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Bacterial dynamics and exchange in plant-insect interactions

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ABSTRACT

In nature, plants and insects engage in intricate interactions. Despite the increasing knowledge of the microbiomes of plants and insects, the extent to which they exchange and alter each other's microbiomes remains unclear. In this work, the bacterial community associated with nymphs of *Philaenus spumarius* (Hemiptera: Aphrophoridae), the stems of *Coleostephus myconis* where the nymphs were feeding, and the foam produced by the nymphs, were studied by culture-dependent and -independent approaches, with an attempt to elucidate the exchange of bacteria between plants and insects. The results suggest that both approaches complement each other, as many bacterial genera identified by metabarcoding were not detected by culturing, and vice versa. Overall, stems and foam exhibited higher bacterial diversity than nymphs, with all the samples showing enrichment in bacteria known to provide diverse benefits to their host. Stems and foam were the most similar in bacterial composition, but *Burkholderiaceae* and *Moraxellaceae* dominated the stems, whereas *Rhizobiaceae* and *Sphingobacteriaceae* dominated the foam. Nymphs exhibit the most distinct bacterial composition, yet more similar to that found in the stem compared to the foam. Indeed, nymphs were enriched on endosymbiotic bacteria, mostly *Candidatus* Sulcia and *Sodalis*, not found in the stem and foam. Nevertheless, during feeding, nymphs appeared to exchange several bacteria genera with *C. myconis*, with a significant number being incorporated into the bacteriome of the nymph. The genera *Curvibacter, Cutibacterium, Methylobacterium, Pseudomonas* and *Rhizobium* are likely the most exchanged. Nymphs also appear to exchange bacteria to the foam, notably species from the *Enhydrobacter, Pseudomonas, Rhizobium* and *Roseomonas* genera. More studies to infer the functions of the shared bacteria between *P. spumarius-C. myconis* are needed.

1. Introduction

Virtually, all eukaryotic organisms are associated with a variety of microorganisms, including fungi and bacteria (Bang et al., 2018). These microorganisms living on and in cell tissues of eukaryotic organisms, form complex communities and play crucial roles in their hosts' health and fitness (Bang et al., 2018). For example, the microorganisms thriving within plant tissues contribute to the plant's ability to tolerate abiotic and biotic stresses, enhance nutrient uptake, and promote plant growth (reviewed in Kumar et al., 2023). Similarly, the insect-associated microorganisms, in particular the group of symbionts, have been shown to aid the host insects with food digestion, acquisition of essential nutrients, detoxification of harmful compounds, defense against pathogens and parasites, and to promote insect growth and reproduction (reviewed in Provorov and Onishchuk, 2018). These microbial communities and their functions within a given habitat are called microbiomes (Berg et al., 2020). In recent years, there have been a considerable number of studies focused on characterizing separately the microbiome of plants (Dastogeer et al., 2020) and insects (Gurung et al., 2019). Such research aims to unravel the diversity, composition and functions of microorganisms within these hosts. However, plants and insects have complex relationships in nature, engaging in various interactions that profoundly impact each other (Giron et al., 2018). Therefore, more integrative studies that examine the interrelation of microbiomes between insects and plants are crucial to gaining a better understanding of how microorganisms influence plant-insect interactions. Specifically, it is crucial to determine the extent of overlap and the specific modifications each organism induces in the other's microbiome, as this remains largely unclear (Pirttilä et al., 2023).

It is well-documented that insects (mostly hemipteran insects) can

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act as vectors for transmitting plant pathogens, when they feed on infected plants and move to healthy ones (Heck, 2018). Although less frequently reported compared to plant pathogens, insects also seem to have the ability to transmit mutualists or entire microbial communities inhabiting plants. For example, the phloem-feeding insect *Scaphoideus titanus* (Hemiptera: Cicadellidae), which is the main vector of *flavescence dorée* phytoplasma, was shown to transfer the entire bacterial endophytic community between grapevine plants (Lòpez-Fernàndez et al., 2017). Similarly, bark beetle vectors (Coleoptera: Curculionidae: Scolytinae) have been reported to transfer a diverse array of fungal symbionts between trees, including Ophiostomatales fungi (Taerum et al., 2013). In contrast, the plant endophytic community was found to influence the microbiome of the leafhopper *S. titanus* (Lòpez-Fernàndez et al., 2017). Plants can also act as intermediaries for the horizontal transmission of bacterial symbionts between insects. For example, there is evidence of the endosymbiotic bacteria *Wolbachia* spp. to be transmitted between *Bemisia tabaci* whiteflies (Li et al., 2017) or *Crioceris* spp. leaf beetles (Kolasa et al., 2017) via the plants on which the insects feed.

In the present work, the meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae) was chosen as an insect model for studying the exchange of microbiomes between plants and insects, aiming to fill critical gaps in our knowledge on this topic. This particular insect, which feeds on xylem sap, has gained considerable attention in Europe because it was recently identified as a vector of *Xylella fastidiosa* (Cornara et al., 2018; Saponari et al., 2014). This bacterial phytopathogen causes serious diseases in a wide range of crops, like Pierce disease in grapevines and olive quick decline syndrome in olives, being also pathogenic for diverse ornamental plant species (Delbianco et al., 2022). Studies on microbial community of *P. spumarius* have been primarily focused on endosymbiotic bacteria (Formisano et al., 2022; Kapantaidaki et al., 2021; Lis et al., 2015). However, recent metabarcoding studies have revealed a broader spectrum and diversity of bacteria inhabiting *P. spumarius* (Cameirão et al., 2024; Kolasa et al., 2023). *Philaenus spumarius* is a univoltine species that develops through three main stages: egg, nymph, and adult. Eggs are laid by adult females in late summer or early autumn, typically within leaf litter, where they spend the winter months in a diapause state (reviewed in Cornara et al., 2018). In the following spring, nymphs emerge and crawl to the closest green plant, primarily herbaceous dicotyledonous species (EFSA et al., 2018). During this period, nymphs undergo five developmental stages, known as instars, with their colour changing from orange to greenish yellow as they progress (Yurtsever, 2000). Nymphs feed on xylem sap using their piercing mouthparts (reviewed in Cornara et al., 2018). While feeding, nymphs excrete surplus sap through their anus and mix air with it, creating frothy spittle masses (Malone et al., 1999). This foam covers the entire nymph body, providing protection from predators or desiccation until molting into a winged adult (Malone et al., 1999). Adults are small (5–7 mm long), winged, and exhibit a wide range of colour variations from yellowish to dark brown or black, often with mottled patterns (Yurtsever, 2000). Also, adults feed on xylem sap, but they have greater mobility when compared to nymphs, which remain stationary within the protective foam (reviewed in Cornara et al., 2018). Nymphs can thrive on all plant species that offer enough moisture for their feeding behaviour (reviewed in Cornara et al., 2018). However, in olive groves in the Northeast of Portugal, *P. spumarius* nymphs have shown a preference for *Coleostephus myconis*, indicating a tendency to primarily feed on the stems of this herbaceous species (Villa et al., 2020). Therefore, this biological system involving *P. spumarius* nymphs feeding on *C. myconis*, with the foam covering the nymphs seems to be a good model for studying the exchange of microbiomes between plants and insects. The foam, composed of excreted plant fluid that has passed through the insect's digestive system, is particularly important in this regard. As a result, the bacterial community associated with *P. spumarius* nymphs, the stems of *C. myconis* where the nymphs were feeding, and the foam covering the nymphs, was studied using metabarcoding and cultural approaches. Specifically, this research aims to address the following questions: (i) How do the diversity and composition of bacterial communities differ between *P. spumarius* nymphs, their host plant, and the foam they produce?; (ii) To what extent do bacterial communities overlap these three biological components? Given the nature of this plant-insect system, it is hypothesized that a certain degree of microbial overlap exists between the insect and their host plant, as the nymphs necessarily feed directly on the xylem sap. The results could provide insights into how insect-associated microbiomes interact with those of host plants, shedding light on the dynamics, and functional implications of these interactions. Overall, this study may contribute to a better understanding of the broader ecological aspects of plant-insect-microbe interactions.

2. Material and methods

2.1. Sample collection and processing

The collection of samples (i.e., *P. spumarius* nymphs, *C. myconis* stems where the nymphs were feeding, and the foam covering the nymphs) was performed during the spring of 2019 at the *Campus* of Instituto Politécnico de Bragança (41° 47′ 53.2″ N, 6° 45′ 51.5″ W; Northeast of Portugal). A total of 48 *C. myconis* plants with a single foam in their stems were sampled. From each plant, all the nymphs feeding on the stem and the foam covering the nymphs were collected into separate microtubes. The stem segments from which the nymphs were feeding, were excised and placed into microtubes using a sterilized scalpel. Samples were transported in a cooler box (≈ 4 °C) to the laboratory for further processing.

