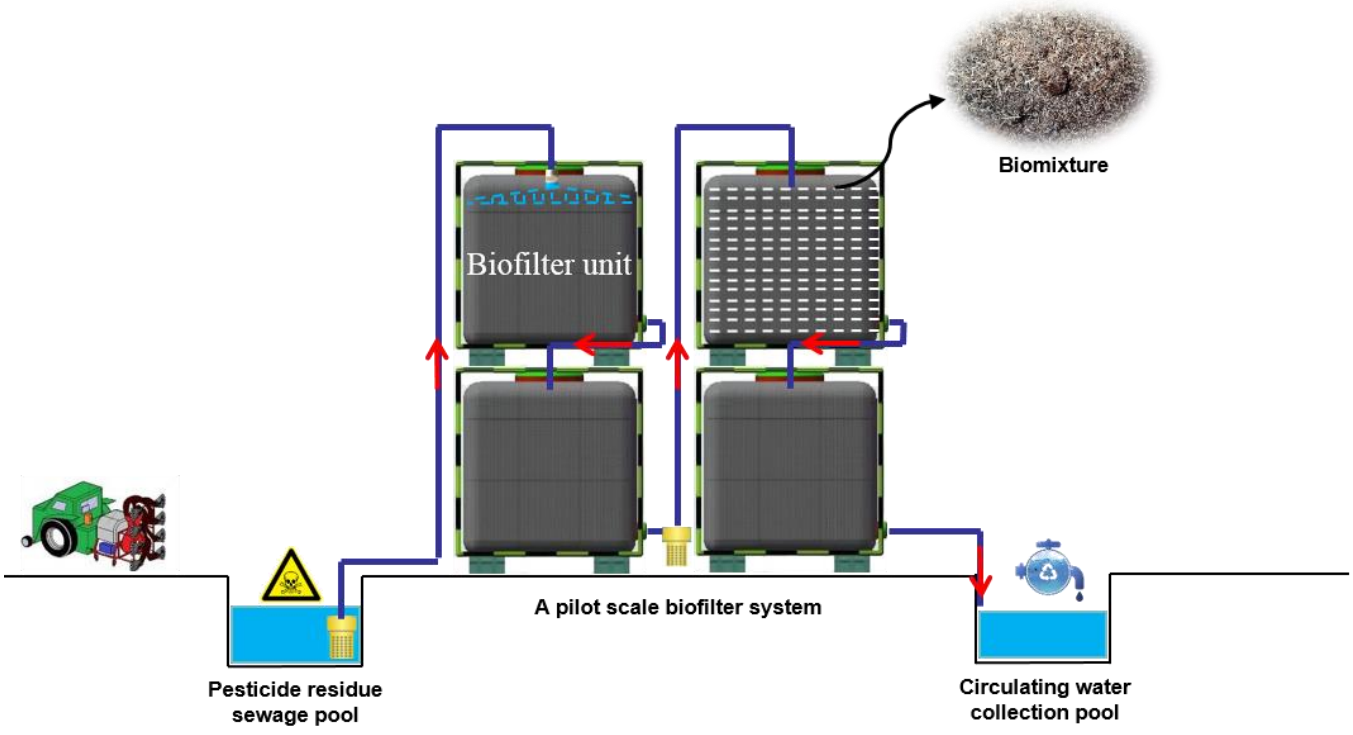

Figure 2. Operating diagram of the biofilter system.

2.4. Water and Biomixture Analyses

The study was conducted between November 2022 and August 2023. Water and biomixture samples were analyzed directly using liquid chromatography–tandem mass spectrometry (LC-MS). The detection method for imidacloprid followed the National Standard of the People's Republic of China (GB 23200.121-2021) "National food safety standard–FSS Determination of 331 pesticide and metabolite residues in foods of plant origin—-liquid chromatography–tandem mass spectrometry method". The analysis of imidacloprid residues in the biomixture was performed in triplicate.

The study focused on pesticide spraying in orchards. We conducted on-site investigations at the production frontline and found that the water dilution ratio of 70% imidacloprid used in Chinese orchards was 1:5000. Therefore, we used the Lidaqing (70% imidacloprid, YongGuan) for experiments, and calculated the residual imidacloprid residue concentration as 140 mg/L (0.7 mg imidacloprid: 5000 mL water), whereas the measured value was 132.6 mg/L. Typical agricultural wastewater samples from the air-assisted sprayers were collected and tested; the concentration range of imidacloprid pesticide residues in the orchard washing water was 3.7–15.3 mg/L.

Based on the concentrations of imidacloprid pesticide residue and pesticide washing water, we simulated the configuration of different concentrations of pesticide-contaminated wastewater entering the biofiltration system with an inflow of 100 L for each test. After each test, 100 mL water samples were collected from the outlet three or four times at irregular intervals to detect the imidacloprid content in the purified water. During the experimental period, samples of the biomixture within the biofiltration unit were collected using a soil auger. The biomixture sampling depth was 0–30 cm, and the sampling depth of some samples was 30–60 cm for analysis of imidacloprid concentrations at different depths. Next, 3–5 samples were mixed and placed in a 100 mL plastic sampling bottle for subsequent detection of the imidacloprid content in the biomixture, which facilitated the exploration of the operational mechanism of the biofiltration system.

2.5. Microbial Sampling and Measurements

Following the aforementioned procedure, biomixture samples from the biofilter system were collected in January, April, June, July, and August 2023 (samples A1, A2, A3, A4, and A5, respectively). Biomixture samples originated from the first connected biofilters unit: A1 from January, where the imidacloprid concentration from the inlet was 2.8 mg/L, A2 from April, where the imidacloprid concentration from the inlet was 8.4 mg/L, A3 from June, where the imidacloprid concentration from the inlet was 11.2 mg/L, A4 from July, where the imidacloprid concentration from the inlet was 33.6 mg/L, and A5 from August, where the imidacloprid concentration from the inlet was 140 mg/L. After collection, biomixture samples were promptly stored at −80 °C. All microbial samples were subsequently sent to Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China) for 16S rDNA sequencing.

