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Effect of Fungicides on Bayberry Decline Disease by Modulating Rhizosphere Soil Properties, Microflora, and Metabolites

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Abstract: Decline disease causes serious damage to bayberry, but the reasons behind this disease are not completely understood, although fungal pathogenicity factors have been proposed. Our past studies have identified that the adversity of this disease is linked to the application of soil fungicide. The objective of this study is to explore the mechanism and alleviation effect of the use of the fungicide prochloraz in this disease by investigating the plant and soil parameters. The results of the current study reveal that the application of prochloraz could improve the tree vigor and fruit quality of decline-diseased bayberry. The beneficial effect of prochloraz on plant growth and fruit quality may be associated with its influence on the rhizosphere soil properties and soil microbiota. Indeed, the application of prochloraz was shown to significantly affect the relative abundance and diversity of the rhizosphere soil microbiota, with it having a greater effect on bacteria than on fungi. Furthermore, the community composition of rhizosphere soil bacteria and fungi at the genus level was found to be significantly affected by the pH, available phosphorus, alkali-hydrolyzable nitrogen, and exchangeable magnesium, which exhibited a greater effect on bacteria than on fungi. In addition, prochloraz significantly affected the metabolic pathways of pyrimidine, galactose, butanoate, arginine, and proline and changed the contents of 58 metabolites, with an 18.59–149.48% increase seen in 51 metabolites and a 31.52–95.21% reduction seen in 7 metabolites. Interestingly, these metabolites were found to be significantly correlated with the rhizosphere soil microbiota at the levels of phylum, order, and genus. Overall, the results of this study provide an alternative choice for protecting bayberry from the damage caused by decline disease.

Keywords: bayberry; fungicide; decline disease; microbial structure; metabolomics

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1. Introduction

Bayberry (*Myrica rubra*) is a fruit tree that grows in southern China. It is an important remedial plant with properties that protect against cancer, inflammation, diabetes, allergies, diarrhea, and bacterial infection [1,2]. Today, the cultivation area of bayberry is about 334,000 hectares, with an approximate yield of 950,000 tons per year [1–3]. Unfortunately, bayberry decline disease has spread widely in recent years, resulting in the inhibition of sprouting, a reduction in the photosynthetic rate, the degradation of soil quality, and

the death of trees 3–4 years later, as well as causing significant changes in the microbial composition and metabolites of rhizosphere soil in decline-diseased trees [4,5]. Therefore, it is very important for us to understand the mechanism of decline development, which will be helpful for the steady development of the industry related to bayberry.

Chemical fungicides are important measures used to control plant diseases in agricultural production. Indeed, our previous studies have found that the application of some fungicides, particularly prochloraz, in rhizosphere soil could reduce the incidence of decline disease and restore the health of decline-diseased trees. These results indicated that some fungal pathogenicity factors may be involved in the development of bayberry decline disease [6]. As we know, prochloraz is a broad-spectrum fungicide that acts by inhibiting the biosynthesis of sterols and is commonly used to control plant fungal diseases [7]. In contrast, it is not easy to elucidate the mode of action of prochloraz in decline disease due to the cause of this disease remaining unclear. Fortunately, our previous studies have shown that decline disease incidence is associated with rhizosphere soil [4,5]; thus, more attention needs to be paid to the soil microbiota.

Microbes can help to maintain the fertility of soil and in the decomposition of organic matter, and the addition of beneficial bacteria into soil ecosystems will enhance the resistance of plants to disease [8,9]. The microbial community is one of the key factors affecting the growth of plants. However, fungicides can affect the microbial communities in soil, resulting in changes in the chemical and physical properties of soil, as well as affecting the development of plants. For example, pyraclostrobin changed soil microbes' enzyme activity and community structure [10], while fludioxonil promoted soybean yield by greatly reducing the relative quantity of *Fusarium* in soil [11]. On the other hand, the ecological associations between other organisms and plants could be mediated by some important soil metabolites, such as sugars, organic acids, and secondary metabolites, which can also participate in the physiological processes, defense mechanisms, growth, and development of plants [12]. Therefore, we hypothesize that the alleviation effect of prochloraz in decline disease may be partially attributed to its effect on optimizing rhizosphere soil properties and the soil microbiota.

To maintain the healthy development of the industry associated with bayberry, the objective of this study was to examine the effects of prochloraz on fruit quality and plant growth, as well as on the microbiota, metabolites, and properties of rhizospheric soil. The results of this study will be of help for the management of decline disease in bayberry trees by improving the micro-environment of rhizospheric soil.

2. Materials and Methods

2.1. Experimental Design

Fifteen-year-old bayberry (cv. Dongkui) trees with similar crown sizes, loads, and disease index of grade 5 were used in this study [4]. The orchard was in Qianjiang Village (30°32′ N; 120°42′ E), which is a typical gentle slope hilly area about 50 m above sea level in Huangwan Town, Haining City, Zhejiang Province, China. Decline disease had infected sixty-five percent of bayberry plants with grades 1–9, while the soil of the orchard was acidic yellow. The spacing of the rows in the orchard was 4 × 5 m, and it was conventionally managed. A total of 15 kg of prochloraz fungicide solution at a 200 times dilution (P, 45% EW) was poured into the rhizosphere soil under each tree canopy before spring shoot pumping, approximately 1.5 m in radius. This study includes decline-diseased trees (D) and prochloraz (P) treatments. A straight-line distance of at least 100 m was left between each treatment. Each treatment had six replicates, with one tree in one replicate.

2.2. Vegetative Growth Parameters Measurement

During the mature fruit period (about 3 months after prochloraz application), twenty twigs were randomly selected from each treatment, and the measurement of twig length was carried out by sampling the 4th–8th leaves underneath the upper vegetative twigs in the middle part of the tree using a ruler (Deli Wenju, Hangzhou, China). Measurements

of photosynthetic rate and chlorophyll content (SPAD) were carried out using a LI-6400 portable photosynthesis instrument (LI-COR Inc. Lincoln, NE, USA) and a SPAD-502 Plus chlorophyll meter (Konica Minolta Optics, Tokyo, Japan), respectively. The length (from the top of the leaf to the base of the petiole) and width (the most) of the leaf were measured using a ruler, while the leaf thickness was examined using a digital Vernier caliper. This experiment was repeated 6 times, and each index had 30 leaves.

2.3. Fruit Economic Characters Measurement

During the period of fruit maturity, 200 mature fruits were randomly selected from each treatment and kept at −20 °C. The average weight was determined by taking measurements of fifteen fruits from all treatments using an electronic balance (known as Shanghai Precision Instrument). The contents of the total soluble solids (TSS) of a single fruit were measured using an ATAGOPR-101a hand-held digital glucometer (Japan). The titratable acid and vitamin C were measured via acid–base titration [13] and 2–6 dichloroindophenol titration, respectively [14].

2.4. Soil Property Measurements

After the harvesting of the fruits, the soil samples were collected using the quartering method from the rhizosphere of 6 trees with constant reproductive and vegetative growth in all treatments, which was carried out by taking about 2 kg of mixed soil samples (0–20 cm) at the drip line around the crown of the bayberry plant and then moving them through a 0.45 mm filter. One-half of the soil samples were used for the DNA extraction and were kept in a freezer at −80 °C. The other samples were naturally air-dried at room temperature for the measurement of soil properties, including the pH, organic matter, alkali-hydrolyzable N, available P, exchangeable calcium, and exchangeable magnesium, which was carried out as described in our previous study [15].

2.5. Soil Genome Sequencing

The extraction of genomic DNA from the soil samples was carried out using a DNA extraction kit. Bacterial diversity was evaluated by targeting the 16S rRNA V3-V4 region, amplified with the primers 343F-5′-TACGGAGGCAGAG-3′ and 798R-5′-AGGGTATCTATCT-3′ [16], whereas, for fungal diversity, the ITSs (ITS1 and ITS2) were amplified using the primers ITS1F-5′-CTTGGTCATTTAGGAAGTAA-3′ and ITS2-5′-GCTGCGTTCTTCATTC GATGC-3′, respectively [17]. Following this process, annotation and BLAST were carried out as reported earlier [5,18]. The alpha diversity of the 16S rDNA and ITS reads was determined using the Chao1 index [19] and Shannon index [20], whereas the QIIME was used with the unweighted distance matrix for phylogenetic trees and the construction of principal coordinate analysis (PCoA).

2.6. GC-MS Metabolomics Analysis

The soil sample used for metabolomic analysis by means of gas chromatography-mass spectrometry (GCMS) was prepared using the method devised by Ren et al. [5]. To validate the authenticity of the whole process, 1 quality control (QC) sample out of every 10 samples was used for comparison, which was achieved by mixing the extract of every sample in an equal proportion. Furthermore, 7890B-5977A GC/MSD GC-MS (Agilent Technologies Inc., Santa Clara, CA, USA) was used to conduct the GC-MS metabolomic analysis of the involved soil samples, as described in a recent publication [5]. A KEGG database search was performed using the resultant metabolite data obtained after comparison with the standard spectrum from the National Institute of Standards.

2.7. Statistical Analysis

Preliminary data analysis was performed using Excel 2010. The Pheatmap software and R 3.6.2 ropls were used to generate the heat map and OPLS-DA, respectively, while redundancy discriminant analysis (RDA), principal coordinates analysis (PCoA), and

community histograms were carried out using R 3.5.1. Furthermore, the Kruskal–Wallis test and SPSS 17.0 software (IBM, Chicago, IL, USA) were used to calculate the α-diversity metrics and carry out the significance test ($p < 0.05$), respectively.