The collected nymphs were observed using a binocular stereoscope to confirm their identity based on dichotomous keys (e.g. Germain et al., 2020) and to determine the instar stage based on their morphological characteristics (Cornara et al., 2018). The collected nymph samples encompassed the first, second, third and fourth nymphal instars, averaging around 1.6 ± 1 nymphs per foam. The nymphs of each sample were then surface sterilized by sequential immersion in 3 % (v/v) sodium hypochlorite for 30 s and sterile distilled water for 2 min (3 times). The stem segments were also surface sterilized by sequential submersion in 70 % (v/v) ethanol for 30 s, followed by 1 min in 3 % (v/v) sodium hypochlorite, and finally three washes with sterile distilled water (1 min each). The success of the sterilization method was confirmed by imprinting the surface of the nymph/stem segments onto the same culture medium used for bacterial isolation.

The stem, nymph, and foam samples were used for bacterial assessment through culture-dependent (strain isolation followed by molecular identification) and culture-independent (metabarcoding) approaches. Among the 48 *C. myconis* plants surveyed, 23 were used on the same day of collection for the culture-dependent technique, while the remaining 25 plants were used to characterize the bacterial community by metabarcoding. For this analysis, five replicates of stems, nymphs, and foams were established, with each replicate being a composite sample obtained from five surveyed plants. Specifically, all nymphs from five plants were pooled into a single sample, and the foam collected from these plants was also combined into a separate single sample. These composite samples were preserved at −80 °C until DNA isolation.

2.2. Bacterial assessment by culture-dependent approach

The bacterial strains were isolated from different samples using Luria Bertani agar (LBA) medium (10 g/L tryptone, 5 g/L yeast extract, 10 g/L sodium chlorite, and 10 g/L agar) and specific methods. This culture medium is considered one of the most robust media to cultivate bacteria, and was selected for its suitability in supporting a broad range of bacterial growth (Yamamoto et al., 2021). The isolation of bacteria from *C. myconis* was performed by plating stem fragments collected specifically from the feeding sites of *P. spumarius* nymphs. Each stem section from this area was cut into seven fragments (ca. 2 mm thick) using a

sterilized razor in the laminar flow hood to prevent contamination. These fragments were directly placed onto the surface of two LBA plates (9 cm diameter). A total of 161 stem segments (23 plants x 7 segments per plant) were used for evaluating the endophytic bacterial communities. For the nymphs and foam samples, the isolation of bacterial strains was performed by the spreading plate method. All nymphs collected from the same plant were pooled together and processed as a single sample for bacterial isolation. Similarly, the foam collected from the same plant was treated as a separate single sample. Each sample was macerated in a microtube using a sterile phosphate-buffered saline (PBS) solution (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) in a proportion of 1:10 (grams of nymph: mL of PBS). The macerate (100 μl) was inoculated into two LBA plates. The inoculated plates of all samples (stems, nymphs and foam) were incubated at room temperature (22–24 °C) in the dark. Plates were checked daily for distinct colonies and were sub-cultured in LBA until pure isolates were obtained. The abundance of the different bacterial colonies was estimated by determination of the frequency of colonization (isolates from stems) or of colony-forming units (CFU) per mL (isolates from nymphs and foam). The frequency of colonization (%) of isolated bacteria was calculated by dividing the number of isolates of a specific taxon by the total number of segments plated.

The purified isolates were grouped based on their cultural similarities, and a representative of each group was randomly selected for molecular identification by sequencing the bacterial barcode (V1-V3 regions of the 16S rRNA). DNA extraction from the isolates was performed using a specific kit (REDExtract-N-Amp™ Plant PCR kit; Sigma, Poole, UK), following the manufacturer's instructions. The V1-V3 region of the 16S rRNA was amplified using the primers *27F*: 5′-AGAGTTTGATCCTGGCTCAG-3′ (Lane, 1991) and *534R*: 5′-ATTACCGCGGCTGCTGG-3′ (Muyzer et al., 1993), in a PCR reaction (20 μl), containing 2 μl of DNA, 1X buffer, 2.5 mM $MgCl_2$, 200 mM dNTPs, 0.2 μM of each primer, and 1.25 U DFS-*Taq* DNA Polymerase (BIORON). PCR was performed in a MyCycler™ Thermocycler (Bio-Rad) with the following conditions: 94 °C for 5 min followed by 25 cycles of 94 °C for 40 s, 54 °C for 50 s and 72 °C for 30 s, and a final cycle at 72 °C for 7 min. The amplified products were purified and sent to Macrogen Inc. (Madrid) to be sequenced. The taxonomic classification was performed using the NCBI database (Coordinators, 2018) and applying the BLAST algorithm (Altschul et al., 1990). Final taxonomic classifications were made based on lower *e*-value and higher percentage of identity (>98 % for species level; 95 % to 97 % for genus level) and query coverage. The 16S rRNA V1-V3 sequences of all bacterial isolates were blasted against each other to prevent redundancy in subsequent analyses. Similarly, a phylogenetic tree was performed using the MEGA software, by aligning the sequences with the MUSCLE algorithm and using the Maximum Likelihood (ML) method based on the Kimura 2-parameter model and Gamma distribution with 1000 bootstrap repetitions (Fig. S1). Out of the 177 sequences obtained, 84 were unique and classified as operational taxonomic units (OTUs). These 177 sequences were deposited in the NCBI database under the accession numbers: ON185007 – ON185183. Detailed information regarding the bacterial sequences is provided in Table S1. A pure culture of each isolate was preserved in 30 % glycerol (v/v), stored at −80 °C and deposited in CIMOCC (Centro de Investigação de Montanha culture collection) at the *Instituto Politécnico de Bragança* (Portugal).

2.3. Bacterial assessment by culture-independent approach

Metabarcoding sequencing was performed on the V4 region of the 16S rRNA to analyze the bacterial microbiome associated with *C. myconis* stem, *P. spumarius* nymph, and foam samples. The frozen samples were ground with liquid nitrogen, and DNA was extracted using the Speedtools Tissue DNA Extraction Kit (BIOTOOLS, Spain), following the manufacturer's instructions. The quality of the extracted DNA was assessed by loading 3 μl of DNA in a 0.8 % (w/v) agarose gel. DNA integrity was further assessed by amplifying the 16S rRNA V1-V3 region, using the primers *27F*: 5′-AGAGTTTGATCCTGGCTCAG-3′ (Lane, 1991) and *534R*: 5′-ATTACCGCGGCTGCTGG-3′ (Muyzer et al., 1993), following the PCR conditions previously described.

The bacterial communities were analyzed using a metabarcoding approach on the Illumina MiSeq platform with paired-end sequencing (2 × 250 bp) targeting the 16S rRNA V4 region. This service was provided by Genoinseq (Portugal). Sequencing was conducted using the primers *515F* (5′-GTGCCAGCMGCCGCGGTAA-3′) and *806R* (5′-GGACTACHVGGGTWTCTAAT-3′) (Caporaso et al., 2011), which incorporated dual indexes and sequencing adapters for further downstream analysis. Raw reads underwent quality control analysis using FastQC (Andrews, 2010). Based on the analysis, read trimming was performed with Sickle (Joshi and Fass, 2011) with default parameters. Error correction of sequencing reads was performed using the BayesHammer module from the SPAdes package (Nurk et al., 2013). Overlapping regions of the paired-end reads were merged using the USEARCH v11 (Edgar, 2010). The size filtering parameters were determined based on a quality report by FastQC (Andrews, 2010) and the expected amplicon size. The filtering was achieved using the ea-utils package (Aronesty, 2011). MICCA (Albanese et al., 2015) was used to cluster reads into Amplicon Sequence Variants (ASVs) and to assign a taxonomic classification using the reference database SILVA (Quast et al., 2013). All sequences from mitochondria and chloroplasts were manually removed and unclassified ASVs were blasted against the NCBI database (Coordinators, 2018) to further understand their taxonomic classification. To account for variations in sampling depth, all datasets were subsampled to match the lowest number of reads present in a sample. This normalization was performed using QIIME (Caporaso et al., 2010), ensuring consistent read counts across all datasets for subsequent comparative analysis. The raw sequencing reads were deposited in the NCBI Sequence Reads Archive (SRA) database under the BioProject PRJNA836511.

2.4. Data analysis

Data analysis and data visualization were performed using the R software version 4.0.2 (R Core Team, 2022). The diversity of bacteria associated with each sample (stem, nymph, and foam) was estimated by calculating the Shannon-Wiener index using the *diversity* function from the vegan package (Oksanen et al., 2018). Differences between means were assessed after checking whether the data met the assumptions for parametric tests using the Shapiro-Wilk test. Since the data did not show a normal distribution, a Kruskal-Wallis test was conducted, followed by pairwise Dunn tests. Statistical significance was determined at *p*-values below 0.05.

To visualize the variation in bacterial community composition among stems, nymphs, and foam, non-metric multidimensional scaling (NMDS) plots were created, based on Bray–Curtis distances. This analysis was conducted using the *metaMDS* function from the vegan package (Oksanen et al., 2018), by using a log-transformed database of bacterial OTUs/ASVs. Kruskal's stress was also calculated to assess the goodness of fit of the model, which is generally acceptable when it is <0.2. Differences in the bacterial composition among samples were tested using permutational multivariate analysis of variance (PERMANOVA) using the *pairwise.adonis* function from the package pairwiseAdonis (Martinez, 2020) with 999 permutations. The analysis was performed on Bray–Curtis dissimilarity measure calculated from log-transformed bacterial OTUs/ASVs community data.

