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Characterization of the Rhizobiome of the Yellow Pitcher Plant (*Sarracenia flava*) in Wild and Restored Habitats of Virginia

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Abstract: The yellow pitcher plant, *Sarracenia flava*, is an insectivorous perennial distributed extensively in southeastern North America. In Virginia, it is restricted to a few wetland ecosystems, with only one natural site known to remain. To uncover whether there were microbial differences in the rhizospheres across natural and reintroduced sites of pitcher plant restoration, shotgun metagenome sequencing was undertaken to characterize the microbiomes of the healthy rhizosphere in the last remaining natural stand in Virginia compared to rhizospheres sampled in two restored habitats where pitcher plants were reintroduced and a nearby control habitat without pitcher plants. Statistical analysis showed no significant differences in rhizobiome communities among the natural, reintroduced, and control sites. Comparison of test rhizobiomes with those of other soil types revealed no significant difference in *S. flava* habitats versus wildland soil types but significant difference from agricultural soils. Indicator species analysis found *Pseudomonas* was a significantly more abundant genus in the *S. flava* habitats. The control site was enriched with iron-reducing bacteria compared to the rest of the sites. Further studies based on gene expression could better facilitate an understanding of the role of *Pseudomonas* in *S. flava* rhizosphere specific to habitats, which will provide better knowledge for local conservation of this plant.

Keywords: pitcher plant; *Sarracenia flava*; rhizosphere; microbiome; wetland; *Pseudomonas*; shotgun metagenome sequencing

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1. Introduction

Carnivorous plants are adapted to live in nutrient-poor environments like bogs or wetlands. They catch and digest small animals, including insects and other arthropods, from which they derive most of their nutrients while also capturing energy by photosynthesis. *Sarraceniaceae* is a phylogenetic family that contains genera *Darlingtonia* (California pitcher plant or cobra lily), *Heliamphora* (sun pitchers), and *Sarracenia* (North American pitcher plant) [1]. These plants inhabit sunny, wet, acidic bogs and wetlands that generally are nutrient-poor [2]. As pitcher plants are considered a well-suited natural system for studying the potential effects of plants and founder microbes on the establishment of a microbial community, the various *Sarracenia* spp. have been widely used in ecological studies of food web structure [3–5], and numerous studies exist of microbial communities, mostly focusing on the pitfall trap fluids [6–9]. Microbial communities in these plants have been shown to vary with the temporal and geographic differences of host habitats [10,11]. Microbiomes of the pitfall trap affect the plant's physiology and development, which plays a crucial role in plant functions [12].

A plant's rhizobiome also affects plant physiology and development and is known to host numerous organisms, including bacteria, fungi, oomycetes, nematodes, protozoa,

viruses, archaea, and arthropods. The rhizosphere is considered one of the most complex ecosystems on Earth [12–15]. Most members of the rhizobiome are part of a complex food web that utilizes the nutrients released by the plant. They provide plants with nitrogen and phosphorus to maintain and boost the productivity of host plants and receive carbon and sugar from them that aid in growth and metabolism [16]. Many studies of microbial communities in the rhizosphere aim to understand the ecological dynamics between plants and the soils they inhabit [12,17]. In contrast to other plants, there is very little known about pitcher plant rhizobiomes, especially in *Sarracenia* spp.

Sarracenia flava is an insectivorous perennial yellow pitcher plant distributed in North America from Virginia southward across the panhandle into eastern Alabama. *S. flava* is secure in North America and currently is listed as "Lower Risk" for endangerment by the International Union for Conservation of Nature [18]. In Virginia, however, *S. flava* is a state-ranked S1 "Critically Rare" species with only one known remaining natural population [19]. Throughout its range, *S. flava* is subject to many threats, including loss and degradation of wetlands, invasive species, collection of mature specimens and seeds by hobbyists, and the effects of herbicides, particularly from road maintenance [20]. In this study, we used shotgun metagenome sequencing analysis to compare the microbiome of a healthy *S. flava* rhizosphere in the last remaining natural stand in Virginia as compared to rhizospheres sampled in restored and a nearby control habitat without pitcher plants. Shotgun metagenome sequencing yields a more comprehensive analysis of microbial composition, including archaea, eukaryotes, bacteria, and viruses, and provides for the identification of functional genes by sequencing from any parts of the microbial genomes that are present in an environmental sample. The shotgun method also avoids amplification biases that can result from the PCR process used for 16S rRNA metabarcoding. We characterized the taxonomy and likely functions of microbes and eukaryotes associated with *S. flava* roots in their natural situation as compared to control and restored sites. To our knowledge, this is the first study investigating the rhizobiome in *S. flava*. The data provided here are useful locally for conservation efforts because they help us to better understand what soil conditions pitcher pants require to persist. This work also informs us of the expected response of soil microbes to conservation practices such as pH and nutrient amendment.