3. Results and Discussion

3.1. Effects of Prochloraz on Vegetative Growth and Fruit Quality

These results showed that the application of prochloraz could significantly improve the tree vigor of diseased trees. Indeed, the application of prochloraz caused increases of 152.84%, 11.11%, 22.30%, 14.53%, 65.78%, and 16.49% in the branch length, leaf length, leaf width, leaf thickness, photosynthetic rate, and chlorophyll content, respectively, as compared to the decline control (Table 1). Similarly, compared with the decline disease control, the application of prochloraz significantly improved fruit quality, with 35.40%, 35.47%, and 13.06% increases seen in the fresh fruit weight, soluble solids, and vitamin C content, respectively, but a 29.46% decrease seen in the titratable acid content (Table 2). Obviously, this result indicated that prochloraz exhibited an alleviation effect on decline-diseased bayberry. Consistent with the results of this study, reductive soil disinfestation (RSD) methods are a promising strategy for supporting plant growth in soil with salinization, acidification, and pathogen accumulation [21].

Table 1. Effects of prochloraz on the vegetative growth of decline-diseased bayberry trees.

Parameters		Parameters	
Length of stem/(mm)		Leaf length/(mm)	
D	13.21 ± 0.54	D	99.51 ± 3.85
P	33.40 ± 1.45 *	P	110.57 ± 1.24 *
Leaf width/(mm)		Leaf thickness/(mm)	
D	27.08 ± 0.41	D	4.06 ± 0.92
P	33.12 ± 0.08 *	P	4.65 ± 0.22 *
Rate of photosynthesis/ ($mg\ CO_2 \cdot 10\ cm^{-2} \cdot h^{-1}$)		Chlorophyll/(SPAD)	
D	1.87 ± 0.28	D	44.62 ± 0.65
P	3.10 ± 0.17 *	P	51.98 ± 0.50 *

D represents decline-diseased trees, while P represents its combination with prochloraz. The * represents significant increases compared to the decline-diseased trees ($p < 0.05$).

Table 2. Effects of prochloraz on the quality of fruits of decline-diseased bayberry.

Parameters		Parameters	
Single fruit weight/g		Soluble solids/%	
D	11.13 ± 0.51	D	8.95 ± 0.25
P	15.07 ± 0.44 *	P	12.07 ± 1.11 *
Titratable acid/%		Vitamin C/(mg/100 g)	
D	1.28 ± 0.12	D	8.73 ± 0.17
P	0.91 ± 0.03 #	P	9.87 ± 0.79 *

D and P represent decline-diseased trees only and in combination with prochloraz, respectively. The * and # represent significant increases or decreases compared to decline-diseased trees ($p < 0.05$).

3.2. The Effect of Prochloraz in Microbial Community Diversity

The number of OTUs in the V3 + V4 region (bacteria) and ITS region (fungi) is shown in Figure 1. The average number of OTUs was 1575 (1203 to 1781) and 1870 (1726 to 2093) in D and P, respectively. In general, P caused 18.76%, 20.05%, and 10.81% increases in the OTU number, Chao1 index, and Shannon index, respectively, of the bacterial community as compared to the diseased trees-only rhizosphere soil (Figure 1). In adverse, no significant difference was observed between the P and D treatments in terms of the OTU number, Shannon index, and Chao1 index of the rhizospheric soil fungal community (Figure 1). This suggests that prochloraz had a greater effect on the bacterial richness and diversity of the rhizospheric soil of trees affected by decline disease compared to those of fungi. Consistent

with the results of this study, biobeds receiving variable pesticide rinsates exhibited a greater bacterial diversity relative to fungal diversity [22].

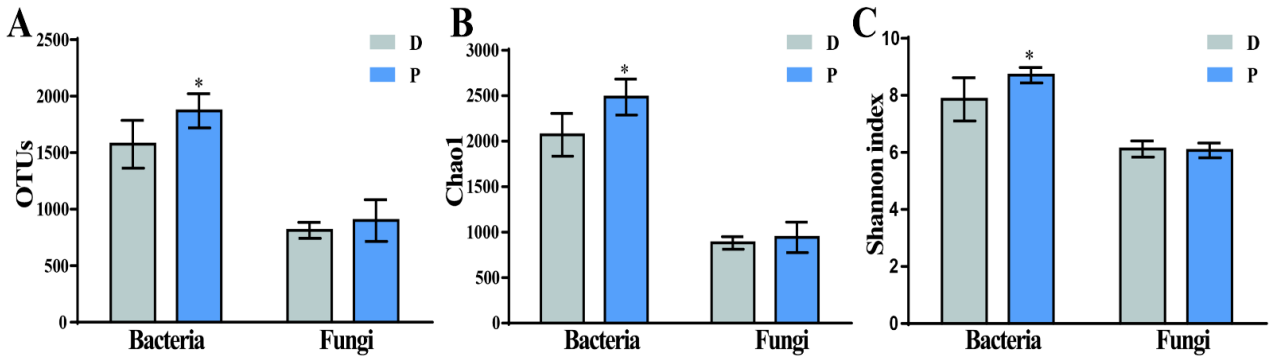

Figure 1. Effect of the fungicide prochloraz on bacterial and fungal community diversity in bayberry rhizosphere soil. (**A**) OTU distribution; (**B**) Chao1 diversity indexes; (**C**) Shannon diversity index. D and P represent decline disease alone and in combination with the fungicide prochloraz, respectively. * on the column indicates that the same parameter of the treatment is significantly larger than that of the control ($p < 0.05$).

3.3. Effect of Prochloraz on Soil Microbial Community Structure

Principal coordinates analysis (PCoA) showed that six replicates of the structure of the bacterial community from the disease control group were grouped into one cluster, while six replicates of the structure of the bacterial community from the prochloraz treatment group were grouped into another cluster, suggesting that the bacterial community structure of the disease control group was quite different from that of the prochloraz treatment group. This indicates that prochloraz significantly changed the bacterial community structure of rhizosphere soil (Figure 2A). Similarly, the six replicates of disease control, as well as prochloraz treatment, were split into two distinct groups based on the PCoA study of the fungal community structure; however, an overlap was found between them (Figure 2B). Overall, compared to the disease control group, the prochloraz treatment caused a greater diversity in the bacterial community structure than that of the fungal community (Figure 2). Consistent with the results of the current study, the soil microorganisms changed in structure and percentage contribution when exposed to Helicur 250 EW fungicide [23].

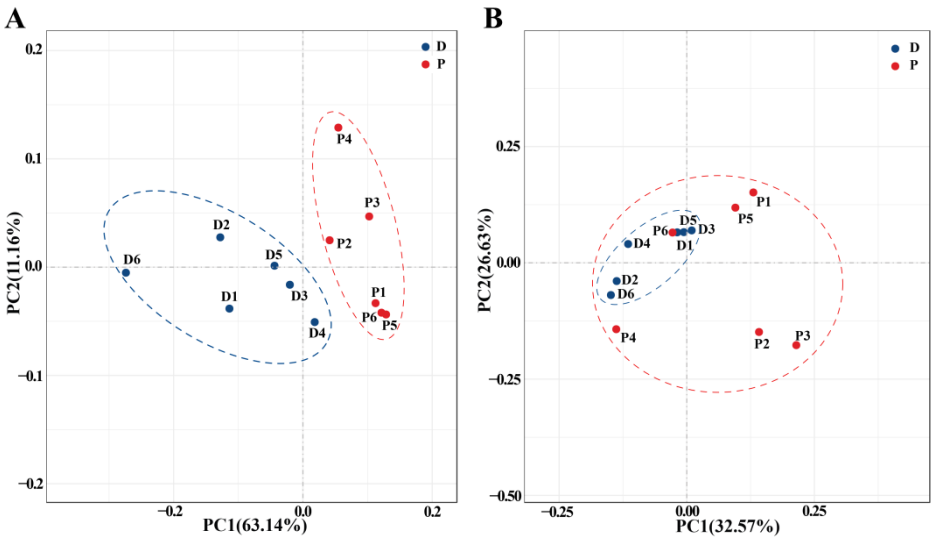

Figure 2. Effect of the fungicide prochloraz on soil microbial OTU abundance based on PCoA. (**A**) Bacteria; (**B**) Fungi. D and P represent decline-diseased trees alone or in combination with the fungicide prochloraz, respectively.

