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Patterns of Insect Distribution in Fruit Trees of South Romania and Their Role as Bacterial Vectors

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Abstract: This study is the first investigation of tree–insect–bacteria interactions in southern Romania, documenting the distribution of 19 insect species across various fruit trees and their insect-associated bacterial diversity. Insect species were identified through DNA barcoding, while bacterial communities in *Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza* insects were analyzed via 16S rRNA gene sequencing. Insect diversity varied across apple, cherry, plum, peach, and quince trees, with most species showing tree-specific distribution, except for *Drosophila melanogaster*, which was found on all tree species. Its presence was primarily influenced by fruit development stages rather than temperature changes. Insect bacterial communities comprised 51 genera across four phyla, predominantly *Pseudomonadota* and *Bacillota*, that varied by tree species rather than insect species, suggesting the potential role of these flies as bacterial vectors. Several potential pathogenic bacterial genera were identified as biomarkers within insect microbiomes, suggesting their involvement in disease transmission, particularly affecting apple and cherry trees. This study also provides the first report of seven insect species in Romania, being the first microbiome characterization of four dipteran species associated with regional fruit trees.

Keywords: insect-associated bacteria; fruit tree; microbiome; pathogen transfer; Romania

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1. Introduction

Insects pose a critical challenge to European fruit cultivation, particularly with the rise of invasive species and climate change. In this context, unraveling the complex interactions between insects, bacterial communities, and host plants is crucial for advancing both ecological and agricultural research [1–3]. Fruit trees function as vital ecosystems where insect activity plays a significant role in plant health and microbial balance. However, the insects' bacterial communities within fruit orchards remain largely understudied, particularly in less investigated European regions such as southern Romania. Some of the most significant cultivated fruit trees in this country include apple (*Malus domestica*), cherry (*Prunus avium*), peach (*Prunus persica*), plum (*Prunus domestica*), and quince (*Cydonia oblonga*) species [4,5].

The distribution of insects in fruit trees is critical for several ecological, agricultural, and economic reasons. Many fruit trees, including apples, peaches, and cherries, depend on insect pollinators to ensure fruit set and optimal yield. Meanwhile, the presence of pests like codling moths, aphids, or cherry fruit flies can directly affect fruit quality and

quantity [6,7]. Therefore, investigation of the presence and distribution of predatory insects known as natural enemies of the fruit trees, and other beneficial species, is essential to unravel their role in ecosystem resilience and natural control of pest populations [1].

Among the insect species that impact fruit trees, several aphids (*Aphididae*) were commonly found on apple and quince trees [8], cherry trees (*Myzus cerasi*) [9], and peach trees (*Myzus persicae*) [10], causing twisted leaves and reducing tree vigor and production [11]. Meanwhile, cherry, peach, and plum trees were often affected by species from the *Drosophila* genus, notably the invasive *Drosophila suzukii* [12–14]. With over 3000 species reported worldwide, except in Antarctica [6,15], *Drosophila* is known to be attracted mainly to rotting fruit, while also reported on various trees [16] and other plant substrates worldwide [6,7,17]. Unlike other *Drosophila* species that target overripe or decaying fruit, *D. suzukii* lays eggs in ripening cherries, leading to larval feeding, fruit collapse, and secondary fungal and bacterial infections [18]. The occurrence of other *Diptera* species on the peach, plum, apple, quince, and cherry trees has also been reported across different regions, including *Rhagoletis indifferens* and *R. pomonella* [19], *Osmia lignaria* [20], *Anthomonas pomorum* [21], *Anastrepha fraterculus* [22], *Phlebotomus papatasi* [23], *Bactrocera dorsalis* [24], and *Hyalopterus* sp. [25] among others.

Studying insect dynamics in Romanian fruit trees is crucial due to its ecological and economic impact, requiring investigation of their diversity, distribution, and microbial relationships to improve tree health, pest control, and environmental management. Among fruit flies reported on various tree species in Romania, *Ceratitis capitata* appeared on cherry, apricot, peach, jujube, and kaki trees from various locations in the South, Southeast, and West parts of the country [26,27]. Moreover, Drosophila melanogaster and *D. suzukii* were retrieved from wild blackberries, apples, figs, grapes, and jujube trees in southern Romania [28,29]. The occurrence of *Hyalopterus* sp. on cherry plum, plum, red plum, apricot, peach, almond, nectarine, and common reed fruit trees was also indicated in various regions of Romania [25]. Investigations of the insect distribution on other host trees also reported that the presence of the common grass-veneer moth (*Anomoia purmunda*) on both *Crataegus monogyna* and *Ziziphus jujube* trees was acknowledged in southern Romania [30], and of *Ips duplicatus*, *Xylosandrus germanus*, and *Neoclytus acuminatus* in both broadleaf and coniferous forests from the mountainous and hilly regions of Romania [31].

The association between specific bacterial species and certain insects could contribute to the transmission of pathogens, while some bacterial exchanges can be mutually beneficial, supporting the insect's health, development, or ecological fitness, highlighting the insect–microbe implications in disease ecology, insect physiology, and ecosystem shaping [1,3,32,33]. Insects like *Drosophila* and aphids serve as vectors, spreading plant pathogens like *Acetobacter* spp. and *Gluconobacter* spp. in fruit trees, which accelerates fruit decay [6,34], as well as viral and bacterial pathogens, causing significant plant damage [35,36]. Other insects, such as *Ceratitis capitata*, also found in Romanian territory [27,28], contribute to disease transmission in orchards, as a potential vector for *Erwinia amylovora*, causing fire blight on apples and pears [37]. Therefore, investigations of insect-associated microbiota can provide valuable insights into their ecological roles and interactions within fruit orchards, with significant implications in the fruit tree industry.

Meanwhile, extensive studies on insect microbiota also revealed its crucial role in their physiology, demonstrating beneficial effects [2]. Among these, *Bacteroidota* species enhanced nutrient availability in *Drosophila*, influencing growth rates, reproductive success, and gut homeostasis [38]. *Bacillota*, a dominant phylum in insect microbiota, contributed to immune enhancement (*Lactobacillus*, *Enterococcus*), digestion of complex carbohydrates and nutrient absorption, and improved stress resistance under starvation or oxidative stress [6,39,40]. In *Drosophila*, members of the phylum *Pseudomonadota*, particularly *Wol-*

bachia spp. [41,42], play a significant role in host immunity, reproduction, and adaptation to environmental conditions. *Actinobacteria* have also been shown to enhance immune function and disease resistance and contribute to dietary fiber digestion in *Drosophila* [43,44].

Although several studies have reported the presence of tree-associated insects in Romania [25–29,31], to our knowledge, no in-depth research has been conducted on insect-associated bacterial communities to explore the dynamics of putative pest insect carriers among fruit trees in this country and beneficial bacteria spreading by insect vectors.

In this context, the current study focused on investigating tree–insect–bacteria interactions within apple, peach, cherry, plum, and quince fruit trees from an orchard located in a southern Romanian region using molecular approaches, to evaluate the insect species distribution among these common fruit tree species of the region and the corresponding dynamics of insect-associated bacteria genera, aiming to assess the potential role of insects in microbial transferring within this habitat.

2. Materials and Methods

2.1. Study Area, Sampling, and Sample Preparation

Insect samples were collected from an orchard located in Clatesti village (Calarasi County) in southern Romania (44°8′37″ N 26°35′52″ E) (Figure 1A–C). Insects were sampled from traps placed in different types of trees (one specimen of each) belonging to the species *Malus pumila* and *Malus domestica* 'Jonagold' (apple tree), *Prunus domestica* (plum tree), *Prunus avium* (cherry tree), *Prunus persica* and *Prunus persica platicarpa* (peach tree), and *Cydonia oblonga* (quince tree), using vinegar and banana slices as attractants placed in the trap [45] (Figure 1D). All specimens collected from the traps 48 h after their installation were placed in 200 µL of Tris-EDTA (TE) buffer, pH 8, and stored at −20 °C for further investigations.

Figure 1. Study site and sampling system: (**A**) Localization on Romanian territory; (**B**) Calarasi County; (**C**) Clatesti village (orange star); (**D**) Insect trap set up in a fruit tree (photo Copoiu D.S.).