2. Materials and Methods

2.1. Sample Collection

Stands of *S. flava* were located at the Meadowview Biological Research Station's Joseph Pines Preserve in Sussex County, Virginia, USA, which contains approximately 200 clumps/hectare (Table 1 and Supplementary Table S1). Samples were collected in 2015 from Sappony Creek, the last native site of yellow pitcher plant in Woodford, Virginia; Addison Bog and Sappony Bog, which were two restored sites with reintroduced yellow pitcher plant populations; and a proximal control site in between a pitcher plant field and a nearby woodland that had no pitcher plants, showed signs of drainage, was likely to have elevated nutrient levels, and therefore not optimal habitat for *S. flava*. Using a 10 mm diameter sediment core, rhizosphere samples were collected from the root zones (top 5 cm) of three live pitcher plants at each of the three sites, and soil samples adjacent to other live plants, including roots, were collected from a nearby control site (no pitcher plants) for microbiome comparison. Three replicate rhizosphere samples were collected from each site (approximately 25 g each). Sediments were placed immediately into 50 mL polypropylene tubes and stored on wet ice for several hours during transport to a −80 °C freezer. Soil samples were also collected from the root zones of rhizosphere sampling sites and later (2022) from a potential pitcher plant reintroduction site at Sappony South drainage for soil characteristics. Complete soil nutrient and texture analyses were performed by WayPoint Analytical (formerly A&L Eastern Laboratories).

Table 1. Information on rhizobiome sampling sites in Virginia.

Sample Number	Sampling Area §	Environmental Condition
1	Addison Bog	Restored
2	Sappony Bog	Restored
3	Sappony Creek	Natural
4	Control Site	Drainage
NA **	Sappony South *	Drainage

§ Latitude and longitude available upon request from the author; * soil collected in 2022 to provide additional context on nutrients; ** NA: Not Available.

2.2. DNA Extraction and Sequencing Analysis

The individual samples of three biological replicates per site were well-mixed, and one gram (1 g) of each sample was used for DNA isolation using the PowerSoil™ DNA extraction kit (MO BIO, Carlsbad, CA, USA) according to the manufacturer's instructions. Nucleic acid quality was checked via Experion™ DNA 12 K Analysis kit (Bio-Rad Laboratories, Inc., Hercules, CA, USA), after which equimolar quantities of the three replicate DNAs for a site were combined to serve as a single sequencing template. At the time this study was performed, sequencing costs were rather steep; therefore, to obtain the best representation of the rhizobiomes while keeping sequencing costs low, it was common to pool replicate biological samples prior to sequencing. The nucleic acid quantity of the pool was verified using the Quant-iT DNA kit (Life Technologies, Grand Island, NY, USA) and adjusted to 50 ng μL^{-1} for library construction. Sequencing libraries were prepared for shogun metagenome sequencing using the Ion Plus Fragment Library kit (Life Technologies, Grand Island, NY, USA). The sequencing process was accomplished on the Ion Torrent PGM™ semiconductor sequencing platform using the Ion PGM™ 200 Sequencing Kit and one 318-chip for each site (Life Technologies, Grand Island, NY, USA).

2.3. Data Analysis for Taxonomic and Functional Metagenome Annotation

Sequence reads <50 bp were removed and quality trimmed to ≥Phred 20 using Genomics Workbench (CLCbio, Cambridge, MA, USA). The filtered reads were assigned with the MetaGenome Rapid Annotation using the Subsystem Technology (MG-RAST version 4.0.3) server with metadata [21]. Taxonomic identification was performed using the lowest common ancestor (LCA) method with the following parameters: a maximum e-value cutoff of 1×10^{-5}, a minimum identity of 60%, and 15 bp as the minimum alignment length [22]. Predicted functional profiles were identified using the SEED subsystems annotation source of the MG-RAST server [23], with a maximum e-value cutoff of 1×10^{-5}, a minimum identity of 60%, and 15 bp as the minimum alignment length [22].

2.4. Selection of Comparative Rhizobiome Shotgun Metagenomes

To compare pitcher plant rhizobiomes with other habitats of similar soil depth, shotgun metagenome data were retrieved for surface sediments from the MG-RAST database (summarized in Supplementary Table S2). As accessions whose environmental metadata perfectly matched the current study were non-existent, we selected eight data sets of surface soils from the National Ecological Observatory Network (NEON) project, whose habitats were similar to the Virginia pitcher plant habitats (woodland, wetland, and mixed forest) in North American eastern regions including Virginia, Alabama, Georgia, Florida, and Massachusetts. In addition, six metagenomes from drainage soils, rhizosphere, and woody wetland soil were used for comparison.

2.5. Statistical Analyses

Taxonomic IDs were normalized to account for differences in read numbers among sites. Data analyses and visualization were performed using the R packages vegan v2.6-4 [24] and ggplot2 v3.4.4, respectively. Bray–Curtis dissimilarities were calculated to assess the genus-level difference in bacterial composition, which was visualized with a

Non-metric multidimensional scaling (NMDS) ordination. The effect of soil nutrients was tested using the ENVFIT function of Vegan [24]. Permutational multivariate analysis of variance (PERMANOVA) was performed with Adonis to test for differences in community compositions among Pairwise.Adonis habitat/soil types and pairwise comparisons were also performed using [25]. Indicator species were identified using the R package indicspecies [26].