This result showed that, compared to the diseased control, prochloraz caused an important modification in the composition of the fungal and bacterial communities at the levels of phylum (Figure S1), order (Figure S2), and genus (Figure 3). Indeed, a relative abundance histogram was generated by selecting the best 15 microbial species in the rhizospheric soil of the bayberry (Figure 3). The results showed that the main bacterial genera with a >1% relative abundance include *Acidibacter, Rhizomicrobium*, *Acidothermus*, and *Burkholderia*. The relative abundance of *Burkholderia* and *Rhizomicrobium* in the rhizosphere soil of the prochloraz treatment group was significantly increased by 141.27% and 11.99%, respectively, compared to the disease control group (Figure 3A), which is the same as the increased change direction of the decline-diseased soil given the treatment with two fertilizers [24]. The relative abundance of *Acidibacter* decreased by 39.29% (Figure 3A), which is the same as the decreased change direction of the decline-diseased soil given the treatment with two fertilizers [24]. The abundance of *Acidothermus* did not change significantly (Figure 3A), which is different from the increased change seen in the compound fertilizer and the decreased change seen in the bio-organic fertilizer treatment on the decline-diseased soil [24]. *Burkholderia,* which has nitrogen fixation and potential growth-promoting effects, can improve plant production; the activities of soil sucrose, catalase, urease, and phosphatase; and the expression of defense-related genes [25,26]. The main genera (average relative abundance > 1%) were *Mortierella*, *Trichoderma*, *Geminibasidium*, and *Cladophialophora*, which were amongst the top 15 dominant fungal genera in terms of the distribution and relative abundance of fungal species in the rhizospheric soil at the genus level, which accounts for >75% of fungal sequences (Figure 3B). The relative abundance of *Geminibasidium* was significantly increased by 99.86%, which is the same as the increased change direction of the compound fertilizer treatment of the decline-diseased soil but different from the decreased change direction seen for the bio-organic fertilizer treatment [24]. The relative abundance of *Trichoderma* and *Cladophialophora* was significantly decreased by 33.51% and 9.95%, respectively, which is the same as the decreased change direction of the compound and bio-organic fertilizer treatment of the decline-diseased soil [24]. There was no significant change in the relative abundance of *Mortierella*, which is different from the increased change direction seen for the two-fertilizer treatment of the decline-diseased soil [24]. The functions of *Geminibasidium* and *Cladophialophora* have not yet been reported and may be harmful to plant growth.

3.4. Effect of Prochloraz on the Soil Nutrient Status

Compared with the decline control, the rhizosphere soil pH, organic matter, and exchangeable calcium of P were significantly increased by 14.71%, 8.21%, and 8.00%, respectively, while the contents of available phosphorus, exchangeable magnesium, and alkali-hydrolyzable nitrogen were significantly decreased by 48.08%, 16.66%, and 8.17%, respectively (Table 3). These results indicated that the application of prochloraz affected the chemical and physical properties, as well as the pH of rhizosphere soil, from decline-diseased bayberry trees. The pH change is different from that seen for the compound and bio-organic fertilizer treatment of the decline-diseased soil [24]. The organic matter, exchangeable calcium, and available phosphorus were the same as those seen for the decline-diseased soil given the bio-organic fertilizer treatment [24]. The exchangeable magnesium and alkali-hydrolyzable nitrogen were the same as those seen for the decline-diseased soil given a compound fertilizer treatment [24].

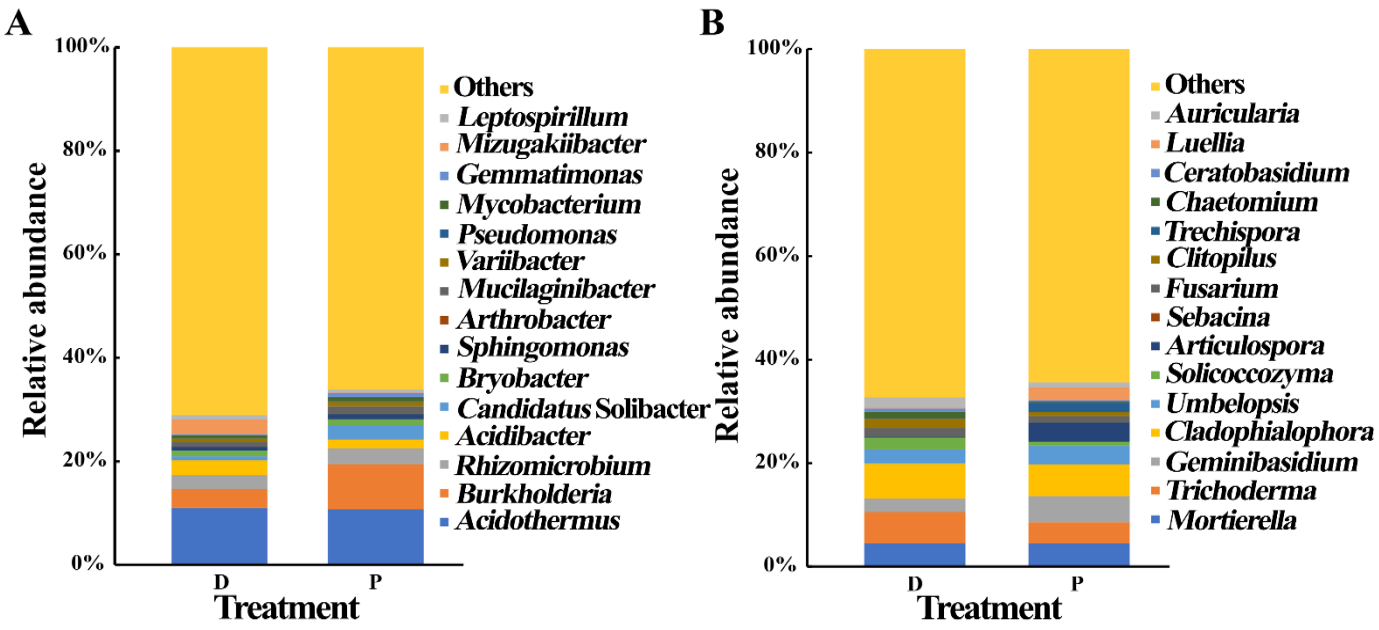

Figure 3. Effect of the fungicide prochloraz on the relative microbial abundance of bayberry rhizosphere soil at the genus level. (**A**) Bacteria; (**B**) Fungi. D and P represent decline-diseased trees only and in combination with the fungicide prochloraz, respectively.

Table 3. The effect of prochloraz on rhizosphere soil pH and physical and chemical properties of decline-diseased bayberry.

Parameters	D	P	Parameters	D	P
pH	4.69 ± 0.39	5.38 ± 0.45 *	AP (mg/kg)	30.78 ± 0.50	15.98 ± 1.72 #
OM (%)	2.80 ± 0.12	3.03 ± 0.24 *	EC (mg/kg)	348.33 ± 9.49	376.20 ± 11.74 *
AHN (mg/kg)	117.90 ± 4.58	108.27 ± 3.84 #	EM (mg/kg)	35.54 ± 1.12	29.62 ± 1.41 #

D and P represent decline-diseased trees alone and in combination with prochloraz, respectively. The * and # represent significant increases or reductions compared to decline-diseased trees ($p < 0.05$). AP: available phosphorus; OM: organic matter; EC: exchangeable calcium; AHN: alkali-hydrolyzable nitrogen; EM: exchangeable magnesium.

3.5. Effect of Prochloraz on RDA

A soil properties analysis shows that prochloraz influenced the fungal and bacterial communities in the rhizospheric soil of bayberry at their genus levels (Figure 4; Table 4). In detail, totals of 59.46% and 25.61% of the cumulative variance were observed at the genus levels of bacteria (Figure 4A) and fungi (Figure 4B), respectively, based on a rhizospheric microbial community-correction factor analysis. The contributions of the four main variables, pH, available phosphorus, alkali-hydrolyzable nitrogen, and exchangeable magnesium, explained 38.4%, 37.7%, 21.0%, and 15.5% of the bacterial communities at the genus level, respectively (Figure 4A, Table 4), whereas the pH, exchangeable calcium, and available phosphorus explained 10.2%, 10.8%, and 10.0% of the species of fungi at the genus level, respectively (Figure 4B, Table 4). Generally, the number of bacteria and fungi in rhizospheric soil was significantly impacted at the level of the genus by the pH, exchangeable magnesium, available phosphorus, and alkali-hydrolyzable nitrogen whereas these four variables exhibited more pressure on bacterial species as compared to fungal species. The pH and available phosphorus were the main two factors that significantly affected the composition of bacteria and fungi in the rhizospheric soil of bayberry trees at the genus level.