Collection was carried out during the warm season, along the months of May, June, July, and August 2021. The minimum, maximum, and average air temperature during the collection intervals were sourced from the National Meteorology Administration (https://www.meteoromania.ro/ accessed on 10 November 2021).

2.2. Insect Identification Based on Morphological Features and DNA Barcoding

Taxonomic identification of the insect specimens was carried out with a Stemi 2000 stereomicroscope (Zeiss, Jena, Germany) based on corresponding morphological identification keys [46,47].

Genetic identification of collected insects was performed by DNA barcoding based on mitochondrial cytochrome c oxidase I (COI) gene amplification [4]. Whole-insect specimens preserved in TE buffer were used for total DNA extraction following a modified protocol with the DNeasy Blood & Tissue Kit (Qiagen, Germantown, MD, USA). An initial cell disruption step was added using 2 mm ZR Bashing Beads (Zymo Research, Irvine, CA, USA) in a SpeedMill PLUS Cell Homogenizer (Analytik Jena, Jena, Germany) [5].

COI amplicons were obtained by PCR in the presence of 200 ng insect DNA as template and 10 pmol/mL of the LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) primers [6] following the previously described protocol [48]. After analysis by electrophoresis using 1% agarose gels, the PCR products were purified using PureLink PCR Purification Kit (ThermoFisher Scientific, Waltham, MA, USA) and sequenced on both strands (Macrogen Genomics, Amsterdam, The Netherlands).

2.3. Identification of Insect Bacterial Communities by 16S rRNA Gene Illumina Sequencing

Purified total DNA obtained from individual insect specimens (Section 2.2) was used for 16S rRNA gene amplification using the 341F/805R primer pair targeting the V3-V4 region [49]. The amplicon library was sequenced using an Illumina MiSeq 300PE platform (Macrogen, Seoul, Republic of Korea).

2.4. Sequence Analyses

The COI sequences were edited using Sequence Assembly and Alignment with CodonCode Aligner 9.0.2 software (CodonCode Corporation, 2003) and identified using BLAST-NCBI Blast-2.16.0+ analysis [50]. Molecular identification of the insects was based on the COI amplicon identity with DNA sequences from the NCBI GenBank database [51] using a 97% threshold.

The resulting DNA sequences were processed with the DADA2 v1.8 package in R (v4.4.2) [52]. After removing the forward and reverse primer sequences using cutadapt (v4.2.2) [53], the sequences were trimmed and filtered, retaining only the forward sequences. Amplicon Sequence Variants (ASVs) were derived from the de-duplicated sequences, and chimeras were removed using the "consensus" approach. The taxonomic classification of the ASVs was carried out with the Silva v138 16S rRNA database (silva.nr.v138). The analyses were performed using MicrobiomeAnalyst 2.0 [54]. The VENN diagram was generated using the Venn Diagram platform [55].

2.5. Statistical Analysis

Alpha and beta diversity were assessed using the pyloseq package [56]. The alpha diversity of the ASVs was measured using the Chao1, Shannon, Evenness, and Fisher indices. Bray–Curtis dissimilarity was employed to evaluate beta diversity and compare the diversity across samples and microbial communities. Non-metric multidimensional scaling (NMDS) was used to represent the 2D matrix, with each point reflecting the full microbiome of a single sample. The statistical significance ($p < 0.05$) of the clustering patterns in the ordination plots was tested using Analysis of Similarities (ANOSIM) and Permutational Multivariate Analysis of Variance (PERMANOVA). All statistical analyses were conducted using MicrobiomeAnalyst 2.0 (Lu et al., 2023) [54]. The data filtering process was carried out using the Low Count Filter, configured with the parameters Minimum Count = 0 and Prevalence in Samples = 10%. Additionally, the Low Variance Filter was applied with Percentage to Remove = 0%, ensuring that no features were excluded based on variance. For normalization, the data were not rarefied, and Total Sum Scaling (TSS) was set to 0. No further data transformation was applied in order to preserve the original

structure and scale of the dataset for downstream analysis. A Student's *t*-test was used to compare the mean gene abundances between the two sample types [57–59].

The COI gene sequences of insects were deposited in GenBank (NCBI) under the accession numbers OK380902-OK380942, PV017878, PV018814- PV018823, and PV153587. The 16S rRNA gene sequences of bacteria present in the insects collected from different fruit trees (Supplementary Table S1) were deposited in the NCBI SRA Sequence Read Archive under the BioProject PRJNA1215968.

3. Results

3.1. Identification and Distribution of Insect Species on Fruit Trees

Investigation of Diptera interactions with seven fruit tree species from three genera (*Malus*, *Prunus*, and *Cydonia*) in an orchard in southern Romania included *Malus pumila* and *M. domestica* 'Jonagold' (apple), *Prunus domestica* (plum), *Prunus avium* (cherry), *Prunus persica* and *P. persica var. platycarpa* (peach), and *Cydonia oblonga* (quince). A total of 51 insect specimens were collected and identified as belonging to Diptera families *Anthomyiidae*, *Drosophilidae*, *Milichiidae*, *Chloropidae*, *Muscidae*, *Aphididae*, *Ulidiidae,* and *Lauxaniidae*, and the *Elateridae* (Coleoptera) family (Supplementary Table S1).

Taxonomic identification was confirmed through cytochrome oxidase I-based DNA barcoding, revealing 18 distinct Diptera species belonging to 14 Diptera genera (*Anthomyia, Botanophila*, *Delia*, *Desmometopa*, *Drosophila*, *Fiebrigella*, *Helina*, *Musca*, *Myzus*, *Neophyllomyza*, *Oscienella*, *Physiophora*, *Sapromyza,* and *Scaptomyza)*, and to one Coleoptera species (*Adrastus rachifer)* (Supplementary Table S1). The majority of insects (49 specimens) were matched to previously reported species (GenBank), based on a $\geq$97% sequence identity threshold, while a lower COI nucleotide sequence identity score was found in the cases of *Fiebrigella* (93.66%) and *Sapromyza* (87.15%) specimens (Supplementary Table S1).

The number of specimens of each insect species varied from 0 to 9 across different tree types (Figure 2A). The highest number of insects (24) was attracted by the cherry tree, followed by the apple tree (12), while fewer insects were collected from the peach (6), plum (4), and quince (4) trees (Figure 2A). Notably, *Drosophila* species dominated the Diptera composition on all tree types, except for the apple tree, which was predominantly visited by *Botanophila fugax* (Figure 2A). Among the more frequently encountered insect species, *Scaptomyza* sp. had a higher presence on the cherry tree during the collection period (Figure 2A). The insects appeared to show preferences for specific tree types, with most species (13) being retrieved from only one type of fruit tree (Figure 2A).

VENN diagram analysis showed that insect diversity varied among the different fruit trees, with most insects showing a tree-specific distribution, except for *Drosophila melanogaster,* which was the only *Diptera* species found on all tree types (Figure 2B). Specific insects belonging to seven species of genera *Delia*, *Fiebrigella*, *Neophyllomyza*, *Myzus*, *Oscienella*, *Drosophila,* and *Scaptomyza* showed preference for the cherry tree, while the apple tree was visited by five other distinct species of genera *Botanophila*, *Helina*, *Musca*, *Anthomyia,* and *Sapromyza* (Figure 2B). Also, *Desmometopa sordida*, *Drosophila simulans,* and *Physiophora alceae* were specifically attracted to the plum tree, and *Adrastus rachifer* and *Scaptomyza elmoi* were present only on the peach tree. No distinct insect species were found on the quince tree. Meanwhile, *Antomyia* species were noted on both apple and cherry trees (Figure 2B).