3. Results

3.1. Soil Characteristics

Soil analysis (Table 2) indicated that the habitats are acidic and chemically depleted, as expected. Data also showed that the natural habitat at Sappony Creek was sandy loam. However, Sappony Bog was closer to silt loam, while Addison Bog showed a sandy loam texture as a natural stand. Soil nutrients in both reintroduced sites closely matched the characteristics of the natural site at Sappony Creek, albeit with slightly higher readings on some macronutrients (Ca, K, Mg, and Na) (Table 2). In contrast, the control location had much higher levels of P, K, Ca, and Mn with higher pH but a lower level of Fe (Table 2). The Sappony South site intended for future restoration activities exhibited nutrient levels that were intermediate between the native and introduced pitcher plant sites and the control area.

Table 2. Summary of physical and nutrient analysis for samples from *S. flava* habitats and a control site.

	Addison Bog	Sappony Bog	Sappony Creek	Control Site	Sappony South Drainage ****
pH	4.5	4.6	4.8	5.1	4.7
P *	8	4	6	21	20
K *	64	55	17	131	101
Ca *	193	231	109	644	440
Mg *	105	49	28	133	69
Na *	20	20	11	10	14
OM **	10.3	5.5	2.8	6.4	8.5
SS ***	0.08	0.08	0.03	0.05	0.05
N *	1	1	1	2	3
Zn *	2.2	1.3	2.1	2.2	2.2
Mn *	6	7	1	39	23
Cu *	0.5	0.5	2.9	1	0.6
Fe *	322	482	257	73	298
B *	0.2	0.3	0.4	0.2	0.3
S *	7	9	8	9	22
sand **	58.4	38.4	50	56.8	46.8
silt **	34.8	50.8	45.2	33.6	43.6
clay **	6.8	10.8	4.7	9.6	9.6
Texture	sandy loam	silt loam	sandy loam	sandy loam	loam

* Nutrient element values are ppm; ** OM and texture values are %; *** SS: soluble salts are mMhos cm^{-1}; **** No sequence data available; site sampled for nutrient comparison and possible future restoration.

3.2. Quality of Sequencing Data

Of the raw data from the four sites, 78–82% of sequences from each site passed quality control screening (Table 3). The number of sequence reads in the Addison Bog sample was approximately half of the read totals of the other sites. Metagenomic rarefaction curves indicated that the species richness did not completely reach saturation, but the number of sequence reads did show sequencing coverage deep enough to compare diversity for all sites (Supplementary Figure S1). Among the sequence reads that passed quality control, >92% of sequences were functionally annotated across all sites, and 44–53% of reads were assigned to species (Supplementary Table S3).

Table 3. Rhizobiome sequence data for Sarracenia flava habitats in Virginia.

Sample ID	Raw Data			Post-QC	MG-RAST ID
	Total Sequence (bp)	Number of Reads	Avg. Read Length (bp)	Number of Reads	
Addison Bog	642,275,452	2,574,063	250	1,995,469	mgm4666289.3
Sappony Bog	1,128,560,650	4,835,722	233	3,948,809	mgm4666288.3
Sappony Creek	1,120,458,672	4,358,588	257	3,399,534	mgm4666291.3
Control Site	1,253,720,035	4,924,299	255	3,957,917	mgm4666290.3

3.3. Archaea and Eukaryota in Rhizobiome of S. flava Habitats

Shotgun metagenomic sequencing data from the four Meadowview Biological Research Station sites were assigned overwhelmingly to the bacteria domain (98%), and both Archaea and Eukaryota each accounted for roughly 1% of reads (Supplementary Table S4a). Archaea were composed primarily of Euryarchaeota, followed by Thaumarchaeota and Crenarchaeota (Figure 1A; Supplementary Table S4b). Out of the total 1% of reads assigned to the Eukaryota domain, the most abundant phylum was Ascomycota, followed by Streptophyta, Chordata, Arthropoda, and various protists (Figure 1B; Supplementary Table S4b). Phylum Streptophyta included the two most abundant eukaryotic genera, Arabidopsis and Ricinus (Supplementary Table S5). Within the Ascomycota, the Order Eurotiales Family Trichocomaceae was the most abundantly assigned, containing the third most abundant eukaryote observed, Aspergillus (Supplementary Table S5).