Before DNA extraction, each sample was centrifuged at 9401.7× *g* for 4 min at 4 °C. Next, total DNA was extracted from each sample using the E.Z.N.A.® soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) according to the manufacturer's instructions. The extracted DNA was detected using 1% agarose gel electrophoresis. DNA concentration and purity were determined using a NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). A polymerase chain reaction (PCR) was used to perform amplification of the V3–V4 region in the 16S rRNA using primers 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) [23]. The PCR mixture contained 4 μL of 5 × Fast Pfu buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of Fast Pfu polymerase, 10 ng of template DNA, and ddH2O to a final volume of 20 μL. Amplification was carried out via denaturation of the DNA at 95 °C for 30 s, 27 cycles at 95 °C for 30 s, 55 °C for 30 s, 72 °C for 45 s, and a single extension at 72 °C for 10 min. Using diluted genomic DNA as a template, the running environment of this PCR adhered to the operating conditions of Sundberg et al. [24] and others, and the PCR (ABI GeneAmp® 9700) amplification was performed using TransGen

AP221-02: TransStart® FastPfu DNA Polymerase; these conditions ensured the accuracy and efficiency of the amplification. The PCR products were recovered and purified using the AxyPrepDNAusingan gel recovery kit and eluted using Tris-HCl. The library was inspected using a NanoDrop 2000 spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and 2% agarose gel. The PCR products were detected and quantified using the QuantiFluor™-ST Blue fluorescence quantification system (Promega, Madison, WI, USA) and then mixed according to the sequencing volume requirements of each sample. Sequencing libraries were generated using a TruSeq™ DNA Sample Prep Kit following the manufacturer's recommendations. Finally, sequencing was performed using the HiSeqS PE250 platform (Illumina, San Diego, CA, USA) according to the standard protocols of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

Raw FASTQ files were de-multiplexed using an in-house Perl script, quality-filtered using fastp version 0.19.6 [25], and then merged using FLASH version 1.2.7 [26]. The optimized sequences were clustered into operational taxonomic units (OTUs) using UPARSE 7.1 [27,28] with a 97% sequence similarity level. The most abundant sequence of each OTU was selected as the representative sequence. To minimize the effects of the sequencing depth on the alpha and beta diversity, the number of 16S rRNA gene sequences from each sample was rarefied to 20,000, yielding an average Good's coverage of 99.09%. The taxonomy of each OTU representative sequence was analyzed using RDP Classifier version 2.2 [29] against the 16S rRNA gene database (e.g., Silva v138) using a confidence threshold of 0.7.

2.6. Statistical Analysis

Based on the OTUs, alpha diversity indices, including observed OTUs, Shannon index, Good's coverage, Chao value, and other metrics, were calculated using the Mothur v1.30.1 software [30]. A bioinformatic analysis of the biomixture microbiota was carried out using the Majorbio Cloud platform, including principal component analysis (PCA), redundancy analysis (RDA), and other analyses.

3. Results and Discussion

3.1. Pesticide Degradation and Biofiltration Performance

Figure 3 shows the imidacloprid depletion of water from the pilot biofilter system. The concentration of imidacloprid in the biofiltration wastewater changed slightly, with an increase in imidacloprid concentration from the inlet. The biofilter system had a high imidacloprid removal efficiency within the pesticide residue wastewater. The removal rates of imidacloprid at the outlet for the different concentrations were all >99%, which is similar to the results of Vischetti et al. [31]; this indicates that the reactor had a high efficiency in removing the pesticide from the water. Subsequent tests found that the imidacloprid concentration from the biofiltration wastewater was always in a low, stable state and even became undetectable despite the initial concentration of imidacloprid increasing through a certain range. The degradation efficiency of the biofilter may have been enhanced via repeated treatment of the specific pesticide [13] because the microbial community using the pesticide for energy proliferated, and the domesticated microorganisms could have potentially degraded the pesticide more easily [32,33]. Alternatively, the degradation efficiency may have been enhanced because the biomixture strongly affected the pesticide via adsorption and degradation, and the biofilter system consisted of four biofiltration units. Furthermore, the substrate from biomixture contained microbial agents, and some research has confirmed that microbial agents had an important impact on pesticide residue degradation and microbial remediation in the soil [34,35]. Therefore, they could be bioaugmentation agents, which could enhance the degradation efficiency of pesticides from the biofilter system. Most of the pesticide was adsorbed onto the biomixture and underwent biodegradation reactions; therefore, there was almost no leaching of the pesticide. Pussemier et al. [15] found that the residues of the five tracked pesticides in the biomixture of a biofilter system reached >95%.

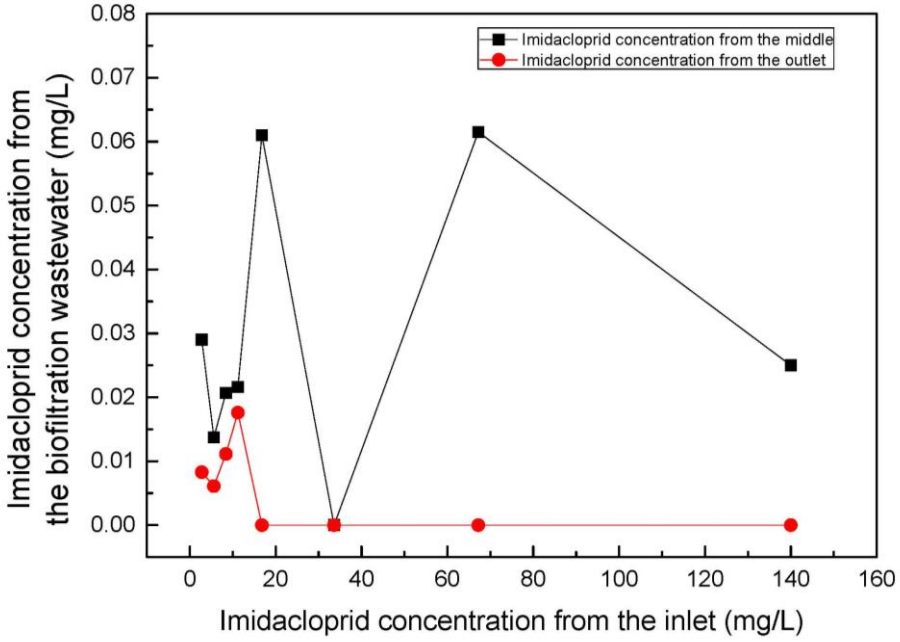

Figure 3. Variation in pesticide residues in wastewater from the biofilter system.

As shown in Figure 4, with an increase in the imidacloprid concentration and cumulative amount of wastewater from the inlet, the imidacloprid adsorbed by the biomixture slowly increased; however, gradually, the imidacloprid in the biomixture decreased and subsequently remained in a relatively stable state. This may have occurred because the first biofilter unit was in a relatively stable saturation state and was unable to adsorb excessive imidacloprid in a short time. Following several tests, the domesticated microorganisms accelerated the degradation of imidacloprid; however, the degradation rate did not change in a short time. The test revealed that the imidacloprid concentration differed at various depths in the same biofilter unit. The imidacloprid concentration in the surface biomixture was relatively low but increased with the depth of the biomixture. The imidacloprid concentration was approximately 2.67 times higher in the deeper biomixture biofilter than in the surface biomixture at the same location, which is consistent with the results of Verhagen et al. [36]. In summary, most pesticide biodegradation activity occurred at the top of the biofilter, and the biodegradation rate thus decreased with increasing biofilter depth.