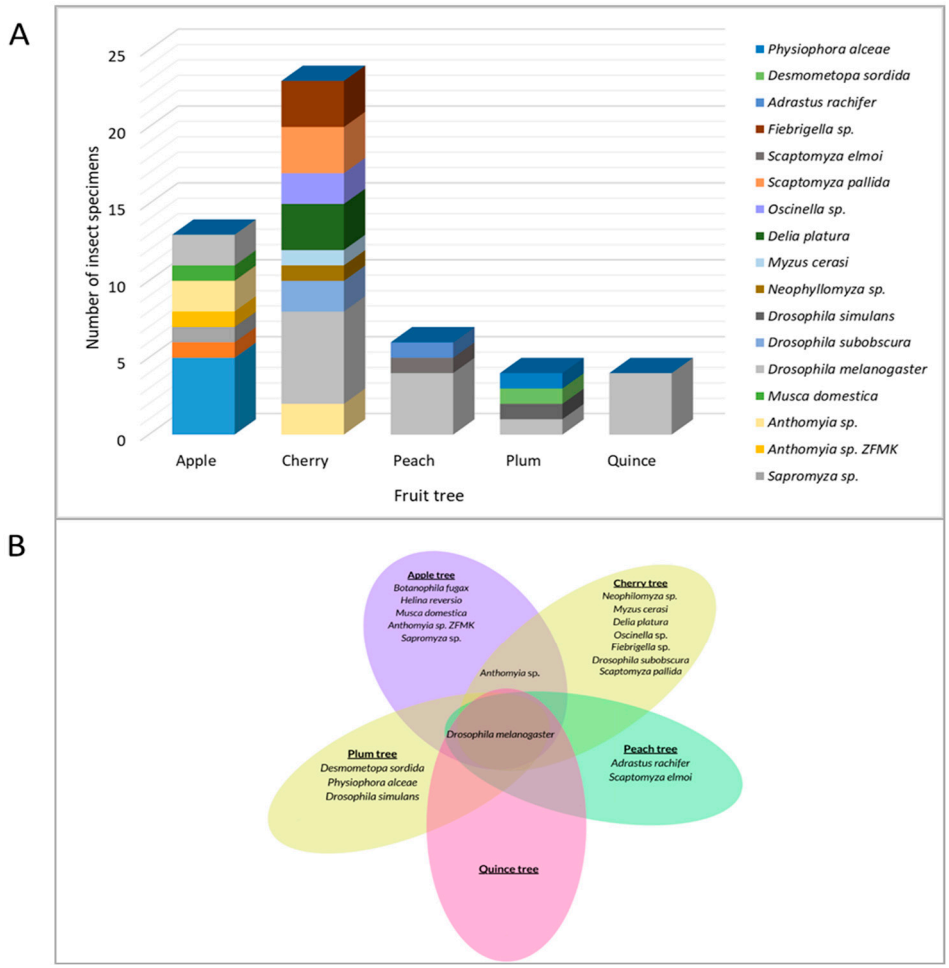

Figure 2. Distribution of insect species on various fruit trees: (**A**) Number of different insect species collected from the investigated apple, cherry, peach, plum, and quince trees. (**B**) Distinct and commonly found insect species on the five types of fruit trees.

3.2. Influence of Temperature and Fruit Developmental Stage on Insect Presence

As expected, most of the insects were collected during July (24 specimens) characterized by higher temperatures in the 16–36 °C interval, followed by June (14 specimens) with temperatures varying between 18 °C and 34 °C, while only two specimens belonging to *Neophyllomyza* sp. and *Myzes cerasi*, respectively, were collected in May from the cherry tree, a month with lower recorded temperatures (10–27 °C) (Supplementary Table S1). Meanwhile, a more reduced number of insects (nine) were retrieved from the investigated fruit trees in August, in spite of comparable temperatures with those of the collection intervals of July, varying between 17 °C and 37 °C (Supplementary Table S1).

The fruit's developmental stage (ripe or green) during the collection season was also considered when assessing insect preferences for the tree types. Thus, the majority of insects present during this survey were found on trees bearing ripe fruit (39 specimens), while only 12 were attracted to trees with green fruit (Supplementary Table S1). Among the latter ones, *Neophyllomyza* sp. and *Myzus cerasi* were only retrieved from cherry trees, and *Scaptomyza elmoi* and *Adrastus rachifer* from the platycarpa subspecies of peach tree (Supplementary Table S1).

In the case of *Drosophila* specimens, which were present on all tree types throughout the warm months (June–August), their distribution revealed a preference for cherry trees in June, apple and plum trees in July, and quince trees in August, while peach trees appeared to be more ubiquitously visited throughout this period (Figure 3A).

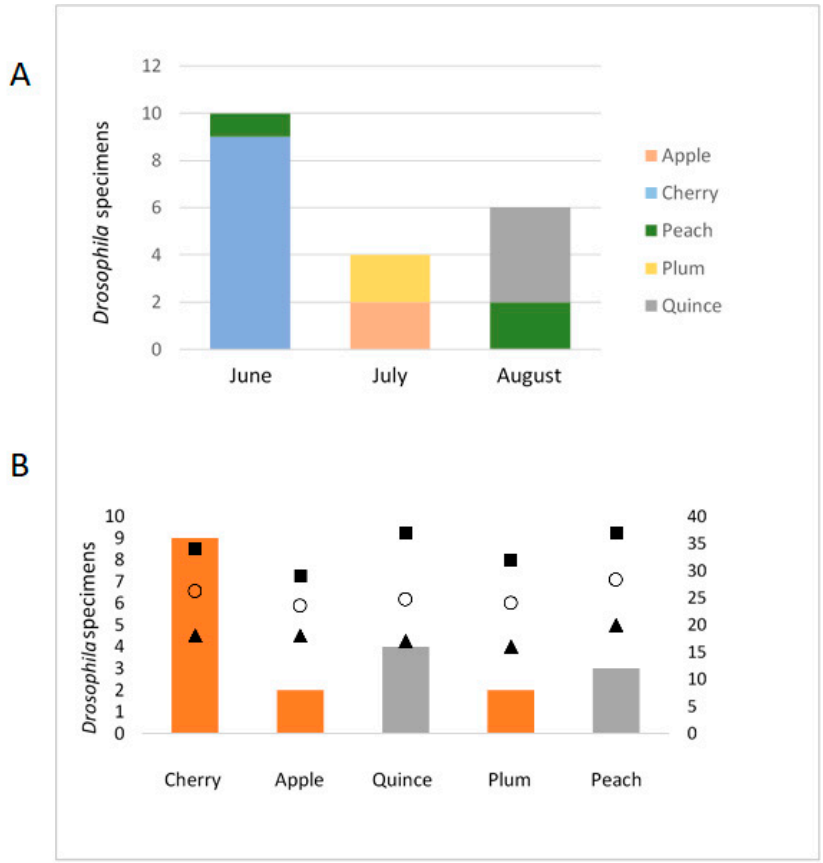

Figure 3. Distribution of *Drosophila* specimens on fruit trees: (**A**): Collection month; (**B**): Preference for ripe (orange) and green (grey) fruit carrying trees. Environmental temperatures (°C) during 48 h collection interval: (▲) minimum value; (■) maximum value; (○) average value.

Regarding the preference for green or ripe fruit carrying trees, *Drosophila* individuals were present during both these developmental stages, being more abundant on ripe fruit carrying trees (13) as compared to green fruit (7), with preference on cherry trees and equal distribution on apple and plum trees, while both plum and peach trees were evenly visited during the green fruit season (Figure 3B). During the collecting periods, minimum and maximum air temperatures ranged from 16 to 20 °C and 29 to 37 °C, respectively, with average values showing little variation in the 23.5–28.3 °C interval (Figure 3B).

Therefore, the environmental temperature did not appear to play a major role in the distribution of this insect on different trees bearing green or ripe fruits. These corroborated data suggest that the shifting preference of *Drosophila* for the investigated fruit trees from southern Romania was primarily influenced by fruit development stages rather than temperature variations during the warm season in southern Romania.

3.3. Bacterial Diversity of the Fruit Tree-Associated Insects

Among the 51 identified insects collected from the various fruit trees (Supplementary Table S1), 19 specimens from genera *Botanophila*, *Antomyia*, *Drosophila*, and *Scaptomyza* encompassing at least 2 individuals from each genus were analyzed using 16S rRNA gene Illumina sequencing to assess the diversity of associated bacterial communities and to examine their variability in relation with the tree type (Supplementary Table S2).

A total number of 1,072,630 post QC filtered reads were obtained and assigned to a median of 991.58 bacterial amplicon sequence variants (ASVs) varying in the 138–1594 interval (Supplementary Table S2).