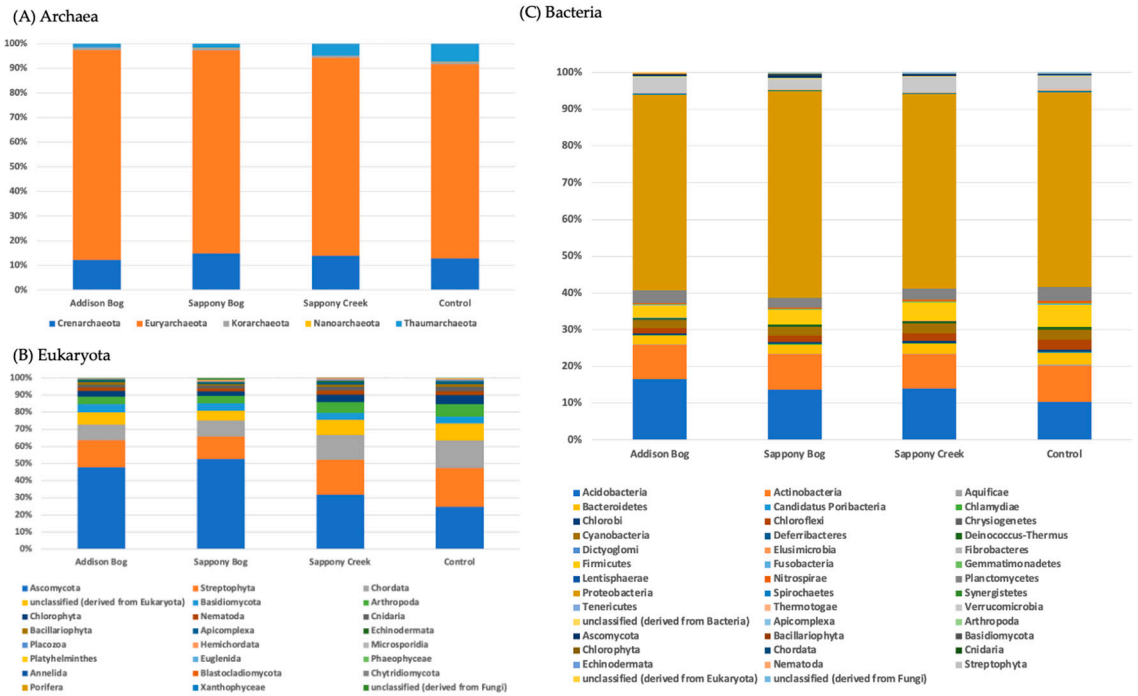

Figure 1. Relative normalized abundance (≥0.01%) of rhizobiome consortia in four Virginia habitats, three with living *Sarracenia flava* stands and one without. (**A**) Archaea proportions; (**B**) Eukaryote; and (**C**) Bacteria. *X*-axis: site; *y*-axis: relative abundance (%).

3.4. Bacterial Community Composition in S. flava Habitats

The most abundant bacterial phylum was Proteobacteria (52–55% of reads) across the four test sites, followed by Acidobacteria, Actinobacteria, Firmicutes, Verrucomicrobia, and Planctomycetes (Figure 1C; Supplementary Table S4b). In the natural habitat, observed Proteobacteria consisted of four major classes, including Alphaproteobacteria (25%), Gammaproteobacteria (10%), Betaproteobacteria (10%), and Deltaproteobacteria (7%) (Figure 2A). Within the predominant class Alphaproteobacteria, Rhizobiales was the predominant order, com-

prising 11 families containing Bradyrhizobiaceae (Supplementary Table S6). Acidobacteria consisted of Acidobacteriaceae and two *Candidatus* groups, including *Candidatus Solibacter* and *Candidatus Koribacter* (Supplementary Figure S2). The Actinobacteria consisted of Actinomycetales, namely Mycobacterium (19%), Streptomyces (17%), and Frankia (11%) (Supplementary Figure S3). The Firmicutes contained roughly equal portions of Clostridia and Bacilli, and nearly half of the Verrucomicrobia were unclassified at the level of Order (Figure 2A). Planctomycetes discovered in these habitats consisted of five similarly abundant genera: *Planctomyces* (31%), *Gemmata* (21%), *Rhodopirellula* (18%), *Pirellula* (15%), and *Blastopirellula* (15%).