3. Results

In this work, the bacterial communities associated with *P. spumarius* nymphs, the stems of *C. myconis* where the nymphs were feeding, and the foam covering the nymphs, were characterized using both culture-dependent and -independent (metabarcoding) approaches.

3.1. Overall bacterial community

From all samples, a total of 177 bacterial isolates were obtained: 86 from the stems, 76 from the nymphs, and 55 from the foam. These isolates were clustered into 84 OTUs (Table S1; Fig. S1), distributed across 23 genera, 18 families, and 4 phyla (Fig. S2a). This community was mostly composed of isolates from the phylum *Proteobacteria* (77 % of the total bacteria isolates), mainly from the family *Pseudomonadaceae* (41 %) and genus *Pseudomonas* (41 %). The next most abundant genera were *Microbacterium* (*Microbacteriaceae, Actinobacteria*), *Erwinia* (*Erwiniaceae, Proteobacteria*), and *Pantoea* (*Erwiniaceae, Proteobacteria*), each comprising approximately 4 % of the total isolates.

Using the metabarcoding approach, a total of 249,869 high-quality bacterial sequence reads (Table S2) were identified in stems, nymphs, and foam. Stem samples consistently exhibited a lower number of high-quality reads (ranging from 688 to 3754 reads per sample) compared to nymphs (19,630 to 28,690) and foam (12,967 to 44,700). To ensure comparability across samples with different sequencing depths, all datasets were standardized by subsampling to 688 reads per sample. This normalization resulted in 2488 ASVs, distributed across 58 genera, 42 families, 29 orders, 13 classes, and 9 phyla (Fig. S2b). The majority of ASVs were classified under the phylum *Proteobacteria* (61 % of classified sequences), followed by *Bacteroidota* (synonym *Bacteroidetes*) (31 %). The most abundant families were *Blattabacteriaceae* (18 % of classified reads), *Enterobacteriaceae* (13 %), *Burkholderiaceae* (10 %), and *Rhizobiaceae* (10 %). The dominant genera were *Candidatus* Sulcia (around 18 % of classified sequences) and *Sodalis* (12 %).

Both approaches used to assess bacterial composition on the three biological samples provided complementary information. Indeed, around 81 % of the bacterial genera detected through culture-independent methods were absent in the culture-dependent analyses (Fig. S2). Similarly, a significant proportion (around 52 %) of genera isolated in culture media were not detected in the culture-independent approach.

3.2. Bacterial diversity and composition in stems, nymphs, and foam

The estimation of bacterial diversity using the Shannon-Wiener diversity index in stems, nymphs, and foam revealed different results depending on the approach used to assess bacterial communities. In the culture-dependent approach, stems exhibited a higher Shannon diversity index compared to nymphs (up to 1.2-fold) and foam (up to 1.3-fold), with statistically significant differences noted for foam ($p < 0.05$; Fig. 1). A different result was obtained when the bacterial community was assessed by culture-independent approach, with foam displaying a significantly higher Shannon-Wiener diversity index compared to stems (up to 1.1-fold, $p < 0.001$) and nymphs (up to 1.1-fold, $p < 0.001$). As expected, the Shannon-Wiener index calculated from metabarcoding results averaged 5.5-fold higher than that from the culture-dependent approach across all samples (Fig. 1).

Both culture-dependent and culture-independent approaches used to assess bacterial communities in stems, nymphs, and foam revealed differences in the overall structure of bacterial communities among the three biological samples, each exhibiting distinct patterns. Using culture-dependent methods, the NMDS plot (Kruskal stress = 0.132) indicated a slight separation among the samples, with statistically significant differences confirmed by PERMANOVA based on Bray-Curtis index ($R^2 = 0.16$, p-value = 0.001) (Fig. 2). Specifically, the most distinct communities were observed between foam and stems ($R^2 = 0.15$, p-value = 0.003), compared to foam vs. nymph ($R^2 = 0.12$, p-value = 0.003) and nymph vs. stem ($R^2 = 0.12$, p-value = 0.003). Using the metabarcoding approach, more pronounced differences in the bacterial structure were evident in the NMDS plot (Kruskal stress = 0.076), corroborated by a higher distinction revealed by PERMANOVA ($R^2 = 0.44$, p-value = 0.001) (Fig. 2). Interestingly, the most distinct communities were found between nymphs and foam ($R^2 = 0.52$, p-value = 0.03), followed by nymphs and stems ($R^2 = 0.41$, p-value = 0.03). Foam and stems exhibited the most similar bacterial communities ($R^2 = 0.20$, p-value = 0.02).

These two methodological approaches also revealed contrasting dominant bacterial compositions among stems, nymphs, and foam, even at higher taxonomic levels. For example, in the culture-dependent approach, the majority of bacterial isolates from stem and foam samples were from the *Pseudomonadaceae* family, accounting for approximately 60 % and 70 % of the total isolates, respectively (Fig. 3). Conversely, nymphs were predominantly colonized by members belonging to the *Rhizobiaceae* family, which comprised 71 % of the isolates from this sample. In contrast, the culture-independent approach showed different patterns of bacterial dominance (Fig. 3). Stem samples were characterized by the presence of *Burkholderiaceae* and *Moraxellaceae* (17 % and 13 % of reads, respectively) while nymph samples had higher abundances of *Blattabacteriacea*, primarily from *Candidatus* Sulcia genus, and *Enterobacteriaceae*, mainly *Sodalis* (53 % and 37 % of the total number of reads, respectively). In contrast, foam samples were dominated by *Rhizobiaceae* and *Sphingobacteriaceae* (22 % and 19 %, respectively).

The bacterial families detected exclusively in stems, nymphs, and foam varied depending on the assessment method. For example, in the culture-dependent approach, some families were exclusively isolated from stems (*Bacillales* and *Yersiniaceae*), others from nymphs (*Comamonadaceae, Flavobacteriaceae, Nocardiaceae, Oxalobacteraceae*, and *Sphingomonadaceae*), and others from foam (*Methylobacteriaceae* and *Micrococcaceae*) (Fig. 3). The culture-independent approach identified different exclusive bacterial families in each sample type (Fig. 3). For

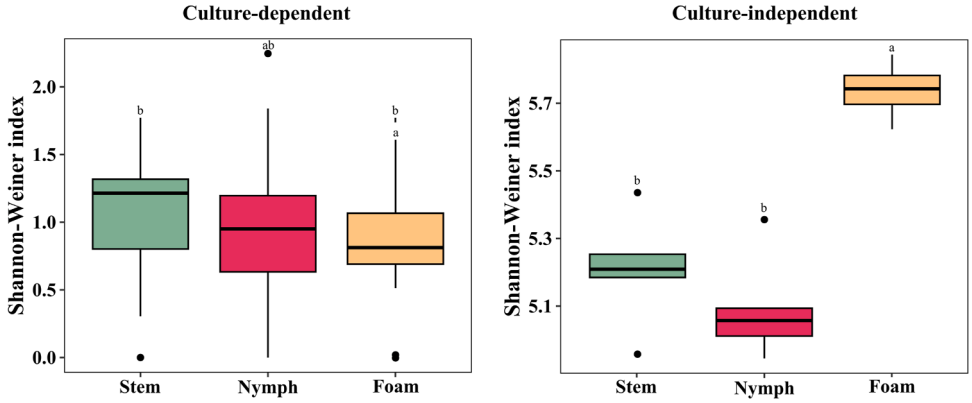
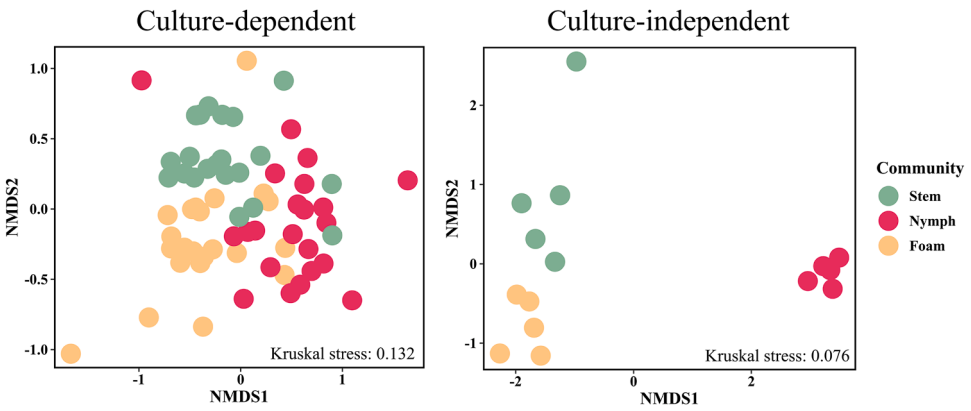


Fig. 1. Boxplots representing the Shannon-Wiener index of bacterial diversity in *Coleostephus myconis* stems, *Philaenus spumarius* nymphs, and foam covering the nymphs, assessed by culture-dependent and culture-independent approaches. The boxplots depict medians (central horizontal lines), the inter-quartile ranges (boxes), 95 % confidence intervals (whiskers), and outliers (black dots). Statistically significant differences ($p < 0.05$) are denoted by different letters.