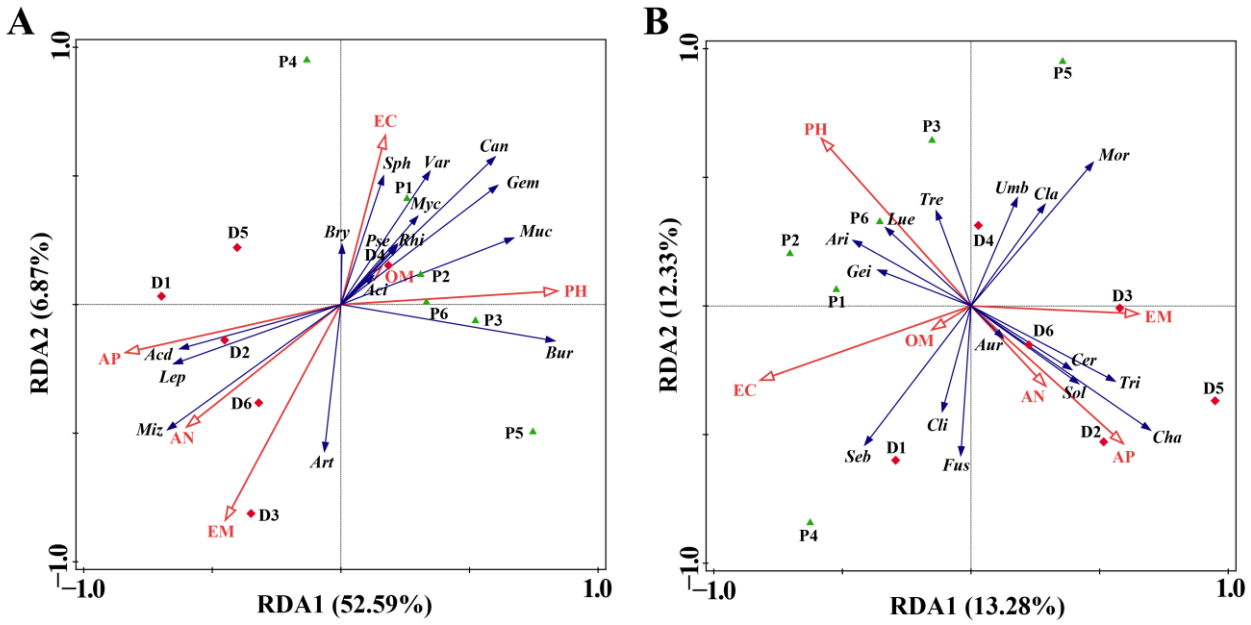

Figure 4. Redundancy discriminant analysis (RDA) of microbial community composition in rhizosphere soil at genus levels. (**A**) Bacteria; (**B**) Fungi. The red diamond and green up triangle icons represent samples from decline-diseased bayberry alone (D) and disease in combination with prochloraz (P). PH (pH); OM (organic matter); AN (alkali-hydrolyzable nitrogen); AP (available phosphorus); EC (exchangeable calcium); EM (exchangeable magnesium); *Acd* (*Acidibacter*); *Aci* (*Acidothermusl*); *Ari* (*Articulospora*); *Art* (*Arthrobacter*); *Aur* (*Auricularia*); *Bry* (*Bryobacter*); *Bur* (*Burkholderia*); *Can* (*Candidatus solibacter*); *Cer* (*Ceratobasidium*); *Cha* (*Chaetomium*); *Cla* (*Cladophialophora*); *Cli* (*Clitopilus*); *Fus* (*Fusarium*); *Gem* (*Gemmatimonas*); *Gmi* (*Geminibasidium*); *Lep* (*Leptospirillum*); *Lue* (*Luellia*); *Miz* (*Mizugakiibacter*); *Mor* (*Mortierella*); *Muc* (*Mucilaginibacter*); *Myc* (*Mycobacterium*); *Pse* (*Pseudomonas*); *Rhi* (*Rhizomicrobium*); *Seb* (*Sebacina*); *Sol* (*Solicoccozyma*); *Sph* (*Sphingomonas*); *Tre* (*Trechispora*); *Tri* (*Trichoderma*); *Umb* (*Umbelopsis*); *Var* (*Variibacter*).

Table 4. Contribution of soil properties to rhizosphere soil bacterial and fungal taxa at the genus level.

Soil Environment	Contribution at Genus Level (%)		Soil Environment	Contribution at Genus Level (%)	
	Bacteria	Fungi		Bacteria	Fungi
pH	38.4	10.2	AP	37.7	10.0
OM	4.0	2.0	EC	6.6	10.8
AHN	21.0	5.0	EM	15.5	6.6

AP: available phosphorus; OM: organic matter; EC: exchangeable calcium; AHN: alkali-hydrolyzable nitrogen; EM: exchangeable magnesium.

Furthermore, the results of the current study helped to identify a complex relationship between fungicide, soil nutrient elements, plant/microbial growth, and soil parameters due to differences between the plants affected by the disease only and the disease in combination with the application of prochloraz in terms of the available soil nutrient elements, such as the contents of available phosphorus, alkali-hydrolyzable nitrogen, exchangeable magnesium, and exchangeable calcium, as well as pH in the rhizospheric environment of bayberry. Consistent with this result, the soil microorganisms were affected by many kinds of ecological components, such as non-target fungicides, alkali-hydrolyzable nitrogen, soil pH, and the contents of organic matter, exchangeable magnesium, exchangeable calcium, and available phosphorus [5,24,27,28].

3.6. Effect of Prochloraz on Rhizosphere Soil Metabolomics

In this study, we identified a total of 223 metabonomics through the GC-MS analysis of rhizospheric soils from the diseased control and prochloraz treatment groups. Indeed, a metabolite score map was obtained (Figure 5) by applying the "orthogonal partial least squares discriminant analysis (OPLS-DA)", which has been considered as a controlled statistical method for the analysis of discriminants [5,24]. Thus, metabonomics could be effectively separated between D and P. The analysis of the differences between the treatments showed that the six sample points of D and P were dispersed in the negative and positive areas of t(1), respectively (Figure 5). The results show that the model values of D and P were R^2X (cum) = 0.592, R^2Y (cum)= 1, and Q^2 (cum)= 0.994, while the R^2 value was 0.772 and the Q^2 value was 0.994. The Q^2 and R^2 values (>0.5) showed that the model had a good interpretation and prediction ability, as the R^2 values were >0.5. The cluster separation effect of D and P indicated that the application of prochloraz fungicide significantly changed the metabolic structure of the rhizosphere soil of decline-diseased bayberry.

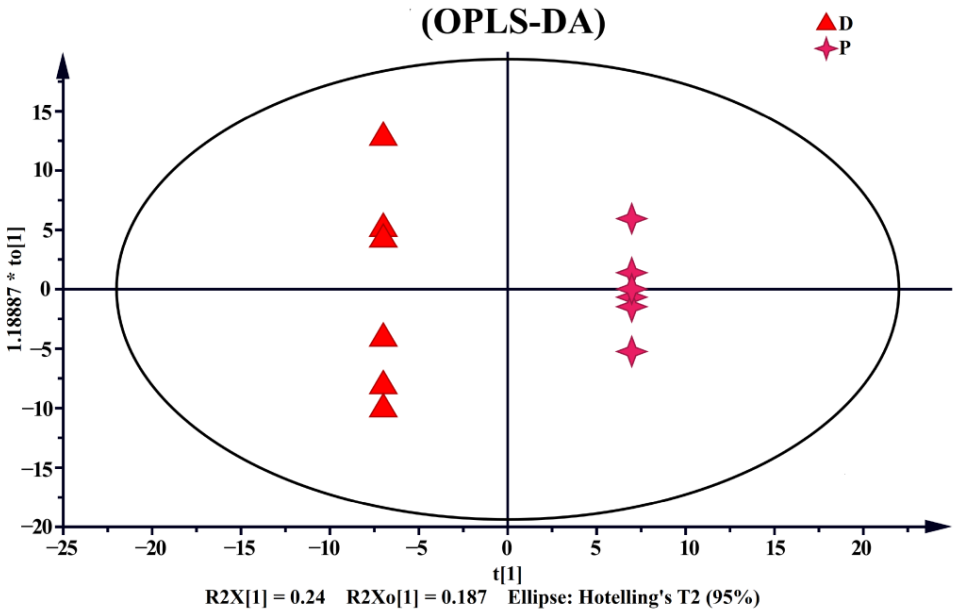

Figure 5. OPLS-DA score map of bayberry rhizosphere soil of the fungicide prochloraz treatment. D and P represent decline-diseased trees alone or in trees affected by decline disease in combination with the fungicide prochloraz, respectively. Effect of the fungicide prochloraz on differential metabolites analysis.

The results of the cluster analysis showed the contents and changes of metabolites in rhizosphere soil under D and P. The main metabolites showing significant changes were sugar, amino acids, secondary metabolites, and organic acids. There were 58 metabolites that showed significant changes between D and P, with 51 being significantly upregulated by 18.59–149.48% (with uridine 5′-monophosphate being the most upregulated) and 7 being significantly downregulated by 31.52–95.21% (with diclofenac being the least downregulated) (Figure 6; Table 5). The seven metabolites with reduced relative contents were diclofenac, 3,4-dihydroxybenzoic acid, formaldehyde acid, glycolic acid, oxamic acid, ornithine, and 4′,5-dihydroxy-7-glucosyloxyflavanone, which may be harmful to plant growth (Figure 6; Table 5). The relative content of diclofenac was also significantly reduced in the decline-diseased rhizosphere soil treated with compound fertilizer and bioorganic fertilizer [24]. Diclofenac is a substance that has polluted the global environment. It has serious effects on plant growth and development, mainly through the activity inhibition of the mitochondrial respiratory ETC (electron transport chain) and respiratory carbon metabolism pathways, resulting in the disorder of the intracellular material and energy metabolisms and an increase in the activity of glutathione S-transferase in the roots, re-

sulting in abiotic stress [29]. The content of diclofenac decreased in P, which may not only reduce the environmental pollution but also be beneficial for the development and growth of bayberry trees via increasing the activities of the mitochondrial respiratory ETC and normal functioning of the carbon metabolism pathway.