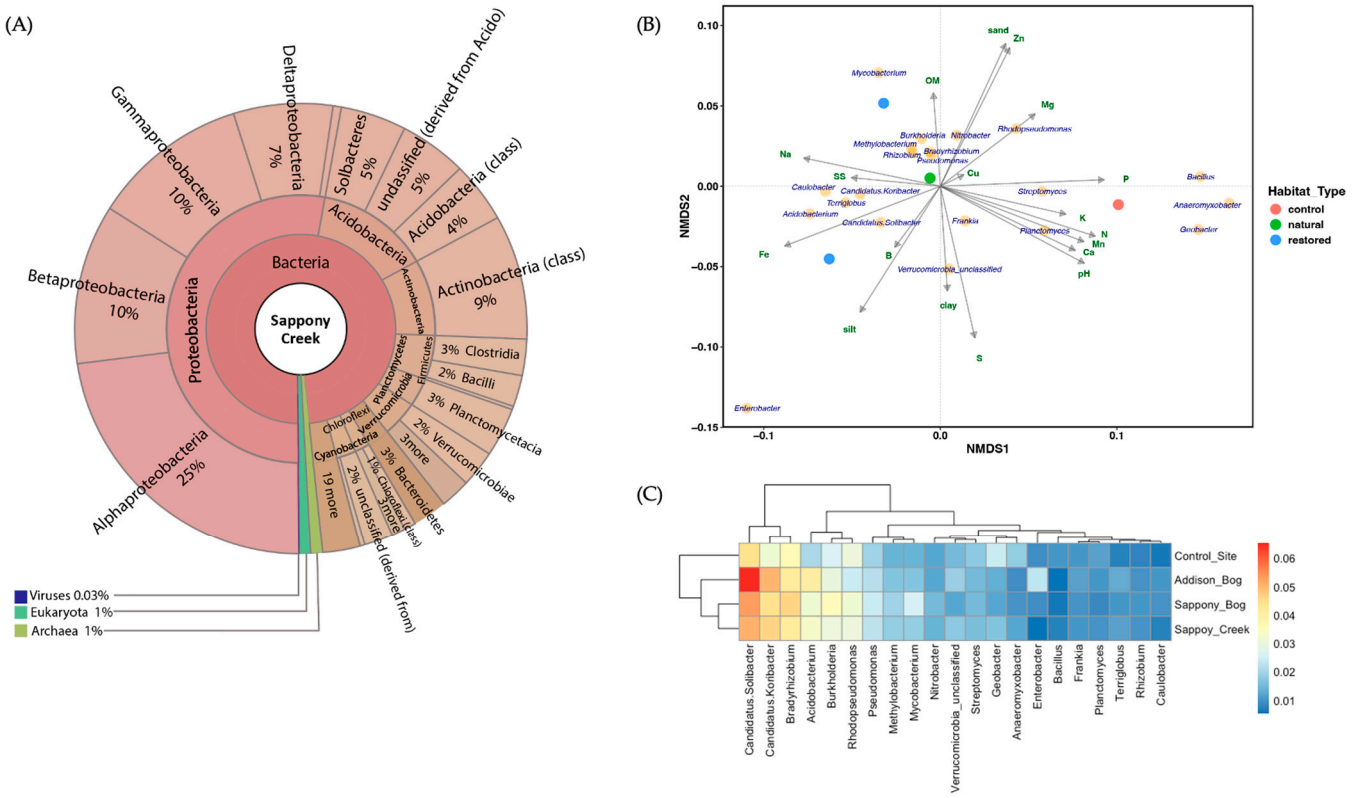

Figure 2. Rhizobiome consortia in *S. flava* at the last known natural stand, restored, and control sites. (**A**) Rhizobiome composition of *S. flava* in Sappony Creek up to the class level; (**B**) NMDS plots of microbiome communities in four different habitats; (**C**) Heap map of the most abundant 21 genera across the habitats.

Twenty-one genera with ≥1.0% normalized abundance at one or more sites were filtered for statistical comparison among sampling sites (Supplementary Table S7), which belonged to six phyla including Proteobacteria, Acidobacteria, Actinobacteria, Planctomycetes, Firmicutes, and Verrucomicrobia; these together accounted for >42% of bacteria at *S. flava* habitats and >38% at the control site (Supplementary Table S7). Statistical analysis of ADONIS showed no significant difference in microbiome communities among natural stand, restored, and control sites (F = 1.485, p = 0.5) and between living plant sites and control sites (F = 4.0811, p = 0.25). Envfit function did not show a significant dominant nutrient among the habitats (R2 = 0.7404; p = 0.5) (Figure 2B). Out of 21 bacterial genera, *Candidatus Solibacter*, *Candidatus Koribacter*, and *Braydyrhizobium* were highly abundant across the habitats (Figure 2C).

3.5. Comparison of Microbial Rhizosphere Communities Among S. flava and Other Selected Habitats

The rhizobiomes of *S. flava* were compared with metagenomes in surface soils from different locations in Virginia and other rhizospheres in eastern North America

(Supplementary Table S2). An NMDS plot of the metagenomes illustrated that sampling sites did not reflect the geographic location at different classification levels but instead clustered according to ecological similarity (Figure 3A). The *S. flava* habitats were clearly separated with most cropland soils, and the control, wetland, and forest rhizobiomes appeared intermediate (Figure 3A). The other two Virginia rhizosphere samples (mixed forest, SCBI.002 and SCBI.003) did not cluster with samples collected for this study; one (SCBI.003) was very similar to the Massachusetts samples (woody wetland, HARV.016) and the other was an outlier due to higher proportions of Eukaryota (6%) and Actinobacteria (30%) and a lower proportion of Proteobacteria (35%) than the other comparative samples (Figure 3A; Supplementary Figure S4). Pairwise Adonis tests showed that bacterial communities were significantly different in agricultural soils from other soil types, except for the control site (Figure 3B). Indicator species analysis revealed that four genera were significantly dominant in agricultural soils (*Nocardioides*: $p < 0.0001$, *Kribbella*: $p < 0.0001$, *Conexibacter*: $p = 0.0051$, and *Salinispora*: $p = 0.0429$). Compared to the other sites, the control site showed three significantly dominant genera: *Bacillus* ($p = 0.0233$), *Geobacter* ($p = 0.0445$), and *Anaeromyxobacter* ($p = 0.027$). *S. flava* habitats and the control site had significantly higher levels of *Pseudomonas* ($p = 8 \times 10^{-4}$) than all other surface soil and rhizosphere sites. Four genera were significantly dominant in soils of non-agricultural sites: *Candidatus Solibacter* ($p = 0.0043$), *Terriglobus* ($p = 0.0053$), *Candidatus Koribacter* ($p = 0.0100$), and *Acidobacterium* ($p = 0.0218$). Analysis of bacterial relative abundance similarly illustrated that agricultural microbiomes were grouped separately from others, and SCBI.002 showed the intermediate pattern of bacterial abundance between agricultural and other soils (Figure 3C).