The test also detected the concentration of imidacloprid in the third biofilter unit connected to the system (0.16 mg/L). This result indicated that the first biofilter unit filtered most of the pesticide in the wastewater under a certain concentration and inflow and that the subsequent biofilter units demonstrated adsorption and degradation within the residual wastewater. However, the overall efficiency was low. Therefore, choosing an appropriate number of biofilter units was important because an excessive combination of biofilter units often leads to high manufacturing costs and low biofiltration efficiency. When reviewing the biofilter system, Castillo et al. [4] showed that (1) the optimal number of units within a biofilter system primarily depends on the amount of wastewater to be treated and the total load of pesticides and (2) a two-unit biofilter is optimal for wastewater with 100 g of active ingredients and <3000 L of water per year. For high loads, systems with three or more units are recommended.

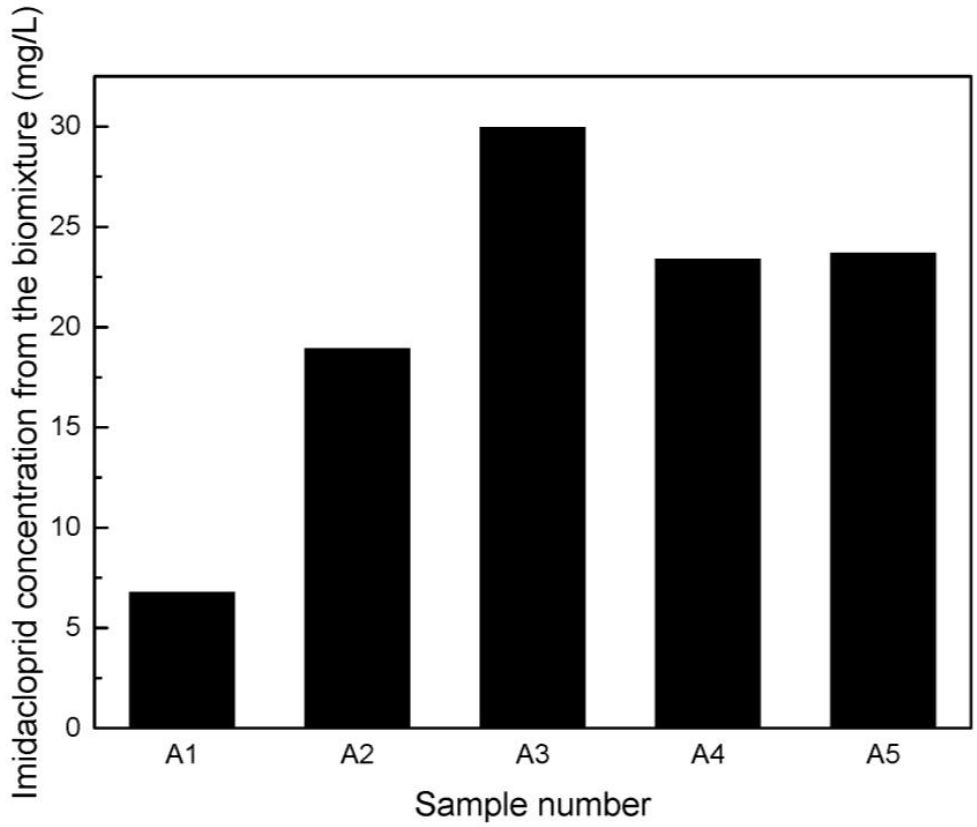

Figure 4. Variation of imidacloprid concentration in the biomixture. Note: Biomixture samples originated from the first connected biofilters unit: A1 from January, where the imidacloprid concentration from the inlet was 2.8 mg/L; A2 from April, where the imidacloprid concentration from the inlet was 8.4 mg/L; A3 from June, where the imidacloprid concentration from the inlet was 11.2 mg/L, A4 from July, where the imidacloprid concentration from the inlet was 33.6 mg/L, and A5 from August, where the imidacloprid concentration from the inlet was 140 mg/L.

3.2. Analysis of Microbial Community at the OTU Level

In this study, 279,200 valid sequences were obtained, resulting in the generation of 5026 OTUs via clustering. Among the five experimental groups, there were 209 common species, accounting for 26.76% of the species of A1, 24.97% of A2, 14.20% of A3, 19.92% of A4, and 23.56% of A5. Additionally, A1, A2, A3, A4, and A5 contained 104, 185, 486, 158, and 200 unique species, respectively.

PCA is a transformation in vector space used to reduce the dimensionality of a dataset [37]. In this method, the response resulting from the processing applied to the samples in the vector space is analyzed based on the correlation between the data extracted from the dataset. To discern the dynamics of the microbial composition during the test process, PCA was performed to cluster OTUs with the maximum variation (40.75% in PC1 and 26.33% in PC2). According to Figure 5, A1 and A2 are close together, as are A4 and A5. This indicates that the microbial community composition within the biofilter system showed minimal variations between A1 and A2 and between A4 and A5. In contrast, A3 was more distant from the other test groups, suggesting significant differences in the microbial community composition within the biofilter system compared with those within the other test groups.