ASV rarefaction curves indicated a complete identification of the insect-associated bacterial diversity for all analyzed specimens, with a distinct ASV richness across the four fly genera (Supplementary Figure S1).

3.3.1. Alpha Diversity

The calculated alpha diversity indices for the bacterial communities associated with the 19 insect specimens displayed considerable variability, influenced by both the type of tree and the insect species (Supplementary Table S2, Figure 4A,B).

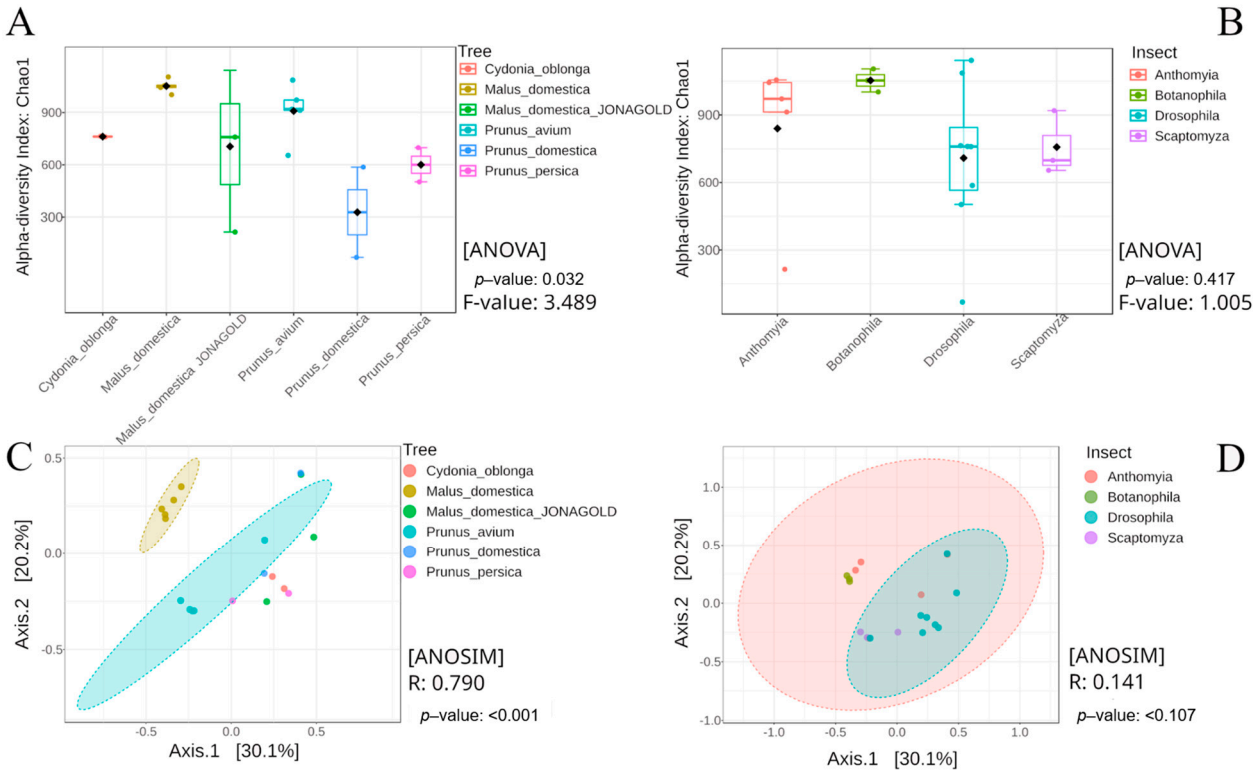

Figure 4. Alpha and Beta diversity of the bacterial communities from tree-associated insects: (**A**) Fruit-tree-dependent bacterial diversity based on Chao1 index; (**B**) Insect-dependent bacterial diversity based on Chao1 index; Non-metric Multidimensional Scaling (NMDS) analysis of bacterial community composition across (**C**) tree species, and (**D**) insect genera.

The Chao1 index distribution demonstrated a significant variation in bacterial diversity among the different fruit tree species (Figure 4A). A large variability was observed between bacterial communities associated with insects collected from *Malus domestica* and *Cydonia oblonga,* characterized by the highest and lowest diversity, respectively (Figure 4A). Also, bacteria from insects collected from the *M. domestica* Jonagold variant displayed a significantly lower Chao1 value and a broader distribution as compared to the regular apple tree (Figure 4A). Furthermore, substantial differences in Chao1 indices were observed between *Prunus persica* (peach tree) and *Prunus avium* (cherry tree), both belonging to the *Prunus* genus. These differences were characterized by wide distributions and high median values, suggesting that even closely related species within the same genus can support bacterial communities with considerable variation in diversity (Figure 4A).

Bacterial diversity, as quantified by the Chao1 index, also varied across insect genera (Figure 4B). *Drosophila* exhibited high variability in bacterial diversity, whereas *Botanophila* and *Anthomyia* displayed higher mean Chao1 values. Meanwhile, *Scaptomyza* insects exhibited an intermediate range of bacterial diversity (Figure 4B). These inter-generic differences

in bacterial diversity among insect species may be influenced by variations in their feeding behaviors and interactions with host plants.

The indices variation revealed significant patterns in species richness, distribution, and evenness. Sample AI1 corresponding to *D. melanogaster* from *Malus domestica* Junagold (Supplementary Table S1) stood out as the most diverse, with the highest values in the Shannon Index (7.02), Fisher's Index (328.8), and Chao-1 estimator (1595), indicating high diversity and species richness, accompanied by notable evenness (0.7016) that highlighted a relatively uniform distribution of microbial species. In contrast, sample PR2 (*D. simulans* from *Prunus domestica*) showed the lowest values in all metrics (Shannon Index: 4.125; Fisher's Index: 15.12; Chao-1: 138), reflecting low diversity and species richness, with moderate evenness (0.4482). Other samples, such as PP2 *(Scaptomyza elmoi* from *Prunus persica* platycarpa), exhibited exceptionally high evenness (0.7252), the highest in the dataset, indicating a very uniform distribution of species, although not necessarily high diversity. Meanwhile, sample G5 (*D. melanogaster* from *Cydonia oblonga)* was characterized by the lowest evenness (0.2735), suggesting strong dominance by a few species, despite moderate Shannon and Chao-1 values. These results highlighted a large variability in the structure of insect-associated bacterial communities, where AI1 represents a critical point of diversity and evenness, and PR2 and G5 indicated a lower diversity and species dominance, respectively (Supplementary Table S2).

3.3.2. Beta Diversity

Principal Coordinate Analysis (PCoA) and Non-Metric Multidimensional Scaling (NMDS) analyses revealed that the distribution of insect-associated bacterial communities varied distinctly based on tree genus and species, as well as insect genus (Figure 4C,D).

The composition of insect-associated bacterial communities varied significantly according to fruit tree species (ANOSIM R = 0.79, $p < 0.001$), indicating a strong and statistically significant separation between the groups (Figure 4C). The high R value, close to one, suggested that the similarity within the groups was much greater than the similarity between them, reflecting clear and robust differences in the bacterial communities associated with samples recovered from each fruit tree species (Figure 4C). Specifically, *Cydonia oblonga* and *Malus domestica* formed distinct clusters, while *Prunus* species exhibited some overlap (Figure 4C). These results indicated that species-specific factors play a crucial role in structuring bacterial communities. The multivariate homogeneity of dispersion analysis confirmed that the assumption of homogeneous dispersion among groups was met for subsequent PERMANOVA testing. Both parametric (ANOVA: F = 1.1482, $p = 0.3844$) and non-parametric (permutation test $p = 0.404$) approaches showed no significant differences in dispersion patterns across the studied groups, including *Cydonia oblonga*, *Malus domestica*, and various *Prunus* species. The principal coordinates analysis revealed that the first three axes (PCoA1 = 1.8685, PCoA2 = 1.2506, PCoA3 = 1.0061) captured the majority of the variation, with average distances to centroids ranging from 0.2268 to 0.4995 across groups. These results collectively demonstrate that the dispersion characteristics satisfy the key assumptions required for valid PERMANOVA interpretation, ensuring that any significant differences detected reflect genuine compositional variation rather than heterogeneous dispersion artifacts. The consistency between analytical methods and the clear structure observed in the PCoA provided strong support for the robustness of the multivariate approach.