3.6. Functional Annotation of the S. flava Rhizobiome Sequences

Functional annotation from the KEGG Orthology (KO) database indicated that 61% of reads were functionally related to Metabolism (map09100), followed by Environmental Information Processing (~17%, map09130) and Genetic Information Processing (~16%, map09120) at Level 1 (Figure 4A). Functional orthologues at Level 2 were predominantly associated with Amino Acid Metabolism (map09105) and Carbohydrate Metabolism (map09101) (Figure 4A). The KO pathways with the highest number of mapped reads, derived from the most abundant bacterial species, were related to ABC transporters (ko02010) (Supplementary Figure S5) and the Two-component system (ko02020) in Environmental Information Processing (map09130) (Figure 4B). Functional annotation comparison showed similar pathway distribution across all of the rhizobiomes in Level 1 and most other levels (Supplementary Figure S6).

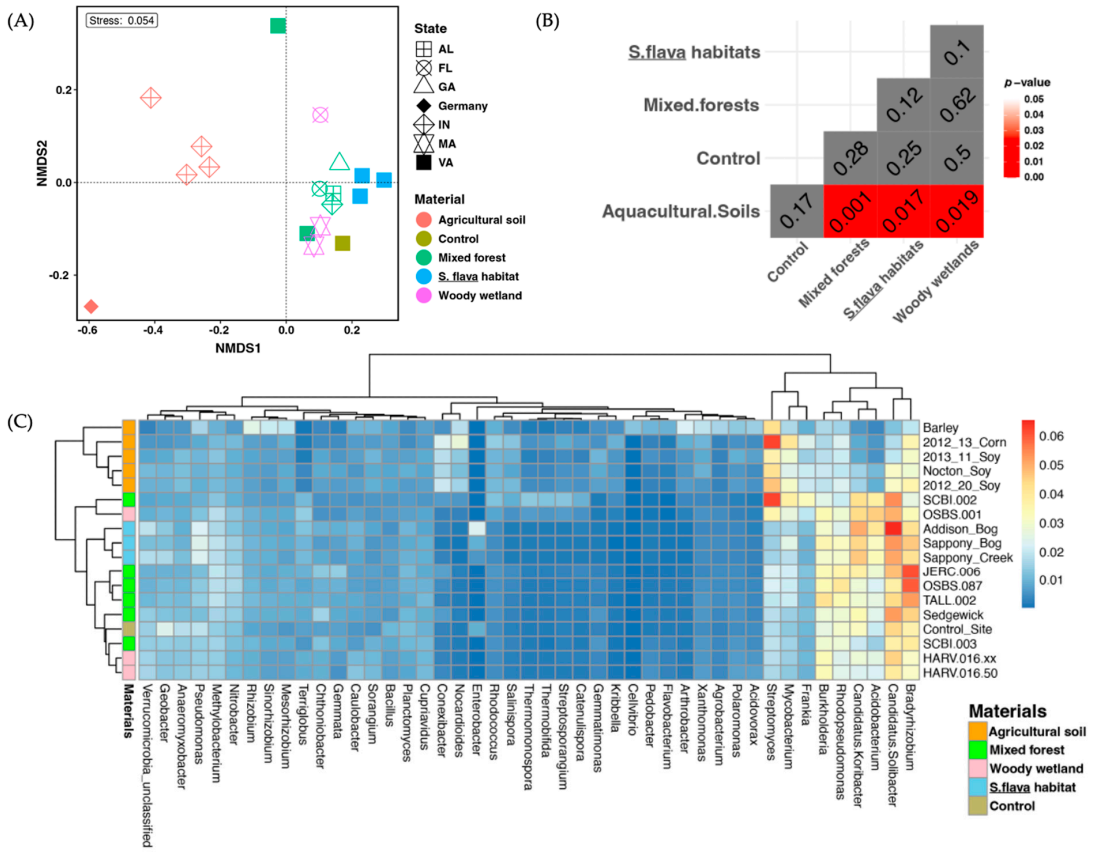

Figure 3. Comparison of microbiome genera across four different S. flava rhizobiomes in Virginia and 14 other soil rhizobiomes accessed through MG-RAST. (**A**) NMDS plots of microbiome composition from different soil types and regions; (**B**) *p*-values of pairwise Adonis analysis based on soil type; (**C**) Relative abundance heat map of most abundant 45 genera in 18 sites.