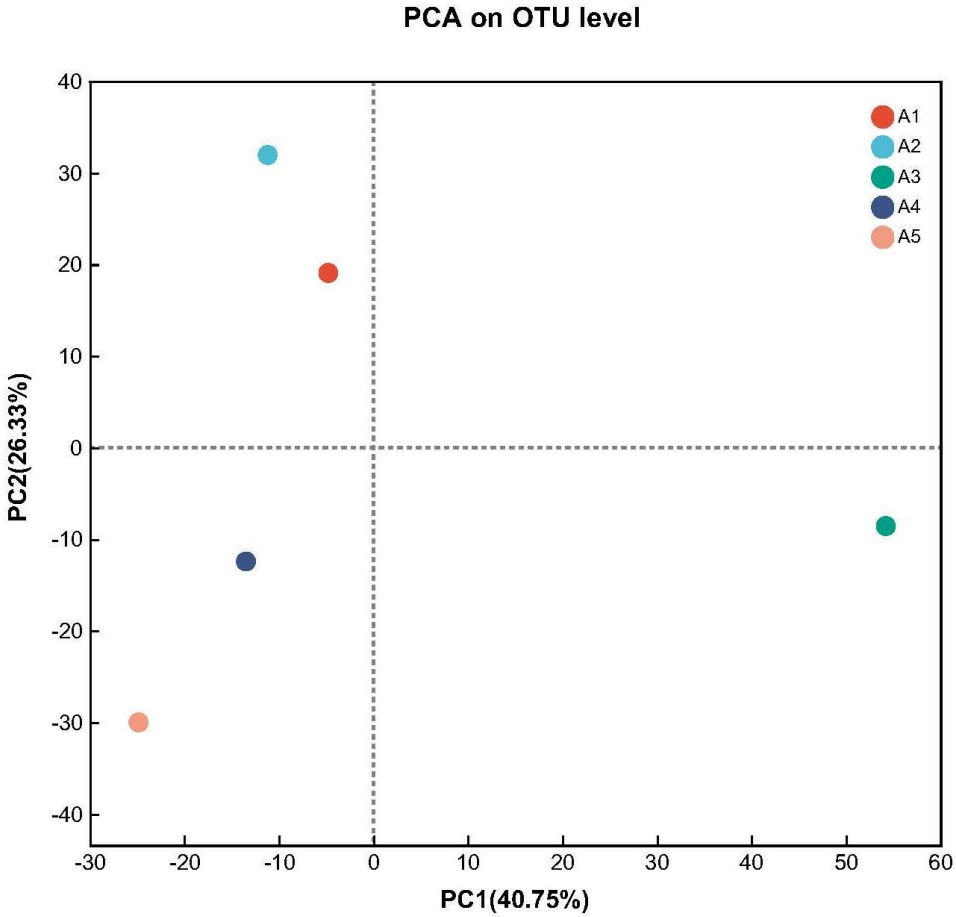

Figure 5. Principal component analysis (PCA) based on operational taxonomic unit (OTU) abundance.

Table 1 shows that the Shannon index, Simpson value, Ace value, and Chao value ranged between 1.16–1.88, 0.22–0.39, 23.49–29.42, and 23.00–31.75, respectively. A lower Shannon value corresponds to a lower α-diversity. As shown in Table 1, the Ace and Chao values were largest in A3, indicating the highest abundance of A3 in the biofilter. The Ace and Chao values were lowest in A2, indicating the smallest abundance of A2 in the biofilter. The Shannon index of the five test groups generally showed a trend of gradual increase and eventual stabilization, with a slight fluctuation in the middle but minimal volatility in the middle. The Shannon index was the largest for A5, which reflected the highest uniformity and diversity of the microbial communities. The Simpson value was always <0.4, indicating a high species richness in each group. The Shannon index and Simpson value were also very different from a previous study [38], this may be related to the different biofilter systems and pesticide residue wastewater. The coverage value was 0.99, indicating that the sampling and sequencing of the samples had high coverage and almost all the OTUs could ensure the comparability of α-diversity [38]. Finally, according to the mean and standard deviation of the index parameters of alpha diversity, the richness and evenness of the species contained in each test sample were different; however, these differences were small. This may have occurred because the biofilter system was primarily utilized to treat wastewater containing residues of the single pesticide imidacloprid and because the concentration and treatment capacity of pesticide residue wastewater were low. This phenomenon resulted in a small change in microorganism diversity in the biofilter system and continuous maintenance of a relatively stable state.

Table 1. Alpha diversity analysis of different samples.

Sample	Shannon	Simpson	Ace	Chao	Coverage
A1	1.16	0.39	25.90	25.20	0.99
A2	1.70	0.23	23.49	23.00	0.99
A3	1.64	0.25	33.71	31.75	0.99
A4	1.64	0.28	26.30	26.00	0.99
A5	1.88	0.22	29.42	28.50	0.99
$\bar{X}$	1.60	0.27	27.76	26.89	0.99
S	0.27	0.069	3.94	3.35	0

3.3. Analysis of Microbial Community Taxonomy

3.3.1. Identification of Dominant Microbes

In the present study, 11 phyla (Figure 6a) and 11 genera (Figure 6b) were identified. Microbes with a relative abundance >5% were defined as dominant microbes, and those with a relative abundance >1% but <5% were defined as subdominant microbes.

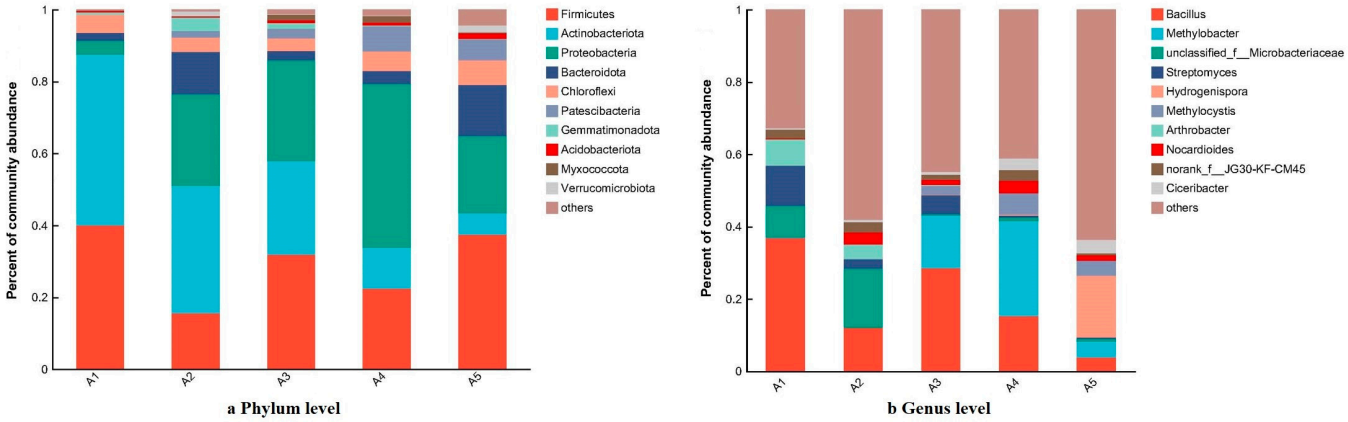

Figure 6. Relative abundances of microbes in the biomixture at the phylum (**a**) and genus (**b**) levels.

The average relative abundances of the seven phyla were >1% (Table 2). As shown in Figure 6a, Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes were the four dominant phyla in the biofilter system under stable operation, with average relative abundances of 29.45%, 25.21%, 24.92%, and 6.87%, respectively. The dominant microbes were similar to those reported by Liu et al. [38]; however, their relative abundances were different, which could be attributed to the different biomixtures and application scenarios. The average relative abundances of Firmicutes, Actinobacteria, and Proteobacteria were relatively high, suggesting that these species integrally influence the degradation of imidacloprid. The subdominant microbial communities at the phylum level were Chloroflexi, Patescibacteria, and Gemmatimonadota, with average relative abundances of 4.97%, 3.45%, and 1.26%, respectively.