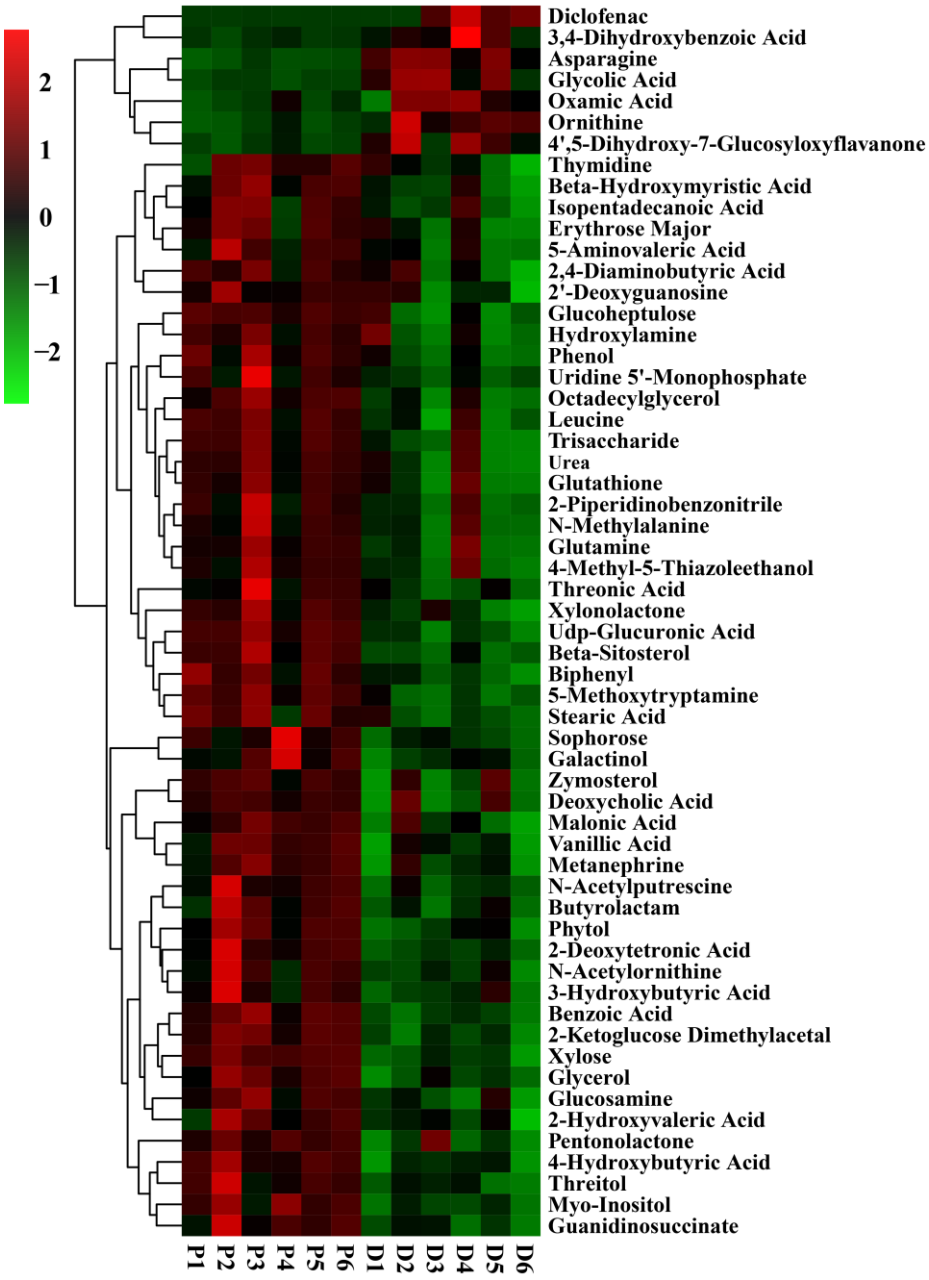

Figure 6. Thermogram analysis of metabolites in the rhizosphere soils of bayberry. D and P represent trees with decline diseased and disease in combination with the fungicide prochloraz, respectively.

Table 5. The relative contents of the metabolites in rhizosphere soil were changed by prochlora on the decline-diseased trees.

Metabolite Name	Relative Content	Metabolite Name	Relative Content
Diclofenac		3,4-Dihydroxybenzoic Acid	
D	130.89 ± 99.65	D	5.52 ± 3.18
P	6.27 # ± 0.89	P	2.13 # ± 0.40
Asparagine		Glycolic Acid	
D	1.28 ± 0.20	D	66.58 ± 12.30
P	0.74 # ± 0.04	P	45.60 # ± 1.26
Oxamic Acid		Ornithine	
D	44.11 ± 14.76	D	0.40 ± 0.14
P	27.90 # ± 5.43	P	0.15 # ± 0.04
4′,5-Dihydroxy-7-Glucosyloxyflavanone		Thymidine	
D	10.72 ± 5.57	D	1.27 ± 0.23
P	2.88 # ± 1.24	P	1.58 * ± 0.20
Beta-Hydroxymyristic Acid		Isopentadecanoic Acid	
D	3.71 ± 0.74	D	0.38 ± 0.11
P	5.21 * ± 0.68	P	0.56 * ± 0.11
Erythrose Major		5-Aminovaleric Acid	
D	1.65 ± 0.40	D	0.33 ± 0.07
P	2.23 * ± 0.33	P	0.45 * ± 0.08
2,4-Diaminobutyric Acid		2′-Deoxyguanosine	
D	0.93 ± 0.201	D	0.25 ± 0.05
P	1.17 * ± 0.10	P	0.31 * ± 0.03
Glucoheptulose		Hydroxylamine	
D	3.90 ± 1.51	D	20.18 ± 7.32
P	6.54 * ± 0.38	P	28.77 * ± 3.39
Phenol		Uridine 5′-Monophosphate	
D	18.94 ± 6.24	D	0.23 ± 0.09
P	33.98 * ± 6.72	P	0.57 * ± 0.25
Octadecylglycerol		Leucine	
D	0.25 ± 0.06	D	1.75 ± 0.40
P	0.38 * ± 0.04	P	2.43 * ± 0.22
Trisaccharide		Urea	
D	1022.82 ± 217.23	D	150.49 ± 36.48
P	1393.56 * ± 117.51	P	198.22 * ± 17.28
Glutathione		2-Piperidinobenzonitrile	
D	0.97 ± 0.27	D	0.37 ± 0.11
P	1.29 * ± 0.14	P	0.54 * ± 0.12
N-Methylalanine		Glutamine	
D	2.55 ± 0.66	D	3.52 ± 0.95
P	3.48 * ± 0.62	P	4.71 * ± 0.55
4-Methyl-5-Thiazoleethanol		Threonic Acid	
D	0.28 ± 0.07	D	0.86 ± 0.14
W	0.37 * ± 0.05	P	1.20 * ± 0.26
Xylonolactone		Udp-Glucuronic Acid	
D	1.28 ± 0.14	D	0.32 ± 0.05
W	1.56 * ± 0.12	P	0.53 * ± 0.05
Beta-Sitostero		Biphenyl	
D	l1.64 ± 0.42	D	0.59 ± 0.14
P	3.50 * ± 0.64	P	1.07 * ± 0.17
5-Methoxytryptamine		Stearic Acid	
D	38.13 ± 12.79	D	1.80 ± 0.53
P	83.51 * ± 11.83	P	3.20 * ± 0.69
Sophorose		Galactinol	
D	8.91 ± 1.63	D	1.18 ± 0.40
P	14.53 * ± 3.52	P	2.22 * ± 0.69

Table 5. *Cont.*

Metabolite Name	Relative Content	Metabolite Name	Relative Content
Zymosterol		Deoxycholic Acid	
D	0.46 ± 0.23	D	0.45 ± 0.21
P	0.73 * ± 0.08	P	0.67 * ± 0.04
Malonic Acid		Vanillic Acid	
D	0.21 ± 0.03	D	3.69 ± 0.83
P	0.25 * ± 0.01	P	5.24 * ± 0.55
Metanephrine		N-Acetylputrescine	
D	0.79 ± 0.35	D	4.19 ± 1.06
P	1.42 * ± 0.23	P	7.34 * ± 1.77
Butyrolactam		Phytol	
D	4.75 ± 0.75	D	1.66 ± 0.45
P	6.70 * ± 1.18	P	2.79 * ± 0.47
2-Deoxytetronic Acid		N-Acetylornithine	
D	2.52 ± 0.63	D	2.52 ± 0.72
P	6.04 * ± 1.79	P	4.35 * ± 1.26
3-Hydroxybutyric Acid		Benzoic Acid	
D	63.10 ± 29.70	D	2.45 ± 0.40
P	126.31 * ± 46.02	P	4.58 * ± 0.61
2-Ketoglucose Dimethylacetal		Xylose	
D	0.16 ± 0.05	D	0.79 ± 0.10
P	0.35 * ± 0.05	P	1.20 * ± 0.05
Glycerol		Glucosamine	
D	195.86 ± 34.98	D	0.70 ± 0.18
P	308.98 * ± 37.76	P	1.06 * ± 0.14
2-Hydroxyvaleric Acid		Pentonolactone	
D	2.79 ± 0.46	D	2.44 ± 0.84
P	3.55 * ± 0.49	P	3.66 * ± 0.27
4-Hydroxybutyric Acid		Threitol	
D	13.86 ± 3.64	D	0.42 ± 0.10
P	23.50 * ± 3.05	P	0.70 * ± 0.15
Myo-Inositol		Guanidinosuccinate	
D	15.64 ± 3.93	D	0.36 ± 0.12
P	32.54 * ± 6.86	P	0.73 * ± 0.20

D and P represent trees affected by decline disease alone and disease in combination with prochloraz, respectively. The * and # represent significant increases or decreases compared to decline-diseased trees ($p < 0.05$).