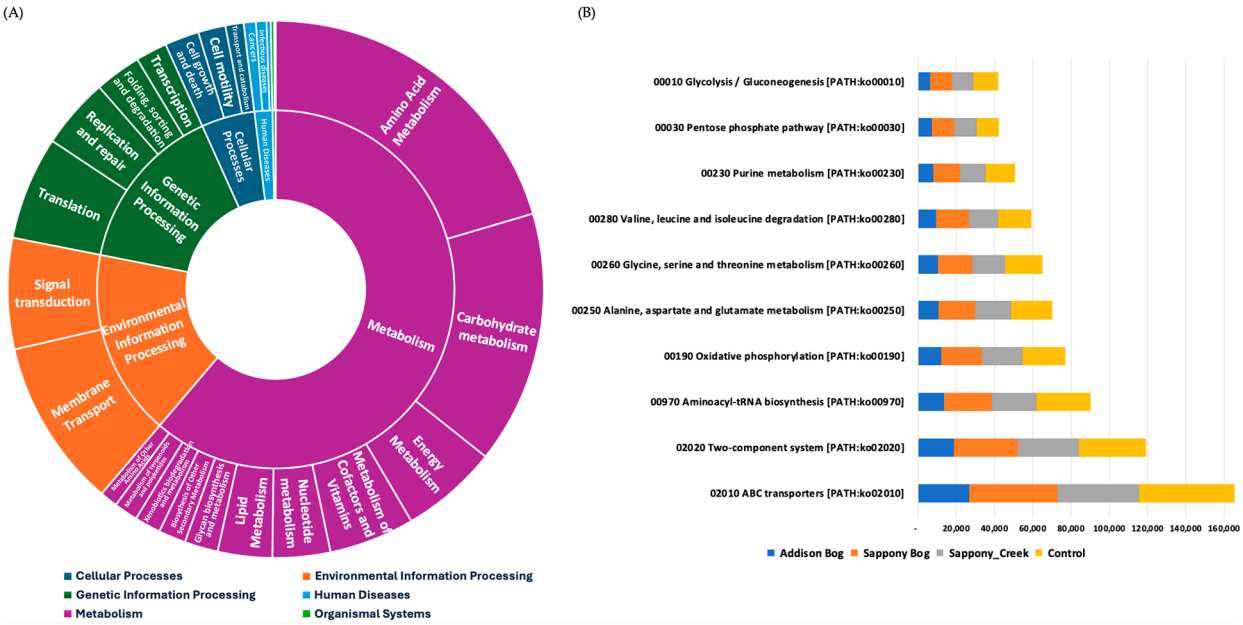

Figure 4. Functional annotation of *S. flava* rhizobiome sequences. (**A**) KEGG Orthology annotation at Level 1 and Level 2; (**B**) The top ten KO pathways annotated by sequence read in each sample.

4. Discussion

Because the rhizosphere is where interactions among plants, soil, and microbes are linked, this region plays a crucial role in nutrient uptake and in defending against biotic and abiotic stresses [27–29]. Over the last decade, metagenome studies have been performed on a diverse suite of rhizospheres in different environmental conditions [12,29–36]. In this study, we characterized the rhizobiome of yellow pitcher plant habitats (natural and restored) and compared them with those in other soil types (woody wetland, mixed forest, and agricultural). Metagenome sequencing analysis of *S. flava* rhizosphere provided a better understanding of the interaction of rhizobacteria with the nutrition status of the habitats.

4.1. Rhizobacterial Features Across Habitats

Most rhizospheric microbes identified at high abundance in this study belonged to Proteobacteria, Acidobacteria, Actinobacteria, and Firmicutes (Figure 2A), which were similar to the dominant rhizobacterial taxa found in diverse plant types [34]. The most abundant bacterial phylum in this study was Proteobacteria (52–56% in this study) (Supplementary Table S4b), and 11 genera were present at >1% (Supplementary Table S7). Proteobacteria are capable of growing and adapting well to soils with low carbon sources, which makes this bacterial group abundant in different plant rhizospheres, including *S. flava* rhizosphere. The most abundant Proteobacteria genus was *Bradyrhizobium*, a common soil-dwelling bacteria that fixes nitrogen in exchange for carbohydrates from host plants. In leguminous plant species, *Bradyrhizobium* increases nodulation, nitrogenase activity, and plant growth [37]. *Burkholderia* strains also play a role in nitrogen-fixing and phosphate-solubilizing capability, which is used for root-knot nematode management in various crops and vegetables [38]. *Methylobacterium* uses one-carbon compounds, including methylamine and methanol, as energy and carbon sources [39] and provides an additional supply of nitrogen in maize and strawberries with nitrogen reduction [40].