	R^2	*p*-value
Foam vs. nymph	0.12	0.003
Foam vs. stem	0.15	0.003
Nymph vs. stem	0.12	0.003

	R^2	*p*-value
Foam vs. nymph	0.52	0.03
Foam vs. stem	0.20	0.02
Nymph vs. stem	0.41	0.03

Fig. 2. Nonmetric multidimensional scaling (NMDS) plots and PERMANOVA tests of the bacterial assemblages in *Coleostephus myconis* stems, *Philaenus spumarius* nymphs, and the foam covering the nymphs, assessed using culture-dependent and culture-independent approaches. The similarity between bacterial communities was measured with the Bray-Curtis coefficient, and Kruskal's stress values are presented (values <0.2 represent good ordination plots). PERMANOVA results include *R*-statistics (R^2) and statistical significance (*p*-value).

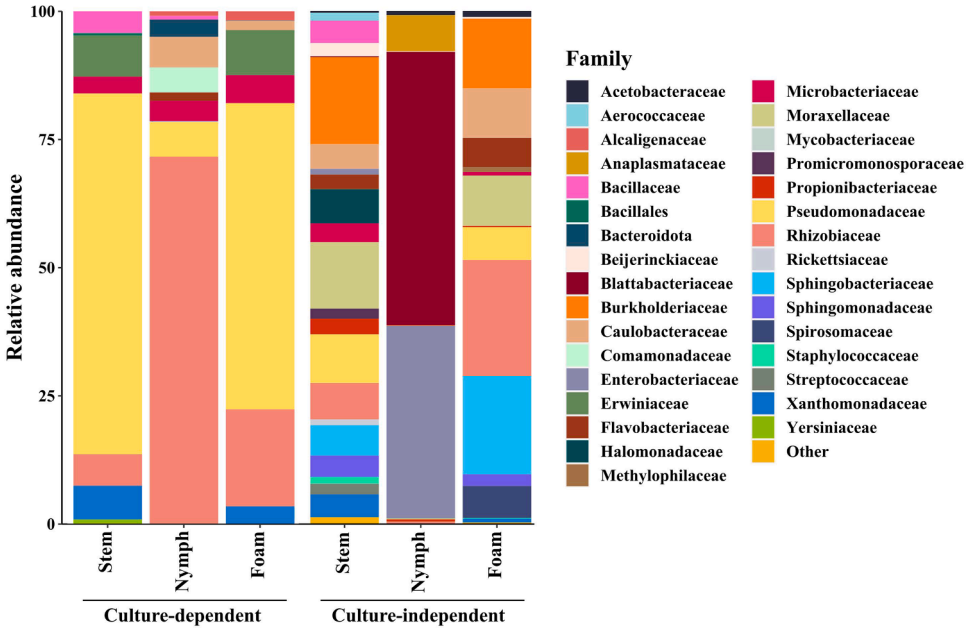


Fig. 3. Relative abundance (%) of bacterial families in *Coleostephus myconis* stems, *Philaenus spumarius* nymphs, and the foam covering the nymphs, assessed by culture-dependent and culture-independent approaches. The "Other" category includes all other OTUs/ASVs that were not classified at the family level or presented an abundance of <0.3 %.

stems, these included *Aerococcaceae, Clostridiaceae, Oligoflexaceae, Promicromonosporaceae, Rickettsiaceae*, and *Veillonellaceae*. Foam had exclusive associations with *Blastocatellaceae, Cellvibrionaceae, Chitinophagaceae, Devosiaceae, Methylophilaceae, Rubritaleaceae, Saccharimonadaceae, Spirosomaceae, Verrucomicrobiaceae*, and *Weeksellaceae*. Notably, only three families - *Anaplasmataceae, Enterobacteriaceae*, and *Blattabacteriaceae* - were exclusively associated with nymphs in the culture-independent approach.

3.3. Distinct and shared bacterial groups in stems, nymphs, and foam

Both culture-dependent and metabarcoding approaches revealed similar results concerning the percentage of common bacterial taxa occurring on stems, nymphs, and foam, particularly at higher taxonomic levels (Fig. 4). In the culture-dependent approach, around 9.5 % of the total bacterial OTUs, 15.6 % of the total genera, and 27.8 % of the total families were shared by the three biological samples. Similarly, in the culture-independent approach, about 0.4 % of total bacterial ASVs, 17.5 % of the total genera, and 26.8 % of the total families were shared across stems, nymphs, and foam. In both approaches, stem and foam showed, in general, the greatest percentage of shared bacteria at either OTUs/ASVs level (21.1 % and 16.5 %, in culture-dependent and –independent approach, respectively) or genus (44.4 % and 28.0 %) or family (31.6 % and 29.6 %) level. However, in the metabarcoding approach, stem and

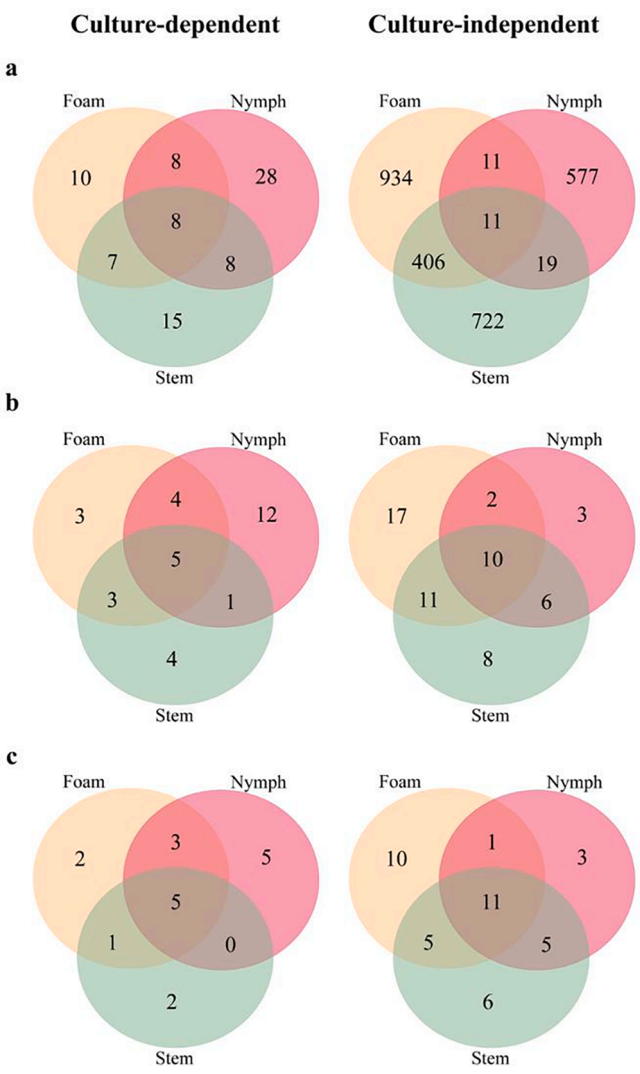


Fig. 4. Venn diagrams displaying the number of shared bacterial OTUs/ASVs (**a**), genera (**b**) and families (**c**) between the *Coleostephus myconis* stems, *Philaenus spumarius* nymphs, and the foam covering the nymphs. The diagrams compare the overlaps as determined by culture-dependent and culture-independent approaches.

nymphs also showed to share an even higher percentage of bacteria at genus (28.6 %) and family (34.0 %) levels than foam vs. nymph.

Among the bacterial families shared between the three biological samples, *Burkholderiaceae, Moraxellaceae, Pseudomonadaceae,* and *Rhizobiaceae* were the most abundant (Fig. 3). The three first families were especially abundant in both stems and foam, when using metabarcoding (*Burkholderiaceae* and *Moraxellaceae*) or cultural methods (*Pseudomonadaceae*). *Rhizobiaceae* was the most prevalent family in the foam when using metabarcoding and in the nymphs when assessed using the culture-dependent approach.

To further explore bacterial exchange between the three biological components (stems, nymphs, and foam), we examined the changes in the relative abundance of bacterial genera within the families identified as common across these samples (Fig. 5). This approach ensured that our assessment focused on truly shared taxa rather than being skewed by the dominance of specific bacterial genera in nymphs. Numerous bacterial genera were detected in all three samples using both methodological approaches, namely *Acinetobacter, Bacillus, Brevundimonas, Cutibacterium, Enhydrobacter, Halomonas, Massilia, Methylobacterium, Microbacterium, Novosphingobium, Pseudomonas, Rhizobium, Staphylococcus* and *Stenotrophomonas*. This overlap supports the occurrence of bacterial exchange among the insect, plant, and foam habitats. However, the overwhelming presence of certain dominant genera in nymphs may have masked some level of shared bacterial diversity.