The average relative abundance of the 10 genera exceeded 1% (Table 3). As shown in Figure 6b, *Bacillus*, *Methylobacter*, and *unclassified_f__Microbacteriaceae* were the three dominant genera during the stable operation of the biofilter system, with average relative abundances of 19.27%, 9.06%, and 5.46%, respectively. The subdominant genera were *Streptomyces*, *Hydrogenispora*, *Methylocystis*, *Arthrobacter*, *Nocardioides*, *norank_f__JG30-KF-CM45*, and *Ciceribacter*.

Table 2. Average relative abundance of microorganisms at the phylum level.

Phylum	Average Relative Abundance
Firmicutes	29.45%
Actinobacteriota	25.21%
Proteobacteria	24.92%
Bacteroidota	6.87%
Chloroflexi	4.97%
Patescibacteria	3.45%
Gemmatimonadota	1.26%
Acidobacteriota	0.79%
Myxococcota	0. 75%
Verrucomicrobiota	0.71%

Table 3. Average relative abundance of microorganisms at the genus level.

Genus	Average Relative Abundance
Bacillus	19.27%
Methylobacter	9.06%
unclassified_f__Microbacteriaceae	5.46%
Streptomyces	3.97%
Hydrogenispora	3.50%
Methylocystis	2.54%
Arthrobacter	2.31%
Nocardioides	2.01%
norank_f__JG30-KF-CM45	2.01%
Ciceribacter	1.74%

3.3.2. Relative Abundance Dynamics of Dominant and Subdominant Microbes

The relative abundance of Firmicutes, which was the dominant phylum, always exceeded 15% with a change in the concentration of the residual pesticide wastewater. Firmicutes participate in xenobiotic degradation in wastewater treatment facilities [12]. Previous studies have shown that Firmicutes can act as an electron donor to promote the co-degradation of pesticides and nitrogen during denitrification [38,39]. With an increase in the concentration of residual pesticide in wastewater, the relative abundance of Actinobacteria decreased sharply, from 47.40% to 5.88%. The relative abundance of Proteobacteria first increased sharply, then decreased and became stable. The variation in its relative abundance ranged from 3.90% to 45.51%. The relative abundance of Bacteroidetes fluctuated considerably, ranging from 2.14% to 14.20%. Actinobacteria, Proteobacteria, and Bacteroidota strongly influence ecosystems and participate in biogeochemical processes such as organic pollutant decomposition and carbon and nitrogen cycling [40,41]. Among these, Bacteroidota has a rich enzyme system and can degrade a variety of plant fibers and other organic substances [40]. This phylum is integral in maintaining the ecological balance and stability of the ecosystem. The relative abundance of the subdominant phylum Chloroflexi varied slightly, ranging from 3.47% to 6.84%. Chloroflexi are typically anaerobic organisms [42] participating in the decomposition of organic substances and circulating elements. Patescibacteria are a group of newly discovered bacteria that may fulfill important roles in ecosystems, including the decomposition and circulation of organic substances.

Bacillus was the dominant genus; its relative abundance was 3.79% in A5 (minimum) and 36.90% in A1 (maximum), indicating its role in the biodegradation of imidacloprid. *Methylobacter* was a non-dominant genus in some groups but dominant in others. The relative abundance of *Methylobacter* was 0.01% in A2 (minimum) and 26.31% in A4 (maximum). With an increase in the concentration of the residual pesticide wastewater, *unclassified_f__Microbacteriaceae* gradually changed from a dominant to a non-dominant microbial community; its relative abundance was 0.35% in A3 (minimum) and 16.32% in A2 (maximum). *Methylobacter* influences ecological processes such as methane production and

degradation of organic matter [43] and is used in environmental and industrial fields. *Unclassified_f__Microbacteriaceae* are particularly abundant in soil and participate in the decomposition and recycling of organic material. Owing to their unknown classification, the ecological and physiological characteristics of these bacteria are not thoroughly understood.

3.3.3. Cluster Analysis for Similarity of Samples Collected in Different Phases

The relative abundance of each genus in each sample was visually measured using a heat map, and a cluster analysis was performed to determine similarities between samples. Figure 7 shows the three main clusters: cluster 1, consisting of A1 and A2; cluster 2, consisting of A3 and A4; and cluster 3, consisting of A5. Taking A1 as an example, it can be seen that the cluster distance between samples gradually increased with increasing pesticide wastewater concentration. Moreover, the diversity of microorganisms changed upon succession, causing the cluster distance between A3 and A4 to be relatively close. The results show that the concentration of pesticide wastewater may influence microbial abundances, and the compositions of the dominant microbial communities from A3 and A4 were similar to those from A1, A2, and A5. The compositions of the top five genera varied among the different test groups.