In contrast, bacterial community composition did not differ significantly among insect genera (R = 0.141, $p = 0.107$) (Figure 4D), suggesting that host–tree interactions or environmental factors have a more substantial influence on microbial assemblages than the insect taxonomy.

3.4. Taxonomic Profile of the Insect-Associated Bacterial Communities

The identified ASVs of the bacterial communities associated with the analyzed insects belonging to *Botanophila*, *Antomyia*, *Drosophila*, and *Scaptomyza* were assigned to 4 phyla, 6 classes, 17 orders, 31 families, and 51 genera (Supplementary Table S3).

Bacterial phyla present in the tree-associated insects encompassed *Pseudomonadota*, *Bacillota*, *Bacteroidota*, and *Actinomycetota*, with a prevalence of *Pseudomonadota* and Bacillota in all insects (Figure 5A). *Pseudomonadota* dominated the insects from *M. domestica* Jonagold (up to 99%), *P. domestica* (up to 99%), and *P. persica* (up to 91%) trees, while *Bacillota* was highly present in *M. domestica* (50%) and *P. avium* (62%), sharing the dominant taxa with *Pseudomonadota* representatives (Figure 5A). A limited occurrence of *Actinomycetota* (up to 2%) was noticed in insects retrieved from quince trees, and incidentally from apple Jonagold (0.4%) and peach (0.3%) trees, identified in the AI1 and N1 insects, respectively. Meanwhile, phylum *Bacteroidota* was majorly present in AI5 collected from apple Jonagold trees (Figure 5A).

Interestingly, at the class level, the microbiome dominated by *Pseudomonadota* (insects collected from quince, apple Jonagold, plum, and peach trees) was distinctively composed of *Alphaproteobacteria* taxa, while the shared *Pseudomonadota–Bacillota* microbiomes from *M. domestica* and *P. avium* fruit trees-associated flies contained only *Gammaproteobacteria* taxa (Figure 5B). Class *Bacteroidia* was identified in insects from *M. domestica* Jonagold trees, and *Clostridia* was present in *M. domestica* and *P. domestica*-associated insects (Figure 5B).

At the genus level, a high variability among the microbiomes of insects associated with these fruit trees was observed, with the 10 most prominent bacterial genera covering 92.2% of the identified bacterial assemblages (Supplementary Table S3). Among these, *Gluconobacter* exhibited a notable prevalence across almost all investigated tree types, with particularly high relative abundances in *P. persica* (65.39%) and *C. oblonga* (45.78%) (Figure 5C). In contrast, genus *Wolbachia* was highly present in *P. domestica* (88.39%) and *M. domestica* Jonagold (32.84%), while being completely absent in *C. oblonga* and *P. persica*. Also, *Fructobacillus* was more frequently detected in *P. avium* (19.62%) and *M. domestica* (20.22%), whereas *Acetobacter* displayed a more uniform distribution, with a notable presence in *M. domestica* Jonagold (11.93%) but a low abundance in *M. domestica* (0.056%) (Figure 5C). Bacteria belonging to genera *Acetobacter*, *Enterococcus*, *Gluconobacter*, *Leuconostoc*, and *Weissella* were consistently detected in all fruit tree species, covering a relative abundance varying in the 0.06% (*M. domestica*)–11.9% (*M. domestica* Jonagold), 0.01% (*M. domestica*)–26.4% (*C. oblonga*), 2% (*M. domestica*)–65.3% (*P. persica*), 0.04% (*P. domestica*)–13.7% (*M. domestica*), and 0.02% (*P. domestica*)–25.7% (*P. avium*) intervals, respectively (Figure 5C, Supplementary Table S3).

Meanwhile, some bacterial genera were absent in insects associated with specific tree species (Figure 5C). For instance, *Apilactobacillus* was absent in specimens collected from *C. oblonga* and *P. domestica*, while *Commensalibacter* was detected in all tree species except *M. domestica* (Figure 5C). Similarly, *Fructobacillus* was not identified in specimens from *Cydonia oblonga*, and *Wolbachia* was absent in insects from both *C. oblonga* and *P. persica* (Figure 5C). These absences indicate that these bacterial genera may not have a strong ecological association with these specific tree species or that their abundance is below detectable thresholds.

Heatmap analysis of the bacterial communities associated with the four fly genera (*Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza*) revealed distinct differences in both composition and relative abundance of bacterial genera across insects (Figure 6A). Thus, the *Drosophila* microbiome was characterized by a high prevalence of *Bacillus*, *Morganella*, and *Pantoea* species, whereas *Scaptomyza* exhibited a distinct microbial profile, with a greater representation of genera *Pseudomonas*, *Corynebacterium*, and *Staphylococcus*. In the case of *Anthomyia* specimens, a more homogeneous bacterial diversity was observed, with a preva-

lence of *Acinetobacter*, *Enterococcus*, and *Bacteroides*, while *Botanophila* flies harbored a relatively high abundance of *Fructobacillus*, *Wolbachia*, and *Lactobacillus* species (Figure 6A).

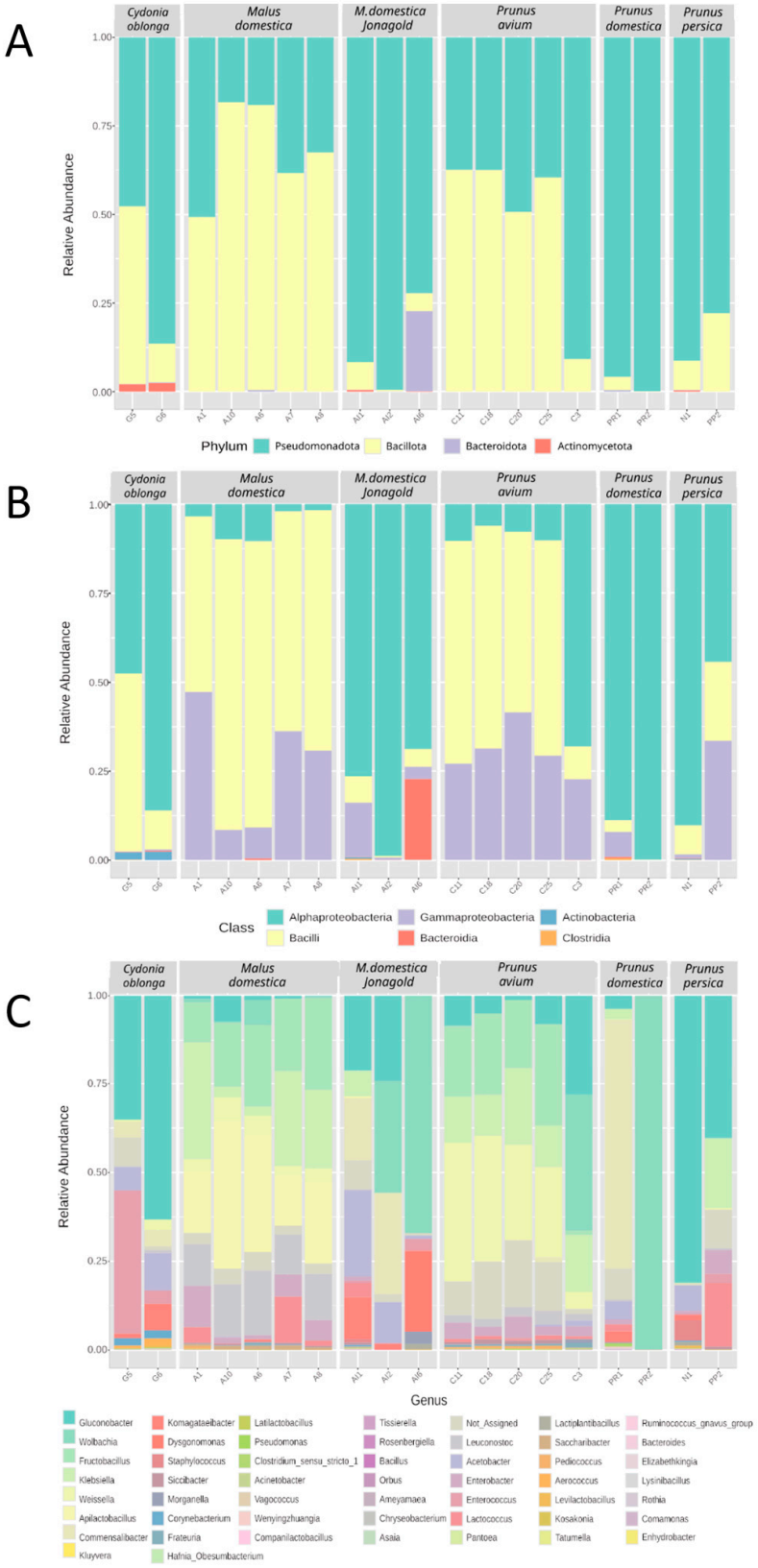

Figure 5. Taxonomy of the insect-associated bacteria collected from apple (*Malus domestica*), cherry (*Prunus avium*), plum (*Prunus domestica*), quince (*Cydonia oblonga*), and peach (*Prunus persica*) trees. Relative abundance of bacterial (**A**) Phyla; (**B**) Classes; (**C**) Genera.