The comparison between stems and foam revealed that the most abundant bacterial genera in stems, such as *Brevundimonas, Pseudomonas, Duganella*, and *Sphingomonas*, were also predominantly found in foam. Curiously, some of these genera were exclusively found in stems and foam (*Duganella* and *Sphingomonas*), together with other five genera (*Alkanindiges, Frigoribacterium, Neorhizobium, Variovorax,* and *Verticia*). Both *Aureimonas* and *Xanthomonas* were found exclusively in stems, while *Curtobacterium* and *Pigmentiphaga* were exclusive to foam. Comparisons of the bacterial communities between stems and nymphs also revealed similarities (Fig. 5). The genera *Cutibacterium* and *Rhizobium*, which were prevalent in nymphs, were also found in relatively high abundance in stems. *Pseudomonas*, a dominant genus in stems, was also dominant in nymphs, although at a lower abundance. Furthermore, some bacterial genera, including *Methylobacterium* and *Curvibacter*, were present in similar relative abundances in both stems and nymphs, with *Curvibacter* being exclusive to these two biological samples. Nevertheless, other genera like *Roseomonas* and *Enhydrobacter*, though dominant in nymphs, were detected in lower abundance in stems. Although less pronounced, similarities in bacteriomes from nymphs and foam were also observed (Fig. 5). In particular, the genera *Enhydrobacter, Pseudomonas, Rhizobium* and *Roseomonas*, which were abundant in nymphs, were also prevalent in foam.

4. Discussion

In this study, the bacterial communities inhabiting *C. myconis* stems, *P. spumarius* nymphs, and the foam surrounding the nymphs were characterized by culture-dependent and -independent methods. It is well known that culture-independent methods provide a more comprehensive view of microbial communities than culture-dependent methods (Cowan et al., 2005). This aspect holds significant importance in studies focusing on the microbial composition of insects, primarily due to the challenges associated with culturing microorganisms, such as symbionts, from insects (Masson and Lemaitre, 2020). Nevertheless, the isolation of bacteria was performed in this study to facilitate their future characterization and potential exploitation in the management of *P. spumarius* population. Although the primary aim was not to compare culture-dependent and culture-independent approaches, it became evident that these methods complement each other by providing a more comprehensive understanding of bacterial diversity, as previously reported for *P. spumarius* adults (Cameirão et al., 2024). Indeed, most of the bacterial genera identified by metabarcoding across the three biological samples were not detected by the culture-dependent method, and many genera isolated in culture were not detected by metabarcoding. This result is likely due to PCR amplification biases and cultivation conditions. Some bacterial genera may remain undetected in metabarcoding due to low abundance in the sample or PCR amplification biases, leading to their underrepresentation in high-throughput sequencing. However, these same taxa can become dominant in culture-based methods if they grow more easily under the given conditions. Additionally, culture media selection strongly influences the diversity of cultivable bacteria, as different media favor different taxa (Wang et al., 2020). In agreement, it is well established that culture-dependent and culture-independent approaches offer complementary yet distinct insights into microbial community composition (e.g., Anguita-Maeso et al., 2020; McIlwaine et al., 2024).

4.1. Foam exhibits greater bacterial diversity than stems and nymphs

Culture–independent approaches showed that foam, followed by stems, harbored a higher diversity of bacteria compared to nymphs. This finding can be attributed to the extensive exposure of foam to the surrounding environment, facilitating the colonization by diverse

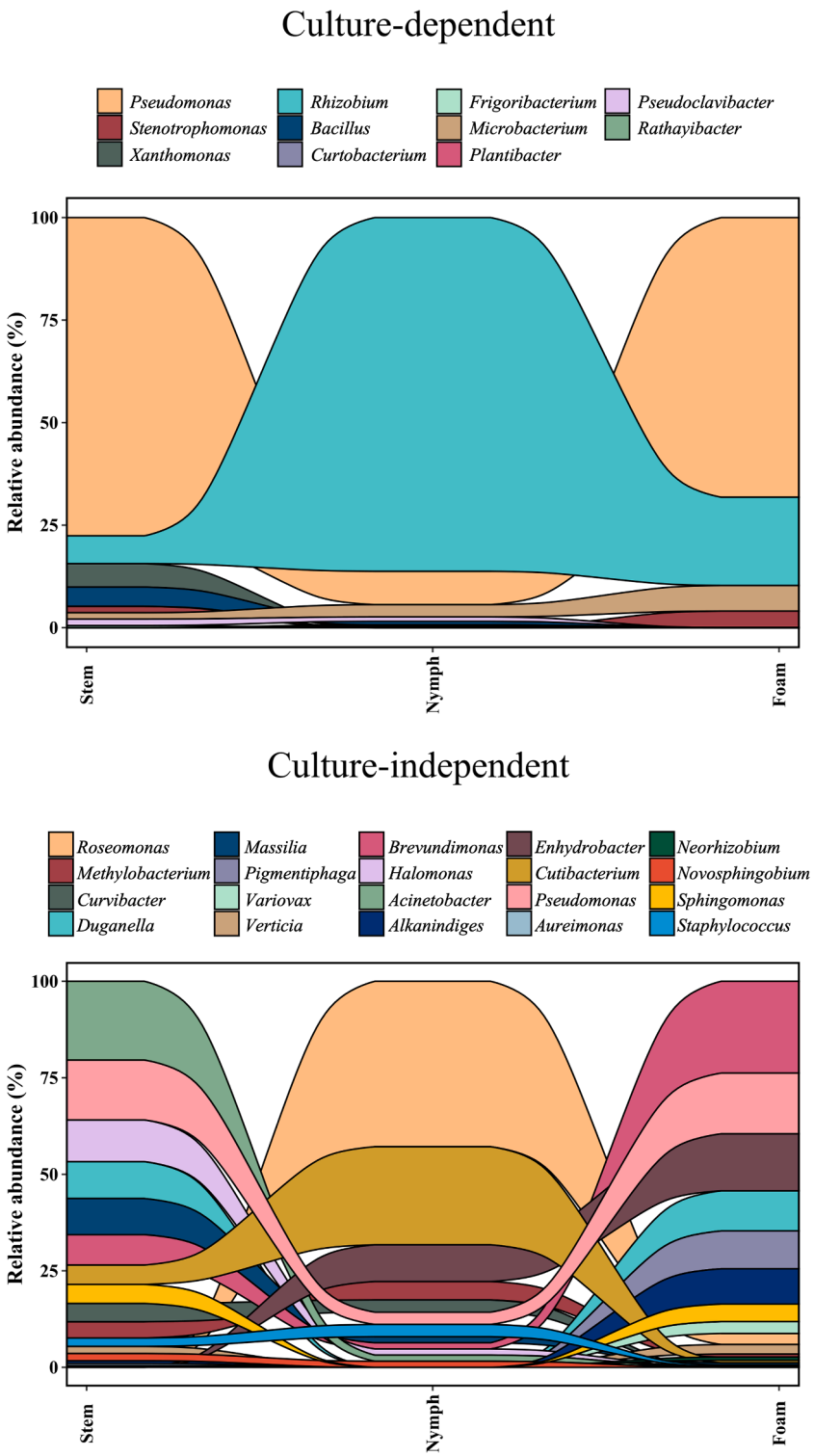


Fig. 5. Relative abundance of the bacterial genera belonging to the bacterial families shared between *Coleostephus myconis* stems, *Philaenus spumarius* nymphs, and the foam covering the nymphs, assessed by culture-dependent and culture-independent approaches.

microorganisms from various sources. Similar findings have been reported for other spittlebug species, such as *Mahanarva fimbriolata*, whose foam harbors a unique microbial community enriched with plant-associated and symbiotic bacteria (Tonelli et al., 2020). Additionally, a recent study by Nencioni et al. (2023) using DGGE analysis identified *Rhizobiaceae* as one of the dominant bacterial families in *P. spumarius* and *Lepyronia coleoptrata* foams, supporting the idea that the foam serves as a microbial niche for plant-associated bacteria. Moreover, foam is a biological matrix rich in proteins, mucopolysaccharides and calcium (Marshall, 1966; Mello et al., 1987). In addition to providing protection against solar radiation (Chen et al., 2018) and assisting in thermoregulation of developing nymphs (Tonelli et al., 2018), this nutrient-rich environment creates an excellent habitat for bacteria growth and supports microbial life. In contrast to foam, the interior of stem or nymph tissues represents a more selective environment with higher selective pressures. Indeed, the successful colonization of plants by endophytes is

a complex and dynamic process shaped by plant defense mechanisms and the adaptive strategies of endophytes (Hu et al., 2024). Similarly, insects undergo selective pressures that influence the colonization of their tissues by microorganisms (Douglas, 2015).