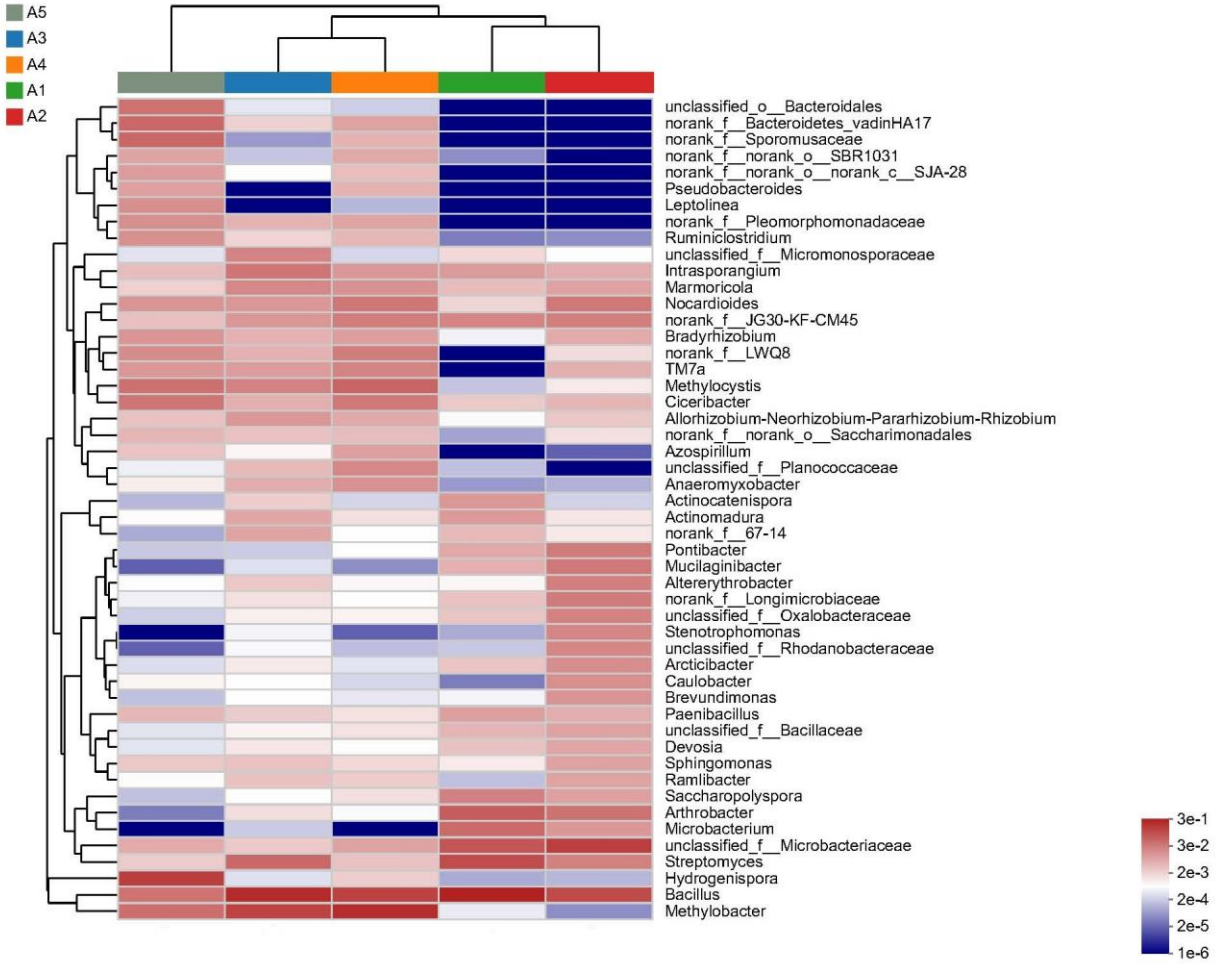

Figure 7. Community heatmap analysis at the genus level.

3.4. Relationship between Imidacloprid Concentration and Microbial Community Structure

The effects of some environmental factors on the top five microbial communities at the phylum level were identified using RDA, as shown in Figure 8. The results indicated that the two axes explained 68.22% and 24.29% of the total variability. Firmicutes were positively correlated with the imidacloprid concentration from the inlet and middle but

negatively correlated with the imidacloprid concentration from the biomixture and outlet. Actinobacteria were positively correlated with the imidacloprid concentration from the middle and outlet but negatively correlated with the imidacloprid concentration from the inlet and biomixture. Proteobacteria were positively correlated with the imidacloprid concentration in the biomixture and inlet but negatively correlated with the imidacloprid concentration in the middle and outlet. Bacteroidota and Chloroflexi were positively correlated with the imidacloprid concentration from the inlet and biomixture but negatively correlated with the imidacloprid concentration from the middle and outlet. The aforementioned results indicated that most of the top five microbes were affected primarily by the imidacloprid concentration of the inlet and biomixture; therefore, the imidacloprid concentration from the inlet and biomixture had significant impacts on the activity of imidacloprid-degrading bacteria as environmental factors. Moreover, some studies have reported that the ambient temperature and humidity in the biomixture significantly affect the microbial community in the biofilter [8,38]. Therefore, to improve the degradation rate of imidacloprid in biofilters, additional attention should be given to the saturation moisture content and operating temperature.

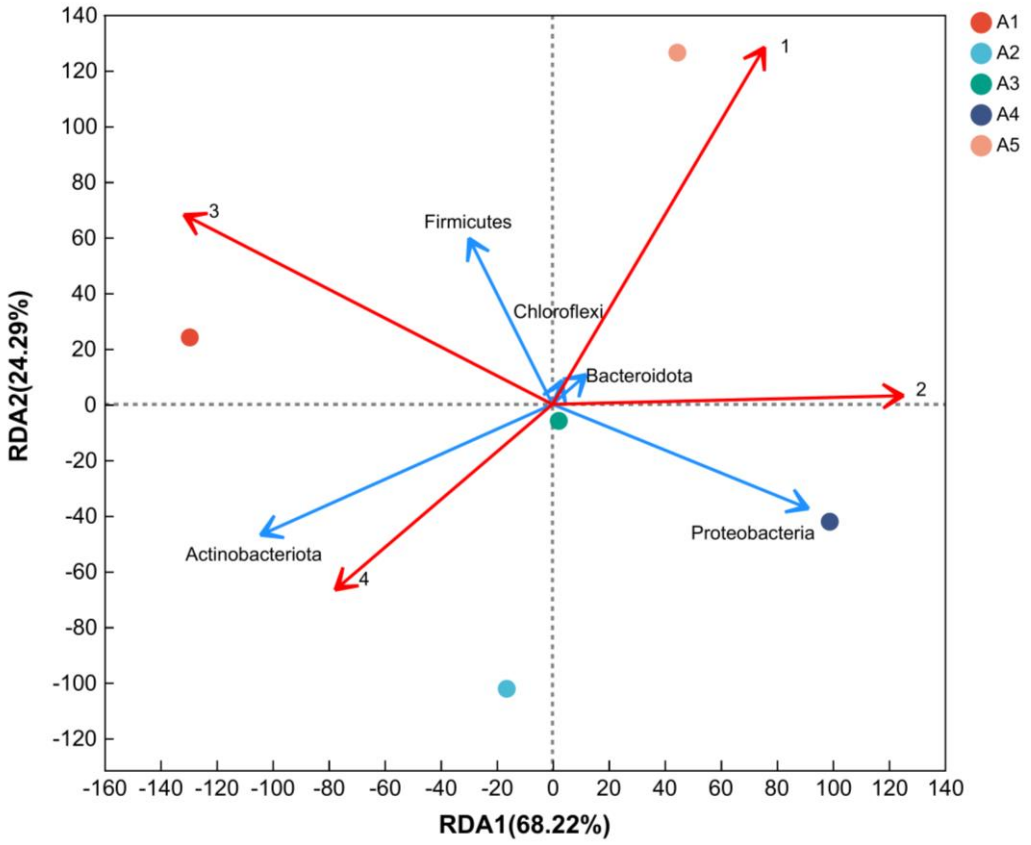

Figure 8. Redundancy analysis (RDA) of some environmental factors and the top 5 microbial communities at the phylum level. Notes: 1: imidacloprid concentration from the inlet, 2: imidacloprid concentration from the biomixture, 3: imidacloprid concentration from the middle, and 4: imidacloprid concentration from the outlet.