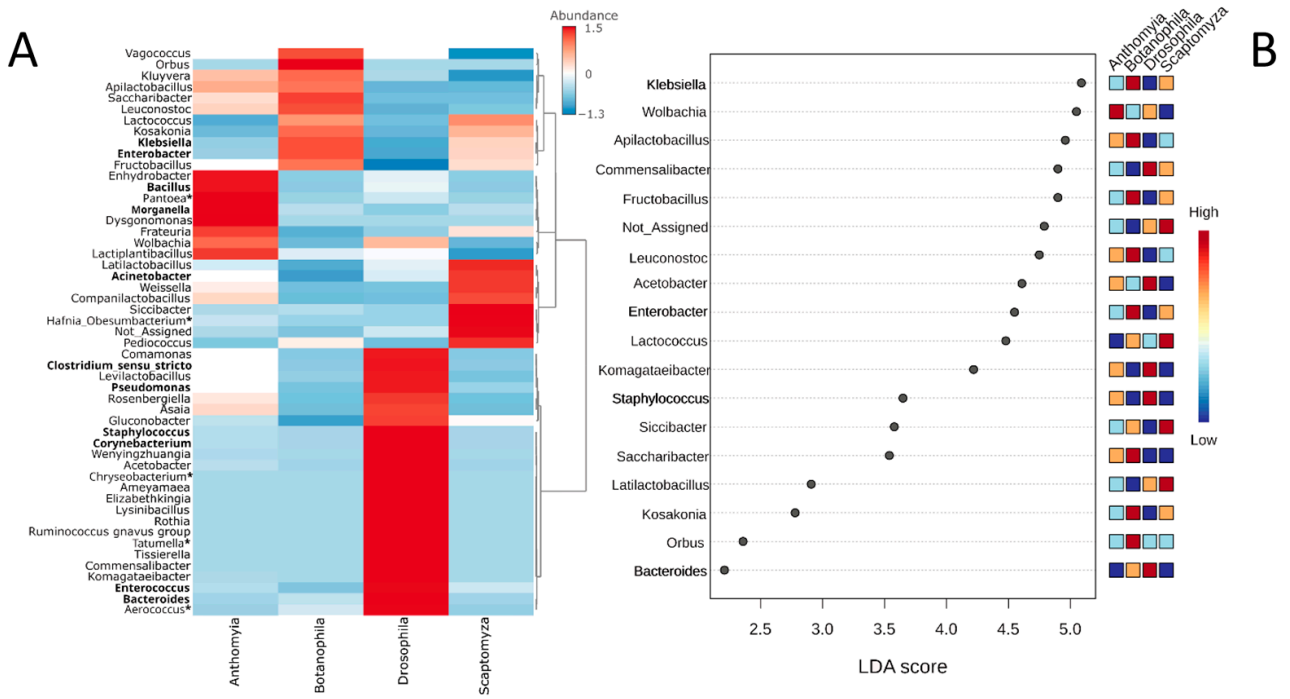

Figure 6. Distribution of bacterial genera in *Anthomyia*, *Botanophila*, *Drosophila,* and *Scaptomyza* insect specimens: (**A**) Heatmap of bacterial abundance associated with the flies; (**B**) LEfSe analysis (LDAscore) of significant bacterial genera associated with the flies; Potential pathogenic taxa (bold); (*) Most abundant taxa.

Among the identified bacterial genera, *Klebsiella*, *Enterobacter*, *Bacillus*, *Acinetobacter*, *Staphylococcus*, *Corynebacterium*, *Enterococcus*, and *Bacteroides,* which are known to contain species with pathogenic potential for humans, were detected at varying levels across the four fly groups (Figure 6A). In the case of the analyzed fruit flies, their prevalence was distinctly shared between insect genera, where *Klebsiella* and *Enterobacter* were highly present in *Botanophila*; *Bacillus* and *Morganella* in *Anthomyia*; and *Acidentobacter* in *Scaptomyza*, while the potentially pathogenic *Staphylococcus*, *Corynebacterium*, *Enterococcus*, and *Bacteroides* were dominant in *Drosophila* specimens (Figure 6A). The clustering analysis profile suggested that the bacterial community composition of these insects is structured differently depending on the host genus, potentially influenced by ecological, dietary, or phylogenetic factors.

To assess bacterial taxa with significant differential abundance among the investigated fruit fly genera, LEfSe (LDA score) analysis was performed (Figure 6B). This analysis identified *Klebsiella*, *Staphylococcus*, *Bacteroides*, and *Clostridium* as statistically significant biomarkers (LDA score > 3.0) that differentiate the four insect genera, indicating their dominance in specific hosts (Figure 6B). These findings revealed the taxonomic specificity of certain bacterial groups within distinct fly genera, suggesting potential ecological interactions shaping their associated microbiome.

4. Discussion

This study represents the first investigation of fruit tree–insect–bacteria interactions in southern Romania, providing novel data on the presence and dynamics of 18 *Diptera* species and one *Coleoptera* across various fruit trees including *Malus pumila* and *Malus domestica* 'Jonagold' (apple), *Prunus domestica* (plum), *Prunus avium* (cherry), *Prunus persica* and *Prunus persica* var. *platicarpa* (peach), and *Cydonia oblonga* (quince). Insect species were identified through genetic DNA barcoding, with further characterization of bacterial

communities associated with 19 specimens belonging to the genera *Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza* by 16S rRNA gene Illumina sequencing, providing an in-depth survey of the distribution pattern of these fruit flies across specific fruit trees in this European region, and their potential role in microbial transfer.

4.1. Fruit Tree Insects in South Romania Orchard

Among the 19 insect species identified in this study, belonging to the *Diptera* families *Anthomyiidae*, *Muscidae*, *Lauxaniidae*, *Drosophilidae*, *Milichiidae*, *Aphididae*, *Chloropidae*, and *Ulidiidae*, as well as the *Coleoptera* family *Elateridae* (Supplementary Table S1), 5 species had not been previously reported in this country according to the Global Biodiversity Information Facility [60]. This study consequently expanded the known distribution range of these insect species in southern Europe (Supplementary Figure S2). Among them, *Helina reversio* (Supplementary Figure S2A) and *Scaptomyza elmoi* (Supplementary Figure S2E) are widely distributed across the continent, while *Drosophila subobscura* (Supplementary Figure S2B) and *Drosophila simulans* (Supplementary Figure S2C) had previously been reported in northern and western European regions. Notably, the occurrence of *Fiebrigella* sp. (Supplementary Figure S2D) across Europe was limited to a few countries that are not geographically connected to Romania, and *D. simulans* (Supplementary Figure S2C) was not reported in any neighboring countries.

Moreover, *Botanophila fugax*, identified in this study, was reported for the first time on Romanian territory in the current investigation, extending the known geographical area of this fruit fly to southeastern Europe.