Foam was found to be enriched with bacterial genera reported from a variety of environments and fulfilling diverse ecological roles. These include *Pseudomonas* spp. (*Pseudomonadaceae* family), well-known as commensal or pathogenic bacteria to plants and animals, including insects (Lalucat et al., 2022; Teoh et al., 2021). Members of this genus are reported to improve plant health by promoting plant growth and suppressing phytopathogens (Sah et al., 2021), and they also form beneficial symbiotic associations with insects (Teoh et al., 2021). Other abundant bacterial genera found in foam, which are also known as plant growth promoters and/or biocontrol agents, include the soil-associated genera *Pedobacter* (*Sphingobacteriaceae* family) (Lee et al., 2019; Yin et al., 2021) and *Rhizobium* (*Rhizobiaceae* family) (Saeed et al., 2021). Interestingly, *Pedobacter* spp. are also known as environmental superbugs due to their resistance to the effects of most known antibiotics (Viana et al., 2018). These microorganisms residing in the foam covering the nymphs of *P. spumarius* are likely to have beneficial effects on the nymphs, although direct evidence to support this hypothesis is currently lacking. This hypothesis is reinforced by reports indicating that spittlebug nymphs are rarely attacked by vertebrates (such as birds) or invertebrates (Balzani et al., 2023). The microbial composition of the foam produced by spittlebug nymphs of the family Aphrophoridae has not been thoroughly analyzed to date. Only recently, denaturing gradient gel electrophoresis (DGGE) profiles of foam samples from nymphs of *P. spumarius* and *Lepyronia coleoptrata* (Hemiptera: Aphrophoridae) have revealed a prevalence of bacterial members of the family *Rhizobiaceae* (Nencioni et al., 2023), similar to the findings of our study. Additionally, a high abundance of bacteria from the genera *Bacillus, Streptomyces, Nocardia*, and *Sphingomonas* was observed in the foam produced by *Mahanarva fimbriolata* (Hemiptera: Cercopidae) nymphs (Tonelli et al., 2020). Despite these findings, the specific functions potentially fulfilled by these microorganisms in the foam produced by spittlebugs remain largely unknown.

Information on the microbial composition of nymphs of *P. spumarius* is very limited. In our work, nymphs of *P. spumarius* were found to be enriched with a range of well-known obligate endosymbiotic genera, namely *Candidatus* Sulcia (from *Blattabacteriaceae* family) and *Sodalis* (from *Enterobacteriaceae* family), both recognized for their capacity to provide essential nutrients and protection to their host (Koga and Moran, 2014; Moran et al., 2005; Roma et al., 2019). These two genera were also previously found in high abundance in *P. spumarius* adults (Cameirão et al., 2024; Kolasa et al., 2023), suggesting they play a key role in providing essential nutrients throughout nymph and adult stages. In a preliminary work, Nencioni et al. (2023) also detected an enrichment of two *Enterobacteriaceae* species, including *Sodalis*, based on DGGE profiles of gut dissections from *P. spumarius* nymphs. In our work, in addition to detecting *Candidatus* Sulcia and *Sodalis* in *P. spumarius* nymphs through metabarcoding, the culture-dependent approach also revealed an enrichment of bacteria belonging to *Rhizobiaceae* family, particularly from the genus *Rhizobium*. Although *Rhizobium* spp. are primarily known for their association with plants (Flores-Félix et al., 2020; Fahde et al., 2023), members of this genus have also been detected in insects (Leite-Mondin et al., 2021), including in *P. spumarius* adults (Cameirão et al., 2024; Kolasa et al., 2023). Since *Rhizobium* spp. are well-known for their role in nitrogen fixation in plants, their presence in insects suggests that they may have a similar function, possibly contributing to nitrogen metabolism or synthesizing essential amino acids that are lacking in the insect's diet. Understanding their function in insects is an intriguing topic that warrants further investigation.

This work also revealed a dominance of bacterial beneficial genera in *C. myconis* stems, including *Pseudomonas, Duganella* and *Acinetobacter*. *Pseudomonas* spp. are known for their capacity to enhance plant health (Sah et al., 2021) and establish symbiotic associations with insects (Teoh et al., 2021). *Duganella* (*Burkholderiaceae* family) is well-known for its antifungal (Haack et al., 2016), antibacterial (Choi et al., 2015), and algicidal (Cai et al., 2024) properties. Similarly, *Acinetobacter* (*Moraxellaceae* family) is noted for its ability to promote plant growth and degrade environmental pollutants (Mujumdar et al., 2023).

4.2. Nymphs exhibit a bacterial community more similar to stems than foam

Overall, the analysis of bacterial communities across the three biological samples revealed that foam exhibited greater similarity to stems than to nymphs. This similarity suggests a significant exchange of bacteria between foam and stems, likely facilitated by the open wounds caused by the nymphs when feeding on stems, a well-established pathway for bacterial entry into plant tissues (Pathak et al., 2022). Our findings highlight that the predominant bacterial genera shared between foam and stems comprise the already mentioned genera (*Pseudomonas* and *Duganella*) with recognized ability to confer beneficial effects on their host. In addition, both biological samples share *Brevundimonas*, which include members known for promoting plant growth and degrading environmental pollutants (Zaim and Bekkar, 2023). Interestingly, a significant number of genera were exclusively found in both foam and stems, suggesting the exchange of these bacteria between these two habitats. Beyond *Duganella*, these exclusive genera include *Sphingomonas, Variovorax, Neorhizobium*, and *Frigoribacterium*, which are also recognized for their roles in plant protection and/or promotion of plant growth (Andrews and Andrews, 2017; Asaf et al., 2020; Gahan and Schmalenberger, 2014; Jamalzadeh et al., 2021). *Verticia* and *Alkanindiges* genera were also exclusively detected in both foam and stem; however, their specific roles in plants are not yet fully understood and require further research.

Another interesting finding from our study was the greater similarity observed in the bacterial community composition between nymphs and stems compared to foam. This suggests that *P. spumarius* nymphs likely ingest bacteria from the stem, incorporating some into their bacteriome, while excreting a few others through the foam. This bacteria exchange between plants and insects was previously demonstrated in *Scaphoideus titanus* (Hemiptera: Cicadellidae) while feeding on grapevine plants (Lòpez-Fernàndez et al., 2017). This exchange may also involve endosymbiotic bacteria, as previously suggested in *Bemisia tabaci* (Li et al., 2017) and *Crioceris* spp. (Kolasa et al., 2017), where the host plant acts as an intermediary for the transmission of bacteria between insects. The most abundant bacteria genera shared between *P. spumarius* nymphs and stems encompass members with recognized beneficial effects on insect or plant hosts. These include species of *Pseudomonas* and *Methylobacterium*, known for their roles as plant growth promoters and biocontrol agents (Madhaiyan et al., 2006; Poorniammal et al., 2009; Sah et al., 2021; Teoh et al., 2021), as well as for supplying essential nutrients and digesting lipids and carbohydrates in insects (Jing et al., 2020). Other shared genera among nymphs and stems include *Curvibacter* and *Cutibacterium*, which have been reported in plants (Faddetta et al., 2021; Mei et al., 2022) and/or insects (Lim and Ab Majid, 2021; Cameirão et al., 2024), but their specific roles in these organisms have not been clearly defined and warrant further research. However, *Curvibacter* members are capable of degrading cellulose by producing cellulase (Zhang et al., 2018), suggesting a potential role in nymph digestion. Similarly, *Cutibacterium* bacteria are predicted to be involved in several functional activities in insect guts, including organic molecule degradation and amino acid metabolism (Kumar et al., 2020). Our results suggest that *Cutibacterium* bacteria may play a more significant role in nymphs compared to *C. myconis* plants, given their greater abundance in nymphs.

Although less apparent, there are similarities between nymphs and foam in terms of certain bacterial genera. Specifically, there appears to be a significant exchange of *Enhydrobacter* bacteria between nymphs and foam, given the comparable abundance of this genus in both samples.

Enhydrobacter species have been documented to colonize plants, including seeds (Araujo et al., 2020), but also the guts of some insects (Moldovan et al., 2023). This genus is relatively understudied, and the role of *Enhydrobacter* species in plants and insects remains poorly documented. Similarly, *Roseomonas* spp. are likely shared between nymphs and foam. The notable enrichment of *Roseomonas* spp. in nymphs suggests they may play essential roles, although these roles are not yet fully understood in insects. However, members of this genus have been found in insecticide-susceptible nymphs of *Nilaparvata lugens* (Hemiptera: Delphacidae), suggesting they may assist insects in adapting to stressful environmental conditions (Malathi et al., 2018).

Several bacterial families were exclusively detected in one of the studied samples. While exclusive foam families are likely acquired from the surrounding environment, bacteria families unique to the stem might be vertically transmitted and/or be acquired from the surrounding environment, including soil, as previously reported (Frank et al., 2017). The three bacterial families exclusively detected in the nymphs (*Anaplasmataceae, Enterobacteriaceae*, and *Blattabacteriaceae*) encompass endosymbiotic bacteria genera strictly associated with insects (Kikuchi, 2009), explaining their absence in stems and foam samples. The family *Anaplasmataceae* includes only the genus *Wolbachia*, a known endosymbiont capable of manipulating host reproduction (Bi and Wang, 2019), and previously detected across different geographic populations of *P. spumarius*, including in the Mediterranean region (Cameirão et al., 2024; Formisano et al., 2022; Kapantaidaki et al., 2021; Kolasa et al., 2023; Lis et al., 2015). Nevertheless, in our study, this genus was detected in a single sample of nymphs, indicating its scarce presence in the Portuguese populations of *P. spumarius*. The rarity of *Wolbachia* in Southern European *P. spumarius* populations has been reported previously (Cameirão et al., 2024; Formisano et al., 2022; Kolasa et al., 2023). This scarcity is potentially due to adverse environmental factors such as hot and dry climates, which may hinder *Wolbachia* transmission (Formisano et al., 2022).