4. Conclusions

In this study, biofilters using straw, substrate, and soil as the biomixture were evaluated for their ability to remove different concentrations of imidacloprid from pesticide residue wastewater. The results showed that the biofilter system demonstrated a high rate of removal of imidacloprid from the biofiltration wastewater, and the removal rates from the outlet were >99% at different concentrations. This study found that the imidacloprid concentration of the inlet and the biomixture, as environmental factors, exerted

significant effects on the activity of imidacloprid-degrading bacteria. The dominant phyla during the stable operation of the biofilter system were Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidota. The dominant genera were *Bacillus*, *Methylobacter*, and *unclassified_f__Microbacteriaceae*. A key finding was that more attention should be paid to pesticide wastewater treatment in orchards in China. Orchard workers should strengthen the treatments of residual pesticide wastewater to mitigate agricultural non-point source pollution. In the future, to improve the performance and applicability of biofilters, additional attention should be focused on the dominant microbial community, the number of biofilter units, and important environmental factors. The popularization and application of biofilters in Chinese orchards will assist in promoting sustainable and green development in agricultural scenarios.

Author Contributions: J.Z. and Q.Y. contributed equally to this research. Conceptualization: J.Z. and X.L. (Xiaolan Lyu); methodology: J.Z. and Q.Y.; software: Q.Y., H.L. and X.L. (Xiaohui Lei); validation: H.L. and W.W.; formal analysis: Q.Y. and W.X.; investigation: W.X. and W.W.; resources: R.X.; data curation: W.X.; writing—original draft preparation: J.Z.; writing—review and editing: Q.Y., W.X., H.L., M.L., X.L. (Xiaohui Lei), W.W., Q.L., X.L. (Xue Li), R.X. and X.L. (Xiaolan Lyu); visualization: Q.L.; supervision: M.L. and Q.L.; project administration: M.L., X.L. (Xiaohui Lei) and X.L. (Xue Li); funding acquisition: J.Z., X.L. (Xue Li), R.X. and X.L.(Xiaolan Lyu). All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Yunnan Provincial Rural Energy Engineering Key Laboratory (Grant No. 2022KF015), Jiangsu Modern Agricultural Machinery Equipment and Technology Demonstration Extension Fund (NJ2023-20), Key Research and Development Program of Shandong Province (2022SFGC0204), and China Agriculture Research System of MOF and MARA (CARS-28-21).

Data Availability Statement: Data are contained within the article.

Acknowledgments: We are grateful to the Yunnan Provincial Rural Energy Engineering Key Laboratory, Jiangsu Modern Agricultural Machinery Equipment and Technology Demonstration Extension Fund, Key Research and Development Program of Shandong Province, and an earmarked fund for CARS for their financial support of this study.

Conflicts of Interest: Author Menghui Li was employed by the company Nanjing Environment Group Co., Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Food of Agriculture Organization of the United Nations. World Food and Agriculture Statistical Yearbook 2022. 2022. Available online: https://www.fao.org/3/cc2211en/cc2211en.pdf (accessed on 8 February 2024).
2. Gao, W.; Liang, J.; Pizzul, L.; Feng, X.M.; Zhang, K.; del Pilar Castillo, M. Evaluation of spent mushroom substrate as substitute of peat in Chinese biobeds. *Int. Biodeterior. Biodegrad.* **2015**, *98*, 107–112. [CrossRef]
3. Fogg, P.; Boxall, A.B.; Walker, A.; Jukes, A. Degradation and leaching potential of pesticides in biobed systems. *Pest Manage. Sci.* **2004**, *60*, 645–654. [CrossRef]
4. Castillo, M.D.P.; Torstensson, L.; Stenström, J. Biobeds for Environmental Protection from Pesticide Use A Review. *J. Agric. Food Chem.* **2008**, *56*, 6206–6219. [CrossRef]
5. De Wilde, T.; Spanoghe, P.; Debaer, C.; Ryckeboer, J.; Springael, D.; Jaeken, P. Overview of on-farm bioremediation systems to reduce the occurrence of point source contamination. *Pest Manag. Sci.* **2007**, *63*, 111–128. [CrossRef]
6. Fait, G.; Nicelli, M.; Fragoulis, G.; Trevisan, M.; Capri, E. Reduction of point contamination sources of pesticide from a vineyard farm. *Environ. Sci. Technol.* **2007**, *41*, 3302–3308. [CrossRef] [PubMed]
7. Karas, P.A.; Perruchon, C.; Karanasios, E.; Papadopoulou, E.S.; Manthou, E.; Sitra, S.; Ehaliotis, C.; Karpouzas, D.G. Integrated biodepuration of pesticide-contaminated wastewaters from the fruit-packaging industry using biobeds: Bioaugmentation, risk assessment and optimized management. *J. Hazard. Mater.* **2016**, *320*, 635–644. [CrossRef] [PubMed]
8. Yang, J.; Liang, J.W.; Gao, W.; Liang, H.T. Introduction of the biobed and the progress of its research application. *Agro-Environ. Dev.* **2012**, *29*, 1–8. (In Chinese)
9. Misra, R.; Satyanarayan, S.; Potle, N. Treatment of agrochemical/pesticide wastewater by coagulation/flocculation process. *Int. J. Chem. Phys. Sci.* **2013**, *2*, 39–51.
10. Guerrero Ramírez, J.R.; Ibarra Muñoz, L.A.; Balagurusamy, N.; Frías Ramírez, J.E.; Alfaro Hernández, L.; Carrillo Campos, J. Microbiology and Biochemistry of Pesticides Biodegradation. *Int. J. Mol. Sci.* **2023**, *24*, 15969. [CrossRef]