From the 19 insect species identified in this survey, the majority (13) exhibited a preference for specific fruit tree types, being observed on only a single tree species. The highest insect diversity was recorded in cherry and apple trees, irrespective of the collection period within the warm season (May–July). Furthermore, while environmental temperature did not appear to influence insect distribution across tree types, fruit ripeness (ripe vs. green) seemed to be a key factor in attracting distinct insect species. The preference of insects for ripe fruits from polyphagous hosts, influenced by factors such as odor and color [61], may be reflected in the present study by the later ripening of quince and peach fruits as compared to apple, cherry, and plum fruits within the experiment carried out in southern Romania between May and August, considering the observed infestation increase based on the time of fruit color change reported the case of different cherry varieties exposed to *Rhagoletis cerasi* [62].

In the current investigation, *Anthomyia* species were observed in apple and cherry trees during the June–July interval, when temperatures varied between 16 °C and 28 °C (Supplementary Table S1). This insect genus (*Anthomyia procellaris*) was previously reported in Arges County, Romania, associated with grapevine plants [63]. Furthermore, a forensic experiment placed in an exposed area in Bucharest, Romania, also revealed the presence of *A. pluvialis*, a related species from the *Anthomyiidae* family, on pig carcasses during spring time when temperatures exceeded 20 °C [64], indicating the interaction of these flies with variable substrates, both vegetal and animal, in the southern region of Romania in warm periods.

Regarding the identified insects belonging to the family *Anthomyiidae*, a recent study in Norway investigating the role of insects as potential vectors of *Enterobacteriaceae* pathogens identified the presence of *B. fugax* and *Delia* species on potato plants in this northern European region [65], suggesting that these *Diptera* also found in our study on apple and cherry trees may play a role in microbial distribution across various plants, including fruit trees, within broader ecosystems.

Among the insect species analyzed for interactions with fruit trees, *Drosophila* stood out for its presence across all tree types, highlighting its adaptability to diverse plant substrates. These flies, recognized as a globally distributed insect genus, are commonly found in various types of orchards and fruit trees [6,7,15,17].

The species *D. subobscura*, *D. simulans,* and *D. melanogaster,* which were identified in the current study in cherry tree, plum tree, and in all five fruit tree types, respectively, from Romania during the warm season, with temperatures reaching 36 °C, have also been observed in association with pear (*Pyrus* spp.) and apple (*Malus domestica*) trees in Chile exposed to average temperatures of 13.3–14.9 °C and variable sunlight conditions [66] (Silva-Lopez et al., 2023), marking their widespread occurrence and versatility.

Among Drosophila representatives, *D. simulans* has also been associated with exotic fruits like bananas and decaying figs, while both *D. simulans* and *D. melanogaster* have been documented on a large variety of fruits and vegetables such as zucchini, watermelon, bananas, grapes, and pears from South Africa [67]. Studies on tree–insect interactions in Hawaii also mentioned *Drosophila* associations with *Cheirodendron*, *Clermontia*, *Pisonia*, and *Acacia* exotic tree species [68]. *D. subobscura* was also present on mixed woodland vegetation from the UK, comprising *Crataegus* (hawthorn), *Sorbus aucuparia* (rowan), and *Solanum dulcamara* (bittersweet nightshade) [69]. Moreover, *D. subobscura*, *D. simulans*, and *D. melanogaster* were observed interacting with fungal organisms, such as summer truffles in northern and southern Italy [70], highlighting the extensive adaptability of these flies to diverse substrates and environmental conditions. Species of *Drosophila*, such as *D. suzukii* and *D. melanogaster*, were also described on Romanian territory in mixed forest fruit plantations such as raspberry and blackberry orchards [27,29] confirming the presence of these commonly found fruit flies on various fruit trees from this region.

4.2. Insect–Bacteria Dynamics on Fruit Trees

The identified bacterial communities from the 19 specimens of *Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza* collected from apple, cherry, peach, plum, and quince trees in southern Romania revealed a high taxonomic diversity, encompassing 51 genera belonging to 4 phyla.

Alpha diversity analysis of the insect-associated 16S rRNA ASVs highlighted a more distinct bacterial community variation at the tree species level, indicating species-specific influences. At the genus level of the fruit trees, the species differences were integrated, reducing variability but highlighting overall diversity trends within each genus. For each insect genus, the corresponding bacterial diversity was influenced by the nature of plant–insect interactions, with genus *Drosophila* showing broader distributions and *Scaptomyza* exhibiting more consistent patterns. Accordingly, Beta diversity NMSD analysis of insects' bacteria confirmed that the most pronounced differences in bacterial community composition occurred at the tree species level, with a moderate and significant R-value indicating species-specific influences. At the tree genus level, bacterial community differences were less distinct but still significant, suggesting broader taxonomic patterns, while no significant differences were detected among insect genera, implying that insect taxonomy has a limited role in structuring bacterial communities. These results suggest that the identity of the host species (in this case, the trees) has a significant impact on the diversity and structure of the microbial communities associated with the insects [71]. The overall data show that the diversity of insect-associated bacteria varied primarily with tree species rather than insect genus, with the exception of *Drosophila*, suggesting that these insects may serve as effective carriers of bacterial communities [72].

A notable preference for specific bacterial taxa was observed among insect communities collected from different tree species. *Bacillota* phylum predominantly colonized flies

from apple and cherry trees, whereas *Pseudomonadota* was more closely associated with insects found in plum, peach, and Jonagold apple trees. Additionally, the bacterial composition at the class level varied significantly, with dominant *Gammaproteobacteria* or *Alphaproteobacteria* depending on the prevalence of this phylum in the insect microbiota. Given the widespread presence of *Drosophila* across all the examined fruit trees, its associated microbiota may be dispersed among different tree species.

In this regard, previous studies on the *Drosophila* microbiome reported a high prevalence of *Acetobacter* and *Lactobacillus* species on the fly surface [73], while the insect gut was dominated by *Enterobacteriaceae* taxa including *Providencia*, *Serratia*, *Shigella*, *Erwinia*, *Pantoea*, and *Enterobacter* species, *Acetobacteraceae* (*Acetobacter* and *Commensalibacter* species), and *Lactobacillales* (*Enteroccocus*, *Lactobacillus*, and *Vagococcus* species) [6]. In our study, *Drosophila* specimens collected from fruit trees from southern Romania exhibited a high relative abundance of *Acetobacter*, *Commensalibacter*, and *Enteroccocus* species, confirming the important metabolic role of these microbes in *Drosophila* feeding on high-energy substrates such as fruits [6,40]. Meanwhile, the major presence of these bacteria associated exclusively with *Drosophila* among the analyzed four insect species could support the microbial shuttle role of this fly between fruit trees in the Romanian investigated habitat.

Bacterial communities associated with *Scaptomyza*, a plant tissue consumer, appeared to be dominated by *Pseudomonadaceae* taxa [71]. Among these, the presence of *Pseudomonas syringae*, a well-known plant pathogen that causes bacterial blight in many crops and fruit trees [74,75], suggests that *Scaptomyza* may serve as a vector for this bacterial pathogen between plants.

4.3. Potential Pathogen Insect Carriers Between Fruit Trees

The association of specific bacterial species with certain insects indicates their potential involvement in disease transmission, while some bacterial exchanges may also confer beneficial effects on the insect host [1,3,32].

In this respect, heatmap analysis of bacterial communities associated with the four insect genera *Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza* collected from fruit trees in southern Romania revealed the normalized abundances of bacterial genera of these *Diptera*, highlighting several taxa of putative clinical relevance. Notably, certain bacterial genera with pathogenic representatives exhibited high abundances. Among them, *Klebsiella*, majorly associated with *Botanophila* and some *Scaptomyza* specimens, includes *K. pneumoniae*, a well-documented human pathogen associated with respiratory and nosocomial infections [76].

A study analyzing *Drosophila* specimens from multiple continents found that bacteria belonging to family *Enterobacteriaceae* make up approximately 60% of various *Drosophila* microbiomes [6]. This bacterial family includes species associated with both animals and plants and can also exist as free-living symbionts in many insects, including *D. melanogaster* [77], which play important roles in insect nutrition, defense, and heat stress tolerance [78,79]. Although, to our knowledge, there are no specific studies documenting diseases associated with *Enterobacter* carried by flies of the genera studied in this research, this genus is known to be associated with nosocomial infections, including bacteremia and sepsis, especially in hospital settings, and to be carried by *Musca domestica* [80,81].