The next most abundant phyla, Acidobacteria (10–16%) and Actinobacteria (9–10%) (Supplementary Table S4b), aid in carbon, sulfur, and nitrogen metabolism in soil. Acidobacteria also play an important role in acid tolerance and transporter systems [34,41]; their abundance is reasonable, given the preference of *S. flava* for acidic soils. Actinobacteria are related to root nodulation and show symbiotic interactions with plants and mycorrhizal fungi to adapt to harsh environmental conditions [34]. The most abundant Actinobacteria genus in this study was *Mycobacterium* (2% of bacteria) (Supplementary Table S4b). Many *Mycobacterium* spp. are known to inhabit rhizospheres of various soil types and water distribution systems [42–46]. High numbers of *Mycobacterium* have been reported in waters, soils, aerosols, and droplets from acidic, brown-water swamps of the southeastern United States coastal plain, including Virginia (Dismal Swamp and Claytor Lake), correlating with warmer temperature, low pH, low dissolved oxygen, high soluble zinc, high humic acid, and high fulvic acid [44]. Rhizobiomes identified here clearly reflected acidic low-nutrient *S. flava* habitats. Thus, the predominance of *Mycobacterium* is reasonable since *S. flava* habitats share numerous environmental factors with other southeastern United States coastal plain habitats.

4.2. Comparison of Rhizosphere Microbes in S. flava Habitats and Other Soil Types

Comparing microbiome communities in *S. flava* habitats with those in other soil types, including mixed forest, woody wetland, and agricultural soil from eight other locales, provided a better understanding of rhizobiome characteristics in *S. flava*. The most abundant genera found in *S. flava* rhizospheres were also shown to be highly abundant in most other soil types (Figures 2C and 3B). Acidobacteria, including *Candidatus Solibacter*, *Terriglobus*, *Acidobacterium*, and *Candidatus Koribacter,* were significantly dominant in all soil types, except for agricultural soil, whose significantly dominant bacteria were Actinobacteria including *Nocardioides*, *Kribbella*, *Conexibacter*, and *Salinispora* (Figure 3B). In the current study sites, including the control, *Pseudomonas* ($p < 0.001$) showed significantly higher relative abundance compared to other soil types, although the control site itself showed that *Bacillus*, *Geobacter*, and *Anaeromyxobacter* were significantly dominant. *Pseudomonas*

spp. are present in a wide array of environments and display great metabolic diversity [47]. *Pseudomonas* spp. directly promote plant growth through phosphate and iron solubilization, nitrogen fixation, phytohormone modulation, and increased abiotic stress tolerance [48–50]. Plant growth-promoting rhizobacteria strains in *Pseudomonas* can inhibit several types of plant pathogens by antibiosis, stimulation of plant defense mechanisms, and competition for niches and nutrients [51,52]. Thus, the significantly higher presence of *Psedumonas* in this study area suggests that there may be some other biotic or abiotic stressors that exist in these *S. flava* areas.

Significantly dominant bacteria at the control site were *Bacillus*, *Geobacter*, and *Anaeromyxobacter*. *Bacillus* are well-known rhizobacteria that promote plant growth, nutrient acquisition, and pathogen biocontrol [50]. *Geobacter* and *Anaeromyxobacter* are, in general, present in paddy soils and function as a potential iron reducer in the environment. Enrichment of these microbes suggests significant iron cycling in the control soils [53–55], which explains the lower iron contents at this site compared to *S. flava* habitats, although most nutrients were more enriched in the control site.

4.3. ABC Transporters to Process Environmental Information

As expected, the most abundant functionally identified sequences across the metagenomes were Metabolism (Level 1 and Level 2) (Figure 4A). KO analysis (Level 3) revealed that the most abundant pathways in the *S. flava* rhizospheres were ABC transporter (map02010) in Environmental Information Processing (Figure 4B). The ABC transporters form one of the largest known protein families across diverse genomes and account for the transport of a wide variety of substrates such as iron, sugar, lipids, and sterols by coupling ATP hydrolysis. The most abundant bacteria species detected in this study were *Candidatus Solibacter* and *Candidatus Koribacter*, both characterized by genomic features of the gene cluster of ABC transporter in a majority of their relatively large genomes [56]. Their genomes contain major facilitator systems for sugar transport and high-affinity ABC transporters, well suited to low-nutrient conditions [56] where *S. flava* thrive.

5. Conclusions

We compared the rhizobiome of the yellow pitcher plant, *S. flava,* in its Natural habitat and restored habitats to facilitate pitcher plant restoration and conservation. Metagenome sequencing analysis of rhizobiomes revealed no significant difference between Natural and restored habitats. Microbiome communities of the *S. flava* habitats were similar to those of mixed forests and woody wetlands and significantly different from agricultural soils. Genomic features of the most abundant genera suggested putative roles in carbon cycling in poor nutrient soil and tolerance of hydration fluctuation. *Pseudomonas* was a significantly dominant genus in the *S. flava* habitat compared to other soil types. Further studies based on gene expression could better facilitate an understanding of the role of *Pseudomonas* in the *S. flava* rhizosphere specific to habitats, which will provide better knowledge for local conservation of this plant.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijpb15040097/s1. Supplementary tables and figures are in the file Supp.Information_PitcherPlant.

Author Contributions: B.L.B. and P.S. devised the study design. B.L.B. and B.-Y.L. analyzed and interpreted the metagenome data. B.L.B. and N.A. performed the library preparation and sequencing. P.S. provided all soil nutrient data. B.-Y.L., B.L.B., and P.S. were major contributors in writing the manuscript. All authors have read and agreed to the published version of the manuscript.

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