The 51 metabolites with an increased relative content may be beneficial to plant growth. The relative contents of galactinol and 2-ketoglucose dimethylacetal were increased by 88.66% and 124.64%, respectively. The relative contents of the various metabolites were also significantly increased in the decline-diseased rhizosphere soil treated with compound fertilizer and bioorganic fertilizer [24]. Sugar and sugar alcohols can be used as energy sources for many microorganisms and as general chemotactic substances [30]. Secondary metabolites include alkaloids (5-methoxytryptamine and metanephrine), phytosterols (beta-sitosterol and zymosterol), and other substances (myo-inositol, guanidinosuccinate, N-acetylputrescine, and uridine 5′-monophosphate). The contents of the above substances were significantly increased by 59.31–149.48% (Figure 6; Table 5). Studies showed that phytosterols may play an important role in seed germination, which explains the low germination efficiency of immature seeds [31]. Thermal oxidation treatment may lead to the formation of phytosterol oxidation products, such as ketones, hydroxyl groups, and epoxy derivatives [32]. Inositol participates in plant response to stress by phosphatidylinositol signal transduction, auxin storage and transport, phytic acid biosynthesis, and cell wall biosynthesis, as well as by reducing membrane lipid peroxidation damage, enhancing antioxidant enzyme activity, and protecting the cell membrane system [33–35]. The inositol content of P increased, which may be beneficial to the growth and development of bayberry and help to enhance its stress resistance. In conclusion, the content of metabolites in the rhizosphere soil of decline-diseased bayberry changed after the application of prochloraz,

and the content of metabolites in P rhizosphere soil was mostly higher than that of decline-diseased bayberry tree, with prochloraz causing the metabolic function of metabolites in decline-diseased rhizosphere soil to be more active.

Consistent with the results of this study, some soil metabolites have been found to play a key role in the rhizosphere soil environment. For example, phenolic compounds and rosmarinic acid benefit plants by changing the soil properties, regulating microbial communities, and controlling abiotic and biological processes [36–38]. The upregulation of fatty acids, small dicarboxylic acids, and polysaccharides might enrich the microbial community of the soil after the application of SiO_2-NP in rhizosphere soil [39]. In contrast, some soil metabolites are found to be harmful to bayberry trees—for instance, esters could hinder the respiratory system of plants, adversely affect the cell structure of plants, and decrease the richness of the bacterial communities in rhizosphere soil [40]; the acid compounds could change the community structures of soil microbiota, as well as showing "low concentration promotion and high concentration inhibition" effects on seedling growth [41]. Therefore, the metabolites in soil are very important for the growth and development of plants and microorganisms.

3.7. Effect of Prochloraz on the Metabolic Pathways

The results of this study showed that D and P differed in four metabolic pathways, including pyrimidine metabolism ($p < 0.01$), arginine and proline metabolism ($p < 0.01$), galactose metabolism ($p < 0.01$), and butanoate metabolism ($p < 0.05$), based on a pathway enrichment analysis of various secondary metabolites based on the "KEGG (Kyoto Encyclopedia of Genes and Genomes) database" (Figure 7). The results showed that the application of prochloraz improved the content of metabolites, promoting metabolic function and that amino acid metabolism and sugar metabolism were the key metabolic pathways of the differential metabolites between the diseased trees and the rhizosphere soil treated with prochloraz.

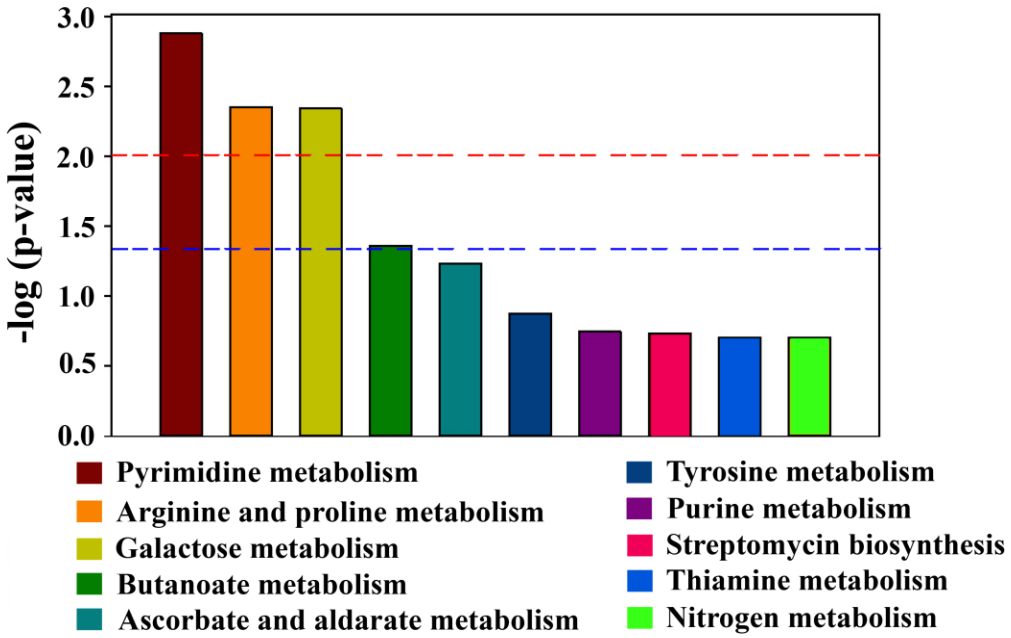

Figure 7. Metabolic pathway enrichment map of different metabolites in bayberry rhizosphere soil. D and P represent trees affected by decline diseased and disease in combination with the fungicide prochloraz, respectively. When the top of the bar is higher than the blue ($p < 0.05$) or red line ($p < 0.01$), the signal pathway is significant.

The pyrimidine metabolic pathway is involved in the formation of pyrimidines, as well as integration into nucleic acids, lipids, and sugars [42], while galactose is crucial

for human metabolism, with various roles in energy delivery and the galactosylation of complex molecules [43]. The pyrimidine pathway and galactose metabolism also show significant changes between decline disease and compound/bio-organic fertilizer [24]. Arginine and proline are important amino acids in plants. Arginine has functions in ammonia detoxification, hormone secretion, immune regulation, and nitrogen storage. At the same time, proline helps to restore growth and functions to maintain protoplasm, maintain the environmental osmotic balance, and prevent water loss. Its metabolism also plays an important role in cold resistance, drought resistance, and salt tolerance [44]. Therefore, prochlora may promote the root growth of decline-diseased bayberry through the pyrimidine pathway, galactose metabolism, and arginine and proline metabolism and improve the stress resistance and cold resistance of decline-diseased bayberry.

3.8. Correlations of Soil Microbial Communities with Plant Secondary Metabolites

The bacterial and fungal genera and metabolites of D and P showing significant differences were significantly correlated (Figure 8; Figures S3 and S4). Among the 58 different metabolites, the bacteria *Burkholderia*, Candidatus *Solibacter*, *Mucilaginibacter*, *Mycobacterium*, and *Gemmatimonas* were positively correlated with the relative contents of main secondary metabolites, in which 20, 31, 37, 11, and 23 species were significantly positively correlated among them, respectively, and only 3, 4, 1, 1, and 3 species were significantly negatively correlated, respectively. The bacteria *Acidibacter*, *Mizugakiibacter*, and *Leptospirillum* and fungus *Chaetomium* were negatively correlated with the relative contents of major secondary metabolites, of which 16, 44, 25, and 10 species were negatively correlated, respectively, and only 3, 2, 1, and 4 species showed a significant positive correlation. Seven metabolites with a reduced relative content are diclofenac, 3,4-dihydroxybenzoic acid, formaldehyde acid, glycolic acid, oxamic acid, ornithine, and 4′,5-dihydroxy7-glucosyloxyflavanone, and these metabolites were negatively correlated with *Burkholderia*, Candidatus *Solibacter*, *Mucilaginibacter*, *Mycobacterium*, and *Gemmatimonason*. On the other hand, they were positively correlated with the bacteria *Acidibacter*, *Mizugakiibacter*, and *Leptospirillum* and the fungus *Chaetomium*. The other 51 metabolites were positively correlated with *Burkholderia*, Candidatus *Solibacter*, *Mucilaginibacter*, *Mycobacterium*, and *Gemmatimonason*; on the other hand, they were negatively correlated with the bacteria *Acidibacter*, *Mizugakiibacter*, and *Leptospirillum* and the fungus *Chaetomium*. This indicates that the species and relative contents of microorganisms in the rhizospheric soil are correlated with the species and relative metabolites. After the application of prochlora, the main metabolites and microbiota in the rhizospheric soil of decline-diseased bayberry trees could interact with each other. The increase in beneficial microorganisms may cause a rise in beneficial secondary metabolites, as well as limit the number of harmful microorganisms that may cause a decrease in harmful metabolites. These two effects enhance the stress resistance of decline-diseased bayberry trees and promote recovery from disease.