5. Conclusions

Our study demonstrated that the foam produced by nymphs of *P. spumarius* exhibited a higher bacterial diversity compared to both the nymphs themselves and the stems of *C. myconis* on which they feed. The most abundant bacterial genera found in these three biological samples include members known to provide diverse benefits to their hosts. The bacterial community composition in nymphs was distinct, yet more similar to that found in the stem compared to the foam. Indeed, nymphs were enriched in endosymbiotic bacteria not found in either the stem or the foam. Nevertheless, many bacterial genera detected in the nymphs were also found in the stems, suggesting a significant exchange of bacteria between these two organisms during nymph feeding. The genera *Methylobacterium, Curvibacter, Cutibacterium, Pseudomonas* and *Rhizobium* were probably the most frequently exchanged, given their higher abundance in both nymphs and stems. Although less evident, nymphs also appear to transfer bacteria to the foam, particularly species from the *Enhydrobacter* genus. However, the greatest similarity in bacterial community composition was found between the stem and the foam, suggesting an exchange of bacteria facilitated by the open wounds caused by nymphs while feeding on the stem. Overall, the results suggest a dynamic transfer of bacteria between the *C. myconis* plant, the nymphs of *P. spumarius*, and the foam produced by the nymphs. Further research is warranted to explore the implications of this bacterial exchange on host fitness. Such insights could inform strategies for managing *P. spumarius* populations through targeted microbiome manipulation.

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Data availability

All data supporting the findings of this study, along with additional results, are available in the supplementary file. Moreover, all 16S rRNA sequences generated in this study have been submitted in the NCBI database under accession numbers from ON185007 to ON185183. Similarly, the raw sequence reads and associated metadata from metabarcoding analysis have been deposited in the NCBI Sequence Reads Archive (SRA) database under the BioProject PRJNA836511.

CRediT authorship contribution statement

Cristina Cameirão: Investigation, Visualization, Writing – original draft, Formal analysis. **José Alberto Pereira:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition. **Rui Tavares:** Formal analysis. **Teresa Lino-Neto:** Conceptualization, Supervision, Writing – review & editing, Data curation, Validation. **Paula Baptista:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition, Data curation, Validation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cris.2025.100110.

Data availability

All data are uploaded to an external repository, and working links to the data are provided in the manuscript

References

Albanese, D., Fontana, P., De Filippo, C., Cavalieri, D., Donati, C., 2015. MICCA: a complete and accurate software for taxonomic profiling of metagenomic data. Sci. Rep. 5, 1–7. https://doi.org/10.1038/srep09743.

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J., 1990. Basic local alignment search tool. J. Mol. Biol. 215 (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Andrews, M., Andrews, M.E., 2017. Specificity in legume-rhizobia symbioses. Int. J. Mol. Sci. 18 (4), 705. https://doi.org/10.3390/ijms18040705, 2017.

Andrews, S. (2010). FastQC - a quality control tool for high throughput sequence data.

Anguita-Maeso, M., Olivares-García, C., Haro, C., Imperial, J., Navas-Cortés, J.A., Landa, B.B., 2020. Culture-dependent and culture-independent characterization of the olive xylem microbiota: effect of sap extraction methods. Front. Plant Sci. 10, 1708.

Araujo, R., Dunlap, C., Franco, C.M.M., 2020. Analogous wheat root rhizosphere microbial successions in field and greenhouse trials in the presence of biocontrol agents *paenibacillus peoriae* SP9 and *streptomyces fulvissimus* FU14. Mol. Plant Pathol. 21 (5), 622–635. https://doi.org/10.1111/mpp.12918.

Aronesty, E. (2011). ea-utis: command-line tools for processing biological sequencing data. In https://github.com/ExpressionAnalysis/ea-utils.

Asaf, S., Numan, M., Khan, A.L., Al-Harrasi, A., 2020. Sphingomonas: from diversity and genomics to functional role in environmental remediation and plant growth. Crit. Rev. Biotechnol. 40 (2), 138–152. https://doi.org/10.1080/07388551.2019.1709793.

Balzani, P., Masoni, A., Samantha, M., Scuola, P., Sant'anna, S., 2023. Spittlebug invisibility cloak: experimental tests on the antipredatory effect of the froth of *Philaenus spumarius*. Bull. Insectol. 76 (1), 21–28. https://www.researchgate.net/publication/369261816.

Bang, C., Dagan, T., Deines, P., et al., 2018. Metaorganisms in extreme environments: do microbes play a role in organismal adaptation? Zoology 127, 1–19. https://doi.org/10.1016/j.zool.2018.02.004.

Berg, G., Rybakova, D., Fischer, D., et al., 2020. Microbiome definition re-visited: old concepts and new challenges. Microbiome 8 (103). https://doi.org/10.1186/s40168-020-00875-0.

Bi, J., Wang, Y.F., 2019. The effect of the endosymbiont *Wolbachia* on the behavior of insect hosts. Insect Sci. 1–13. https://doi.org/10.1111/1744-7917.12731.

Cai, G., Yang, X., Yu, X., Zheng, W., Cai, R., Wang, H., 2024. The novel application of violacein produced by a marine *Duganella* strain as a promising agent for controlling *Heterosigma akashiwo* bloom: algicidal mechanism, fermentation optimization and agent formulation. J. Hazard. Mater. 466. https://doi.org/10.1016/j.jhazmat.2024.133548.

Cameirão, C., Costa, D., Rufino, J., Pereira, J.A., Lino-Neto, T., Baptista, P., 2024. Diversity, composition, and specificity of the *Philaenus spumarius* bacteriome. Microorganisms 12 (298). https://doi.org/10.3390/microorganisms12020298.

Caporaso, J.G., Kuczynski, J., Stombaugh, J., et al., 2010. QIIME allows analysis of high-throughput community sequencing data. Nat. Methods 7 (5), 335–336. https://doi.org/10.1038/nmeth0510-335.

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. PNAS 108 (SUPPL. 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107.

Chen, X., Meyer-Rochow, V.B., Fereres, A., Morente, M., Liang, A.P., 2018. The role of biofoam in shielding spittlebug nymphs (Insecta, Hemiptera, Cercopidae) against bright light. Ecol. Entomol. 43 (3), 273–281. https://doi.org/10.1111/een.12496.

Choi, S.Y., Kim, S., Lyuck, S., Kim, S.B., Mitchell, R.J., 2015. High-level production of violacein by the newly isolated *Duganella violaceinigra* str. NI28 and its impact on *Staphylococcus aureus*. Sci. Rep. 5. https://doi.org/10.1038/srep15598.

Coordinators, N.R., 2018. Database resources of the national center for biotechnology information. Nucl. Acids Res. 46 (D1), D8–D13. https://doi.org/10.1093/nar/gkx1095.

Cornara, D., Bosco, D., Fereres, A., 2018. *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. J. Pest Sci. 91 (3), 957–972. https://doi.org/10.1007/s10340-018-0966-0.

Cowan, D., Meyer, Q., Stafford, W., Muyanga, S., Cameron, R., Wittwer, P., 2005. Metagenomic gene discovery: past, present and future. Trends Biotechnol. 23 (6), 321–329. https://doi.org/10.1016/j.tibtech.2005.04.001.

Dastogeer, K.M.G., Tumpa, F.H., Sultana, A., Akter, M.A., Chakraborty, A., 2020. Plant microbiome–an account of the factors that shape community composition and diversity. Curr. Plant Biol. 23 (100161). https://doi.org/10.1016/j.cpb.2020.100161.

Delbianco, A., Gibin, D., Pasinato, L., Boscia, D., Morelli, M., 2022. Update of the *Xylella* spp. Host plant database – systematic literature search up to 31 December 2021. EFSA J. 20 (6), 7356. https://doi.org/10.2903/j.efsa.2022.7356.

Douglas, A.E., 2015. Multiorganismal insects: diversity and function of resident microorganisms. Annu. Rev. Entomol. 60, 17–34. https://doi.org/10.1146/annurev-ento-010814-020822.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26 (19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

EFSA, Jeger, M., Caffier, D., Candresse, T., Chatzivassiliou, E., Dehnen-Schmutz, K., Gilioli, G., Grégoire, J.C., Jaques Miret, J.A., MacLeod, A., Navajas Navarro, M., Niere, B., Parnell, S., Potting, R., Rafoss, T., Rossi, V., Urek, G., Van Bruggen, A., Van der Werf, W., Bragard, C., 2018. Updated pest categorization of *Xylella fastidiosa*. EFSA J. 16 (7). https://doi.org/10.2903/j.efsa.2018.5357.