In the current study, most of the potentially harmful bacterial taxa were associated with *Drosophila* specimens, suggesting that this insect could serve as an effective, widespread carrier, as it was collected from all types of fruit trees. Among these bacteria, *Staphylococcus*, particularly *S. aureus*, is implicated in antibiotic-resistant skin infections and sepsis [82]. This opportunistic pathogen has been shown to infect *Drosophila*, leading to immune modulation and a reduced lifespan [83].

Bacteroides, an opportunistic genus, has been linked to intra-abdominal infections and intestinal dysbiosis, while *Enterobacter* is a common multidrug-resistant pathogen in hospital environments [84]. *Clostridium* includes species such as *C. difficile* and *C. perfringens*, both of which are associated with severe gastrointestinal and systemic infections [85], and *Aerococcus* has been reported as a causative agent of urinary tract infections and bacteremia [86].

A potential pathogen from the genus *Acinetobacter* was identified in *Scaptomyza* specimens collected from both cherry and peach trees. While no documented evidence currently links *Acinetobacter* species specifically to *Scaptomyza*, this bacterial genus is well known for its pathogenicity in humans, particularly in hospital environments, where infections caused by *A. baumannii* are frequently associated with immunocompromised patients, contributing to pneumonia, bacteremia, wound infections, and urinary tract infections [87,88].

Anthomyia specimens collected from apple and cherry trees were carrying a high content of both *Bacillus* and *Morganella* bacterial species. These genera are known for having some representatives that are pathogenic to humans. While *Bacillus* species are generally non-pathogenic and widely distributed in soils, plants, and various environments due to their ability to form resilient endospores, certain species, such as *B. anthracis* and *B. cereus*, are responsible for anthrax and food poisoning, respectively [89,90]. Among *Morganella* species, *M. morganii*, a facultative anaerobic Gram-negative bacterium, is recognized as a human pathogen associated with urinary tract infections and also associated with wound infections from animal bites, indicating its role in zoonotic diseases [91]. However, despite also being a lethal pathogen for the larvae of the Mexican fruit fly (*Anastrepha ludens*) [92], there is no available information on *Morganella* species associated with *Anthomyia*, *Botanophila*, and *Scaptomyza*.

Myzus cerasi, recognized as a significant pest of cherries worldwide, and identified in central and eastern parts of Romania [25,93], was also detected on cherry trees in the southern region of this country during this study, further confirming its widespread affinity for this host. A previous study on pathogen transmission among fruit trees in Romania identified five aphid species from plum trees, including a fly belonging to the same *Diptera* genus (*M. persicae*) as a vector of the plum pox virus [94], suggesting the potential role of these insects in bacterial transfer between different fruit trees.

4.4. Potential Dynamics of Beneficial Bacteria

Meanwhile, some bacterial genera associated with the fruit tree insects in southern Romania might play a beneficial role for their host. Among these, fructose-associated *Fructobacillus* spp., highly present in *Botanophila* specimens, were reported to play a major role in insects' digestive processes, immune modulation, and protection against pathogenic microorganisms [95]. Also, *Klebsiella* and *Enterobacter* species, mainly found in *Botanophila* and *Scaptomyza* insects, are known to facilitate sugar metabolism [96]. Moreover, acetic acid bacteria from the genera *Acetobacter* [97] and *Saccharibacter* [98] have been identified as key microbial symbionts of *Diptera* that primarily rely on sugar-based diets [88]. These well-adapted bacteria to sugar- and ethanol-rich environments are known to play a crucial role in larval development and adult fitness by enhancing nutrient availability and shaping microbial interactions within the gut [99]. Consequently, their high relative abundance in *Drosophila* and *Botanophila* specimens collected from fruit trees in this study suggests a beneficial role in host physiology. Previous studies on *Drosophila* microbiome composition revealed the crucial role of *Bacteroidetes* species for the digestion of complex carbohydrates from plants, making nutrients available to the fly [73], and to the immune function and gut health by shaping the host's overall microbial environment [6].

Wolbachia, also identified in these insects, is a non-pathogenic symbiont in humans, including species that can provide metabolic advantages such as vitamin synthesis, and enhanced survival under nutritional stress in certain host organisms like the fruit fly (*Drosophila melanogaster*) [42,100]. *Bothanophila* species from Europe and the USA were also reported to have an increased rate of larval infection by *Wolbachia* [101]. This endosymbiont can manipulate host reproduction to enhance its vertical transmission through strategies such as cytoplasmic incompatibility, feminization, and male-killing, favoring the proportion of female offspring that can transmit the bacteria [102–104]. While these mechanisms do not directly cause disease, they can alter host population dynamics and potentially reduce evolutionary fitness in non-adapted species [102]. This bacterial genus associated with both the *Anthomyia* and *Drosophila* insect species identified in this study might be widespread among fruit trees, in particular apple and cherry trees, where the insects were predominantly found, with both beneficial and harmful potential effects.

Such a dual effect was also highlighted in the case of some *Enterococcus* species, known as well-established members of the *Drosophila* microbiome. While these bacteria can assist in digestion and help maintain a balanced gut microbiota [40], under conditions of immune compromise or stress, they can become opportunistic pathogens, posing a risk to the host [105].

The remarkable diversity of bacterial genera associated with *Anthomyia*, *Botanophila*, *Drosophila*, and *Scaptomyza* specimens collected from five fruit tree genera indicates that these *Diptera* species may play a role in facilitating microbial transfer among apple, cherry, peach, plum, and quince trees. This hypothesis suggests a multifactorial mechanism of microbial dissemination, influenced by the characteristics of the plant substrate, among other environmental and ecological factors.

5. Conclusions

This pioneering investigation of tree–insect–bacteria interactions conducted in southern Romania using molecular techniques such as COX1 DNA barcoding for insect identification and 16S rRNA gene Illumina sequencing for bacterial profiling revealed a distinct distribution pattern of 19 insect species across apple, cherry, peach, plum, and quince trees. This distribution was influenced by the developmental stage of the fruit, with the exception of *Drosophila* species, which were found ubiquitously across all tree types throughout the warm season (May–August). Notably, five of these insect species were reported for the first time in Romania, according to the GBIF platform [60].

The identified bacterial communities associated with Anthomyia, Botanophila, *Drosophila*, and *Scaptomyza* represent, to our knowledge, the first comprehensive overview of microbiota linked to these fruit flies. The results indicate that tree species, rather than insect genus, have a greater influence on bacterial diversity, with the exception of *Drosophila*, suggesting that this insect genus may function as an efficient vector for bacterial communities. The investigation revealed a distinct taxonomic profile, comprising 51 bacterial genera across four phyla, associated with the examined fruit flies. Several bacterial genera with potential pathogenic effects, as well as some with potentially beneficial properties, were predominantly identified in *Drosophila* specimens, highlighting the widespread potential of this species to facilitate bacterial transmission across various tree types.

These findings contribute to a broader understanding of the interplay between tree hosts, insect vectors, and microbial diversity, with implications for disease transmission and fruit crop management.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17040295/s1, Table S1: Identification of insect species from trees

based on COI gene sequence; Table S2: Number of 16S rRNA reads, filtered ASVs and alpha diversity indices of bacterial communities from tree-associated insects; Figure S1: Rarefaction curves of the bacterial ASVs associated with Anthomyia, Botanophila, Drosophila, and Scaptomyza insects collected from fruit trees; Figure S2: First records of Diptera species on Romanian territory according to GBIF https://www.gbif.org/ (accessed on 21 February 2025); Table S3: Relative abundance bacteria taxa (xls).

Author Contributions: D.S.C. performed the insect sampling and the experimental steps for insect identification, DNA extraction, PCR, COI sequence analysis, and contributed to manuscript editing; P.L. performed the bioinformatics and statistical analyses and contributed to data interpretation and manuscript writing; C.I. contributed to multiparameter data analysis; C.P. performed data interpretation, and wrote and reviewed the final manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data supporting the conclusions of this article are included in the manuscript. The COI gene sequences of insects were deposited in GenBank (NCBI) under the accession numbers OK380902-OK380942, PV017878, PV018814-PV018823, and PV153587. The 16S rRNA gene sequences of bacteria present in the insects collected from different fruit trees (Supplementary Table S1) were deposited in the NCBI SRA Sequence Read Archive under the BioProject PRJNA1215968.

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