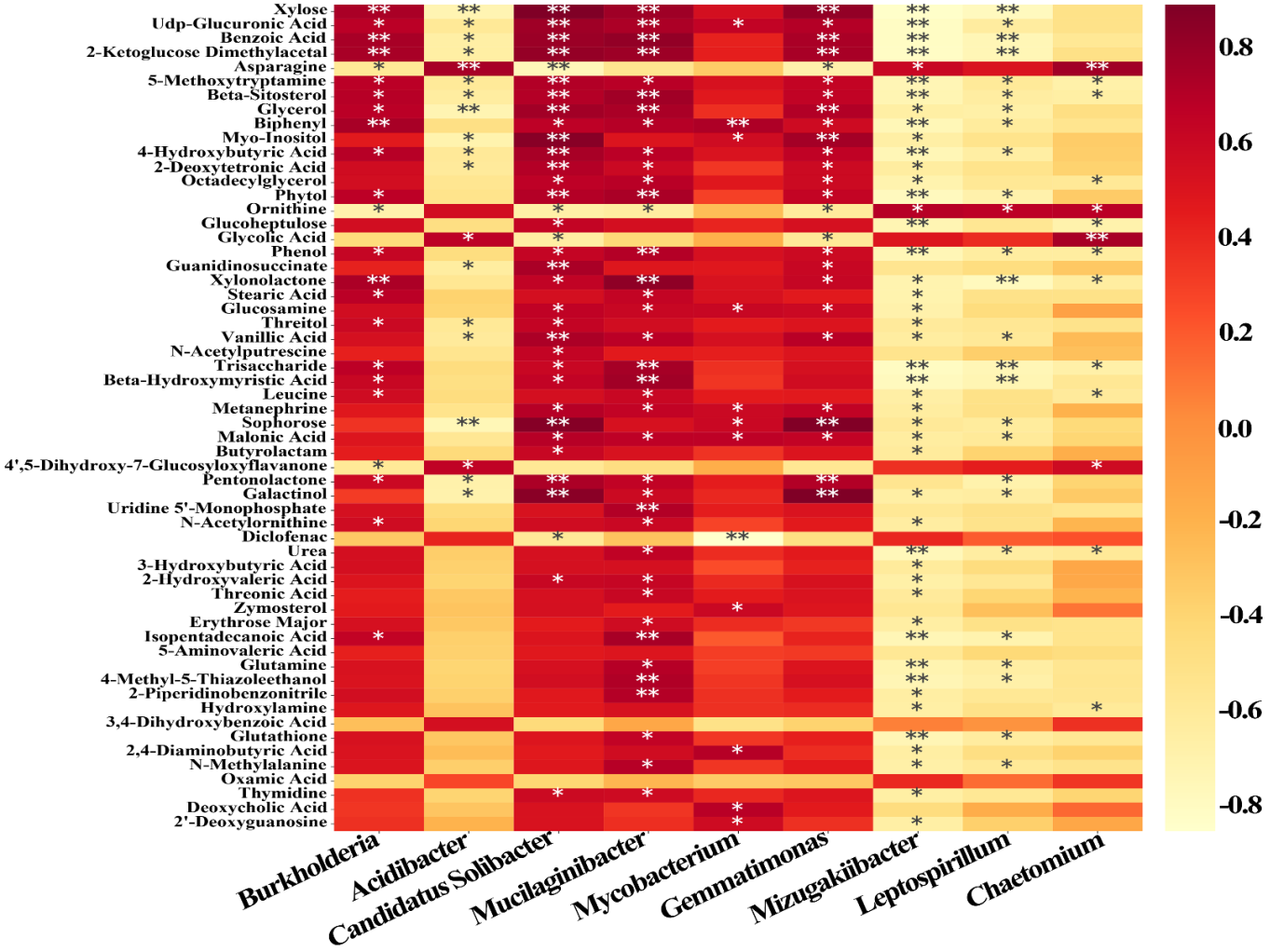

Figure 8. Correlation analysis between the relative abundances of the soil microbiota at the genus level and the metabolite relative contents in the fungicide prochloraz treatment. * and ** mean significance at $p < 0.05$ and $p < 0.01$, respectively. The depth of the orange scale indicates the magnitude of the correlation coefficient; the lighter the color is, the greater the negative correlation is, while the darker color is, the greater the positive correlation is.

4. Conclusions

These results indicate that prochloraz affected the plant growth, microbiota, fruit quality, secondary metabolites, and rhizosphere soil characteristics. The use of prochloraz improved the tree vigor and fruit quality of bayberry with decline disease. The diversity of the rhizosphere soil of trees with decline disease and the bacterial richness was greatly affected by prochloraz, in contrast to the lesser impacts seen on fungi. In the rhizosphere soil of P, the content of *Burkholderia*, *Rhizomicrobium*, and *Geminibasidium* was significantly increased; however, the content of *Acidibacter*, *Trichoderma*, and *Cladophialophora* was decreased compared with that of D. Furthermore, the community composition of bacteria and fungi in the rhizosphere soil was greatly influenced at the genus level by the pH, available phosphorus, alkali-hydrolyzable nitrogen, and exchangeable magnesium. These four variables also exhibited a significant influence on the bacterial population in comparison to the fungal population. The available phosphorus and pH were the two main factors influencing the composition of the fungal and bacterial population present in the rhizosphere soil of bayberry at the genus level. In addition, the application of prochloraz fungicide significantly modified the metabolic pattern of the rhizosphere soil of the decline-diseased bayberry. Among the 58 metabolites that were significantly changed in

comparison to the control, 51 were highly upregulated, with uridine 5′-monophosphate being the most upregulated, while 7 were significantly downregulated, with diclofenac being the least downregulated. A significant difference was found between D and P in terms of the metabolic pathways of pyrimidine, galactose, proline, arginine, and butanoate. The microbial groups and metabolites were significantly correlated at the levels of the phylum, order, and genus. In conclusion, these results provide knowledge of an alternative means to protect bayberry from the negative impact of decline disease.

Supplementary Materials: The supplementary data related to this article can be found online at https://www.mdpi.com/article/10.3390/agronomy12030677/s1. Figure S1: Relative abundance of rhizosphere soil bacteria (A) and fungi (B) at the phylum level; Figure S2: Relative abundance of rhizosphere soil bacteria (A) and fungi (B) at the order level; Figure S3: Heatmap of correlation analysis between the relative abundances of rhizosphere soil microbiota at the phylum level and the metabolite relative contents; Figure S4: Heatmap of correlation analysis between the relative abundances of rhizosphere soil microbiota at the order level and the metabolite relative contents.

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References

1. Ren, H.Y.; Yu, H.Y.; Zhang, S.W.; Liang, S.M.; Zheng, X.L.; Zhang, S.J.; Yao, P.; Zheng, H.K.; Qi, X.J. Genome sequencing provides insights into the evolution and antioxidant activity of Chinese bayberry. *BMC Genom.* **2019**, *20*, 458. [CrossRef] [PubMed]
2. Sun, C.; Huang, H.Z.; Xu, C.J.; Li, X.; Chen, K.S. Biological activities of extracts from Chinese bayberry (*Myrica rubra* Sieb. et Zucc.): A review. *Plant Foods Hum. Nutr.* **2013**, *68*, 97–106. [CrossRef] [PubMed]
3. Erickson, D.L.; Hamrick, J.L. Genetic and clonal diversity *Myrica cerifera* for along a spatiotemporal island chronosequence. *Heredity* **2003**, *90*, 25–32. [CrossRef] [PubMed]
4. Ren, H.Y.; Zheng, X.L.; Zhang, S.W.; Liang, S.M.; Wu, C.W.; Wang, K.Q.; Yu, Z.P.; Qi, X.J. The symptoms and nutritional status of decline diseased bayberry. *Zhejiang Agri. Sci.* **2020**, *61*, 2043–2048. (In Chinese)
5. Ren, H.Y.; Wang, H.Y.; Qi, X.J.; Yu, Z.P.; Zheng, X.L.; Zhang, S.W.; Wang, Z.S.; Zhang, M.C.; Ahmed, T.; Li, B. The damage caused by decline disease in bayberry plants through changes in soil properties, rhizosphere microbial community structure and metabolites. *Plants* **2021**, *10*, 2083. [CrossRef] [PubMed]
6. Ren, H.Y.; Wang, J.; Qi, X.J.; Yu, Z.P.; Zhang, Q. The rejuvenation effect of 10 fungicides on decline disease bayberry. *Zhejiang Agri. Sci.* **2021**, *62*, 27–30. (In Chinese)
7. Zhang, R.; Xu, Q.R.; Zhang, Y.C.; Zhu, F.X. Baseline sensitivity and toxic actions of prochloraz to *Sclerotinias clerotiorum*. *Plant Dis.* **2018**, *102*, 2149–2157. [CrossRef] [PubMed]
8. Frąc, M.; Lipiec, J.; Usowicz, B.; Oszust, K.; Brzezińska, M. Structural and functional microbial diversity of sandy soil under cropland and grassland. *PeerJ* **2020**, *8*, e9501. [CrossRef]
9. Zhang, Y.; Tian, C.; Xiao, J.L.; Wei, L.; Tian, Y.; Liang, Z.H. Soil inoculation of *Trichoderma asperellum* M45a regulates rhizosphere microbes and triggers watermelon resistance to *Fusarium* wilt. *AMB Express* **2020**, *10*, 189. [CrossRef] [PubMed]