11. Sviridov, A.; Shushkova, T.; Epiktetov, D.; Tarlachkov, S.; Ermakova, I.; Leontievsky, A. Biodegradation of organophosphorus pollutants by soil bacteria: Biochemical aspects and unsolved problems. *Appl. Biochem. Microbiol.* **2021**, *57*, 836–844. [CrossRef]
12. Guarin, T.C.; Pagilla, K.R. Microbial community in biofilters for water reuse applications: A critical review. *Sci. Total Environ.* **2021**, *773*, 145655. [CrossRef] [PubMed]
13. Vischetti, C.; Monaci, E.; Cardinali, A.; Casucci, C.; Perucci, P. The effect of initial concentration, co-application and repeated applications on pesticide degradation in a biobed mixture. *Chemosphere* **2008**, *72*, 1739–1743. [CrossRef] [PubMed]
14. Pigeon, O.; De Vleeschouwer, C.; Cors, F.; Weickmans, B.; De Ryckel, B.; Pussemier, L.; Debongnie, P.; Culot, M. Development of biofilters to treat the pesticides wastes from spraying applications. *Commun. Agric. Appl. Biol.* **2005**, *70*, 1003–1012.
15. Pussemier, L.; De Vleeschouwe, C.; Debongnie, P. Self-made biofilters for on-farm clean-up of pesticides wastes. *Outlooks Pest Manag.* **2004**, *15*, 60. [CrossRef]
16. Ni, M.D.; Cui, S.R. The development and prospect in application of the biofilter in treating agriculture odor. *J. Agric. Mech. Res.* **2005**, *3*, 221–223. (In Chinese)
17. Hellman, M.; Hubalek, V.; Juhanson, J.; Almstrand, R.; Peura, S.; Hallin, S. Substrate type determines microbial activity and community composition in bioreactors for nitrate removal by denitrification at low temperature. *Sci. Total Environ.* **2021**, *755*, 143023. [CrossRef] [PubMed]
18. Torstensson, L. Experiences of biobeds in practical use in Sweden. *Pestic. Outlook* **2000**, *11*, 206–211. [CrossRef]
19. De Wilde, T.; Spanoghe, P.; Sniegowksi, K.; Ryckeboer, J.; Jaeken, P.; Springael, D. Transport and degradation of metalaxyl and isoproturon in biopurification columns inoculated with pesticide-primed material. *Chemosphere* **2010**, *78*, 56–60. [CrossRef] [PubMed]
20. Lescano, M.; Fussoni, N.; Vidal, E.; Zalazar, C. Biodegradation of pesticide-contaminated wastewaters from a formulation plant employing a pilot scale biobed. *Sci. Total Environ.* **2022**, *807*, 150758. [CrossRef]
21. Castillo, M.D.P.; Torstensson, L. Effect of biobed composition, moisture, and temperature on the degradation of pesticides. *J. Agric. Food Chem.* **2007**, *55*, 5725–5733. [CrossRef]
22. Ministry of Agriculture and Rural Affairs of the People's Republic of China. Scheme for Promoting Pilot Projects for the Resource Utilization of Agricultural Waste. 2016. Available online: https://www.moa.gov.cn/govpublic/FZJHS/201609/t20160919_5277846.htm (accessed on 8 February 2024).
23. Liu, C.; Zhao, D.; Ma, W.; Guo, Y.; Wang, A.; Wang, Q.; Lee, D.J. Denitrifying sulfide removal process on high-salinity wastewaters in the presence of *Halomonas* sp. *Appl. Microbiol. Biotechnol.* **2016**, *100*, 1421–1426. [CrossRef] [PubMed]
24. Sundberg, C.; Al-Soud, W.A.; Larsson, M.; Alm, E.; Yekta, S.S.; Svensson, B.H.; Sørensen, S.J.; Karlsson, A. 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. *FEMS Microbiol. Ecol.* **2013**, *85*, 612–626. [CrossRef] [PubMed]
25. Chen, S.; Zhou, Y.; Chen, Y.; Gu, J. fastp: An ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **2018**, *34*, i884–i890. [CrossRef] [PubMed]
26. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef]
27. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef]
28. Stackebrandt, E.; Goebel, B.M. Taxonomic note: A place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int. J. Syst. Bacteriol.* **1994**, *44*, 846–849. [CrossRef]
29. Wang, Q.; Garrity, G.M.; Tiedje, J.M.; Cole, J.R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **2007**, *73*, 5261–5267. [CrossRef] [PubMed]
30. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef]
31. Vischetti, C.; Capri, E.; Trevisan, M.; Casucci, C.; Perucci, P. Biomassbed: A biological system to reduce pesticide point contamination at farm level. *Chemosphere* **2004**, *55*, 823–828. [CrossRef]
32. Kirkland, K.; Fryer, J. Degradation of several herbicides in a soil previously treated with MCPA. *Weed Res.* **1972**, *12*, 90–95. [CrossRef]
33. Torstensson, N.; Stark, J.; Göransson, B. The effect of repeated applications of 2, 4-D and MCPA on their breakdown in soil. *Weed Res.* **1975**, *15*, 159–164. [CrossRef]
34. Zhu, J.W.; Zhao, Y.; Fu, L.; Li, X.L.; Meng, Z.L. Isolation of two isocarbophos-degrading bacteria and development of a microbial agent for bioremediation of organophosphorus pesticides contamina ted soil. *Fresenius Environ. Bull.* **2022**, *31*, 6000–6007.
35. Zhou, Y.X.; Pan, S.W. Assessment of the efficiency of immobilized degrading microorganisms in removing the organochlorine pesticide residues from agricultural soils. *Environ. Monit. Assess.* **2023**, *195*, 1274. [CrossRef] [PubMed]
36. Verhagen, P.; Destino, C.; Boon, N.; De Gelder, L. Spatial heterogeneity in degradation characteristics and microbial community composition of pesticide biopurification systems. *J. Appl. Microbiol.* **2015**, *118*, 368–378. [CrossRef] [PubMed]
37. Behbahani, B.A.; Yazdi, F.T.; Shahidi, F.; Mortazavi, S.A.; Mohebbi, M. Principle component analysis (PCA) for investigation of relationship between population dynamics of microbial pathogenesis, chemical and sensory characteristics in beef slices containing Tarragon essential oil. *Microb. Pathog.* **2017**, *105*, 37–50. [CrossRef] [PubMed]

38. Liu, H.; Wang, X.; Ou, Y.; Cheng, L.; Hou, X.; Yan, L.; Tian, L. Characterization of acetochlor degradation and role of microbial communities in biofilters with varied substrate types. *Chem. Eng. J.* **2023**, *467*, 143417. [CrossRef]
39. Sun, J.; Chen, L.; Rene, E.R.; Hu, Q.; Ma, W.; Shen, Z. Biological nitrogen removal using soil columns for the reuse of reclaimed water: Performance and microbial community analysis. *J. Environ. Manag.* **2018**, *217*, 100–109. [CrossRef] [PubMed]
40. Thomas, F.; Hehemann, J.H.; Rebuffet, E.; Czjzek, M.; Michel, G. Environmental and gut Bacteroidetes: The food connection. *Front. Microbiol.* **2011**, *2*, 93. [CrossRef] [PubMed]
41. Zheng, Y.; Dzakpasu, M.; Wang, X.; Zhang, L.; Ngo, H.H.; Guo, W.; Zhao, Y. Molecular characterization of long-term impacts of macrophytes harvest management in constructed wetlands. *Bioresour. Technol.* **2018**, *268*, 514–522. [CrossRef]
42. Yamada, T.; Sekiguchi, Y.; Imachi, H.; Kamagata, Y.; Ohashi, A.; Harada, H. Diversity, localization, and physiological properties of filamentous microbes belonging to *Chloroflexi* subphylum I in mesophilic and thermophilic methanogenic sludge granules. *Appl. Environ. Microbiol.* **2005**, *71*, 7493–7503. [CrossRef]
43. Yao, X.Z.; Chu, Y.X.; Wang, C.; Li, H.J.; Kang, Y.R.; He, R. Enhanced removal of methanethiol and its conversion products in the presence of methane in biofilters. *J. Clean. Prod.* **2019**, *215*, 75–83. [CrossRef]