10. Zhang, C.; Zhou, T.; Zhu, L.; Juhasz, A.; Du, Z.; Li, B.; Wang, J.; Wang, J.; Sun, Y. Response of soil microbes after direct contact with pyraclostrobin in fluvo-aquic soil. *Environ. Pollut.* **2019**, *255 Pt 1*, 113164. [CrossRef]
11. Wang, L.G.; Shu, N.L.; Sun, K.G. The process of soybean continuous cropping obstacle and control teachnology. *Soil Fert. Sci. Chin.* **2001**, *5*, 3–8.
12. Lucie, M.; Jakub, R.; Marketa, P.; Tomas, M.; Ondrej, U. Effects of secondary plant metabolites on microbial populations: Changes in community structure and metabolic activity in contaminated environments. *Int. J. Mol. Sci.* **2016**, *17*, 1205.
13. Sogi, D.S.; Siddiq, M.; Dolan, K.D. Total phenolics, carotenoids and antioxidant properties of tommy atkin mango cubes as affected by drying techniques. *LWT-Food Sci. Technol.* **2015**, *62*, 564–568. [CrossRef]
14. Jiang, Y.; Nie, W.J. Chemical properties in fruits of mulberry species from the Xinjiang province of China. *Food Chem.* **2015**, *174*, 460–466. [CrossRef]
15. Yin, H.; Zheng, H.; Zhang, B.; Tariq, A.; Lv, G.; Zeng, F.; Graciano, C. Stoichiometry of C:N:P in the roots of alhagi sparsifolia is more sensitive to soil nutrients than aboveground organs. *Front. Plant Sci.* **2021**, *12*, 698961. [CrossRef]
16. Wu, L.Y.; Wen, C.Q.; Qin, Y.J.; Yin, H.Q.; Tu, Q.C.; Nostrand, J.D.V.; Yuan, T.; Yuan, M.T.; Deng, Y.; Zhou, J.Z. Phasing amplicon sequencing on Illumina Miseq for robust environmental microbial community analysis. *BMC Microbio.* **2015**, *15*, 125. [CrossRef]
17. Mukherjee, P.K.; Chandra, J.; Retuerto, M.; Sikaroodi, M.; Brown, R.E.; Jurevic, R.; Salata, R.A.; Lederman, M.M.; Gillevet, P.M.; Ghannoum, M.A. Oral mycobiome analysis of HIV-infected patients: Identification of pichia as an antagonist of opportunistic fungi. *PLoS Pathog.* **2014**, *10*, e1003996. [CrossRef]
18. Reyon, D.; Tsai, S.Q.; Khayter, C.; Foden, J.A.; Sander, J.D.; Joung, J.K. FLASH assembly of TALENs for high-throughput genome editing. *Nat. Biotech.* **2012**, *30*, 460–465. [CrossRef]
19. Chao, J.; Bunge, J. Estimatin the number of species in a stochastic abundance model. *Biometrics* **2002**, *58*, 531–539. [CrossRef]
20. Hill, T.C.; Walsh, K.A.; Harris, J.A.; Moffett, B.F. Using ecological diversity measures with bacterial communities. *FEMS Microbiol. Ecol.* **2003**, *43*, 1–11. [CrossRef]
21. Meng, T.; Ren, G.; Wang, G.; Ma, Y. Impacts on soil microbial characteristics and their restorability with different soil disinfestation approaches in intensively cropped greenhouse soils. *Appl. Microbiol. Biotechnol.* **2019**, *103*, 6369–6383. [CrossRef] [PubMed]
22. Bergsveinson, J.; Perry, B.J.; Sheedy, C.; Braul, L.; Reedyk, S.; Gossen, B.D.; Yost, C.K. Identifying the core bacterial and fungal communities within four agricultural biobeds used for the treatment of pesticide rinsates. *J. Appl. Microbiol.* **2018**, *125*, 1333–1342. [CrossRef] [PubMed]
23. Baćmaga, M.; Wyszkowska, J.; Kucharski, J. Response of soil microorganisms and enzymes to the foliar application of Helicur 250 EW fungicide on *Horderum vulgare* L. *Chemosphere* **2020**, *242*, 125163. [CrossRef] [PubMed]
24. Ren, H.Y.; Wang, H.Y.; Yu, Z.P.; Zhang, S.W.; Qi, X.J.; Sun, L.; Wang, Z.S.; Zhang, M.C.; Ahmed, T.; Li, B. Effect of two kinds of fertilizers on growth and rhizosphere soil properties of bayberry with decline disease. *Plants* **2021**, *10*, 2386. [CrossRef] [PubMed]
25. Gao, M.; Zhou, J.J.; Wang, E.T.; Chen, Q.; Xu, J.; Sun, J.G. Multiphasic characterization of a plant growth promoting bacterial strain, *Burkholderia* sp 7016 and its effect on tomato growth in the field. *J. Int. Agric.* **2015**, *14*, 1855–1863. [CrossRef]
26. Maqsood, A.; Shahid, M.; Hussain, S.; Mahmood, F.; Basit, F. Root colonizing *Burkholderia* sp. AQ12 enhanced rice growth and upregulated tillering-responsive genes in rice. *Appl. Soil. Ecol.* **2020**, *157*, 103769. [CrossRef]
27. Santísima-Trinidad, A.B.L.; Montiel-Rozas, M.D.M.; Diéz-Rojo, M.A.; Pascual, J.A.; Ros, M. Impact of foliar fungicides on target and non-target soil microbial communities in cucumber crops. *Ecotoxicol. Environ. Saf.* **2018**, *30*, 78–85. [CrossRef] [PubMed]
28. Li, X.Q.; Su, Y.; Ahmed, T.; Ren, H.Y.; Javed, M.R.; Yao, Y.L.; An, Q.L.; Yan, J.L.; Li, B. Effects of different organic fertilizers on improving soil from newly reclaimed land to crop soil. *Agriculture* **2021**, *11*, 560. [CrossRef]
29. Pierattini, E.C.; Francini, A.; Huber, C.; Sebastiani, L.; Schröder, P. Poplar and diclofenac pollution: A focus on physiology, oxidative stress and uptake in plant organs. *Sci. Total Environ.* **2018**, *15*, 944–952. [CrossRef] [PubMed]
30. Zhu, S.S.; Vivanco, J.M.; Manter, D.K. Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. *Appl. Soil Ecolo.* **2016**, *107*, 324–333. [CrossRef]
31. Zhou, W.X.; Branch, W.D.; Gilliam, L.; Marsh, J.A. Phytosterol composition of arachis hypogaea seeds from different maturity classes. *Molecules* **2018**, *24*, 106. [CrossRef] [PubMed]
32. Scholz, B.; Guth, S.; Engel, K.H.; Steinberg, P. Phytosterol oxidation products in enriched foods: Occurrence, exposure, and biological effects. *Mol. Nutr. Food Res.* **2015**, *59*, 1339–1352. [CrossRef] [PubMed]
33. Xue, H.; Chen, X.; Li, G. Involvement of phospholipids signaling in plant growth and hormone effects. *Curr. Opin. Plant Biol.* **2007**, *10*, 483–489. [CrossRef]
34. Patra, B.; Ray, S.; Richter, A.; Majumder, A.L. Enhanced salt tolerance of transgenic tobacco plants by co-expression of PcINO1 and McIMT1 is accompanied by increased level of myo-inositol and methylated inositol. *Protoplasma* **2010**, *245*, 143–152. [CrossRef] [PubMed]
35. Mukherjee, R.; Mukherjee, A.; Bandyopadhyay, S.; Mukherjee, S.; Sengupta, S.; Ray, S. Selective manipulation of the inositol metabolic pathway for induction of salt-tolerance in indica rice variety. *Sci. Rep.* **2019**, *9*, 5358. [CrossRef] [PubMed]
36. Ghimire, B.K.; Ghimire, B.; Yu, C.Y.; Chung, I.M. Allelopathic and autotoxic effects of medicago sativa-derived allelochemicals. *Plants* **2019**, *8*, 233. [CrossRef] [PubMed]
37. Shibl, A.A.; Isaac, A.; Ochsenkuhn, M.A.; Cardenas, A.; Fei, C.; Behringer, G.; Arnoux, M.; Drou, N.; Santos, M.P.; Gunsalus, K.C.; et al. Diatom modulation of select bacteria through use of two unique secondary metabolites. *Proc. Natl. Acad. Sci. USA* **2020**, *117*, 27445–27455. [CrossRef] [PubMed]

38. Tian, L.Y.; Shen, J.P.; Sun, G.X.; Wang, B.; Ji, R.; Zhao, L.J. Foliar application of SiO_2 nanoparticles alters soil metabolite profiles and microbial community composition in the pakchoi (*Brassica chinensis* L.) rhizosphere grown in contaminated mine soil. *Environ. Sci. Technol.* **2020**, *54*, 13137–13146. [CrossRef]
39. Zhao, L.J.; Zhang, H.L.; Jason, C.W.; Chen, X.Q.; Li, H.B.; Qua, X.L.; Ji, R. Metabolomics reveals that engineered nanomaterial exposure in soil alters both soil rhizosphere metabolite profiles and maize metabolic pathways. *Environ. Sci. Nano* **2019**, *6*, 1716–1727. [CrossRef]
40. Zhang, Y.; Tao, Y.; Zhang, H.; Wang, L.; Sun, G.Q.; Sun, X.; Erinle, K.O.; Feng, C.C.; Song, Q.X.; Li, M. Effect of di-n-butyl phthalate on root physiology and rhizosphere microbial community of cucumber seedings. *J. Hazard. Mater.* **2015**, *289*, 9–17. [CrossRef] [PubMed]
41. Wang, Y.C.; Wu, B.D.; Jiang, K. Allelopathic effects of Canada goldenrod leaf extracts on the seed germination and seedling growth of lettuce reinforced under salt stress. *Ecotoxicology* **2019**, *28*, 103–116. [CrossRef] [PubMed]
42. Tiwari, K.; Dubey, V.K. Fresh insights into the pyrimidine metabolism in the trypanosomatids. *Parasit. Vectors* **2018**, *11*, 87. [CrossRef] [PubMed]
43. Coelho, A.I.; Berry, G.T.; Rubio-Gozalbo, M.E. Galactose metabolism and health. *Curr. Opin. Clin. Nutr. Metab. Care* **2015**, *18*, 422–427. [CrossRef]
44. Appleton, J. Arginine: Clinical potential of a semi-essential amino acid. *Altern. Med. Rev.* **2002**, *7*, 512–522. [PubMed]