Faddetta, T., Abbate, L., Alibrandi, P., Arancio, W., Siino, D., Strati, F., De Filippo, C., Fatta Del Bosco, S., Carimi, F., Puglia, A.M., Cardinale, M., Gallo, G., Mercati, F., 2021. The endophytic microbiota of *Citrus limon* is transmitted from seed to shoot highlighting differences of bacterial and fungal community structures. Sci. Rep. 11 (7078). https://doi.org/10.1038/s41598-021-86399-5.

Fahde, S., Boughribil, S., Sijilmassi, B., Amri, A., 2023. Rhizobia: a promising source of plant growth-promoting molecules and their non-legume interactions: examining applications and mechanisms. Agriculture 13 (1279). https://doi.org/10.3390/agriculture13071279.

Flores-Félix, J.D., Menéndez, E., Peix, A., García-Fraile, P., Velázquez, E., 2020. History and current taxonomic status of genus *agrobacterium*. Syst. Appl. Microbiol. 43 (126046). https://doi.org/10.1016/j.syapm.2019.126046.

Formisano, G., Iodice, L., Cascone, P., Sacco, A., Quarto, R., Cavalieri, V., Bosco, D., Guerrieri, E., Giorgini, M., 2022. *Wolbachia* infection and genetic diversity of Italian populations of *Philaenus spumarius*, the main vector of *Xylella fastidiosa* in Europe. PLoS ONE 17 (e0272028). https://doi.org/10.1371/journal.pone.0272028.

Frank, A.C., Guzmán, J.P.S., Shay, J.E., 2017. Transmission of bacterial endophytes. Microorganisms 5 (70). https://doi.org/10.3390/microorganisms5040070.

Gahan, J., Schmalenberger, A., 2014. The role of bacteria and mycorrhiza in plant sulfur supply. Front. Plant Sci. 5 (723). https://doi.org/10.3389/fpls.2014.00723.

Germain, J.F., Lester, K., Cornara, D., EPPO, 2020. PM 7/141 (1) *Philaenus spumarius, Philaenus italosignus* and *Neophilaenus campestris*. EPPO Bull. 50 (1), 32–40. https://doi.org/10.1111/epp.12610.

Giron, D., Dubreuil, G., Bennett, A., et al., 2018. Promises and challenges in insect–plant interactions. Entomol. Exp. Appl. 166 (5), 319–343. https://doi.org/10.1111/eea.12679.

Gurung, K., Wertheim, B., Falcao Salles, J., 2019. The microbiome of pest insects: it is not just bacteria. Entomol. Exp. Appl. 167 (3), 156–170. https://doi.org/10.1111/eea.12768.

Haack, F.S., Poehlein, A., Kröger, C., Voigt, C.A., Piepenbring, M., Bode, H.B., Daniel, R., Schäfer, W., Streit, W.R., 2016. Molecular keys to the janthinobacterium and *Duganella* spp. Interaction with the plant pathogen *fusarium graminearum*. Front. Microbiol. 7 (1668). https://doi.org/10.3389/fmicb.2016.01668.

Heck, M., 2018. Insect transmission of plant pathogens: a systems biology perspective. MSystems 3 (2). https://doi.org/10.1128/msystems.00168-17.

Hu, Y., Wei GY, G., Wen, J., Chang, L., Chen, Y., Wang, Y., Nizamani, M., 2024. Endophytic fungi: tracing the evolutionary roots and exploring the diversity of plant-fungal symbioses. Curr. Res. Environm. Appl. Mycol. 14 (1), 1–48. https://doi.org/10.5943/cream/14/1/1.

Jamalzadeh, A., Darvishnia, M., Khodakaramian, G., Bazgir, E., Zafari, D., 2021. Genetic diversity and plant growth-promoting activity of the dominant bacteria from canola plants in Western Iran. Egypt. J. Biol. Pest Control. 31 (1). https://doi.org/10.1186/s41938-021-00442-1.

Jing, T.Z., Qi, F.H., Wang, Z.Y., 2020. Most dominant roles of insect gut bacteria: digestion, detoxification, or essential nutrient provision? Microbiome 8 (38). https://doi.org/10.1186/s40168-020-00823-y.

Joshi, N., Fass, J. (2011). Sickle: a sliding-window, adaptive, quality-based trimming tool for FastQ files. In https://github.com/najoshi/sickle.

Kapantaidaki, D.E., Antonatos, S., Evangelou, V., Papachristos, D.P., Milonas, P., 2021. Genetic and endosymbiotic diversity of Greek populations of *Philaenus spumarius, Philaenus signatus* and *Neophilaenus campestris*, vectors of *Xylella fastidiosa*. Sci. Rep. 11, 3752. https://doi.org/10.1038/s41598-021-83109-z.

Kikuchi, Y., 2009. Endosymbiotic bacteria in insects: their diversity and culturability. Microbes Environ. 24 (3), 195–204. https://doi.org/10.1264/jsme2.ME09140S.

Koga, R., Moran, N.A., 2014. Swapping symbionts in spittlebugs: evolutionary replacement of a reduced genome symbiont. ISME J. 8 (6), 1237–1246. https://doi.org/10.1038/ismej.2013.235.

Kolasa, M., Montagna, M., Mereghetti, V., Kubisz, D., Mazur, M.A., Kajtoch, Ł., 2017. Preliminary evidence of the horizontal transmission of *Wolbachia* between *Crioceris* leaf beetles (Coleoptera: chrysomelidae) and their *Asparagus* host plants. EJE 114. https://doi.org/10.14411/eje.2017.057. Article 446-454.

Kolasa, M., Kajtoch, Ł., Michalik, A., Maryańska-Nadachowska, A., Łukasik, P., 2023. Till evolution do us part: the diversity of symbiotic associations across populations of *philaenus* spittlebugs. Environ. Microbiol. 25 (11), 2431–2446. https://doi.org/10.1111/1462-2920.16473.

Kumar, V., Raghuvanshi, N., Kumar, A., Rithesh, L., Rai, A., Pandey, A.K., 2023. Deciphering phytomicrobiomes for sustainable crop production: recent findings and future perspectives. Plant Stress 10 (100285). https://doi.org/10.1016/j.stress.2023.100285.

Kumar, V., Tyagi, I., Tyagi, K., Chandra, K., 2020. Diversity and structure of bacterial communities in the gut of spider: *thomisidae* and *Oxyopidae*. Front. Ecol. Evol. 8 (588102). https://doi.org/10.3389/fevo.2020.588102.

Lalucat, J., Gomila, M., Mulet, M., Zaruma, A., García-Valdés, E., 2022. Past, present and future of the boundaries of the *Pseudomonas* genus: proposal of *Stutzerimonas* gen. Nov. Syst. Appl. Microbiol. 45 (1). https://doi.org/10.1016/j.syapm.2021.126289.

Lane, D.J., 1991. 16S/23S rRNA sequencing. In: Stackebrandt, E., Goodfellow, M. (Eds.), Nucleic Acid Techniques in Bacterial Systematics. John Wiley & Sons, pp. 115–175.

Lee, S.A., Kim, H.S., Kim, T.W., Sang, M.K., Song, J., Weon, H.Y., 2019. Complete genome sequence of plant growth-promoting bacterium *pedobacter ginsengisoli* T01R-27 isolated from tomato (*Solanum lycopersicum* L.) rhizosphere. Korean J. Microbiol. 55 (4), 425–427. https://doi.org/10.7845/kjm.2019.9116.

Leite-Mondin, M., DiLegge, M.J., Manter, D.K., Weir, T.L., Silva-Filho, M.C., Vivanco, J. M., 2021. The gut microbiota composition of *trichoplusia ni* is altered by diet and may influence its polyphagous behavior. Sci. Rep. 11 (5786). https://doi.org/10.1038/s41598-021-85057-0.

Li, S.J., Ahmed, M.Z., Lv, N., Shi, P.Q., Wang, X.M., Huang, J.L., Qiu, B.L, 2017. Plant–mediated horizontal transmission of *Wolbachia* between whiteflies. ISME J 11 (4), 1019–1028. https://doi.org/10.1038/ismej.2016.164.

Lim, L., Ab Majid, A.H., 2021. Characterization of bacterial communities associated with blood-fed and starved tropical bed bugs, *Cimex hemipterus* (F.) (Hemiptera): a high throughput metabarcoding analysis. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-021-87946-w.

Lis, A., Maryańska-Nadachowska, A., Kajtoch, Ł., 2015. Relations of *Wolbachia* infection with phylogeography of *Philaenus spumarius* (Hemiptera: aphrophoridae) populations within and beyond the Carpathian contact zone. Microb. Ecol. 70, 509–521. https://doi.org/10.1007/s00248-015-0570-2.

Lòpez-Fernàndez, S., Mazzoni, V., Pedrazzoli, F., Pertot, I., Campisano, A., 2017. A phloem-feeding insect transfers bacterial endophytic communities between grapevine plants. Front Microbiol. 8, 834. https://doi.org/10.3389/fmicb.2017.00834.

Madhaiyan, M., Suresh Reddy, B.V., Anandham, R., Senthilkumar, M., Poonguzhali, S., Sundaram, S.P., Sa, T., 2006. Plant growth-promoting *methylobacterium* induces defense responses in groundnut (*Arachis hypogaea* L.) compared with rot pathogens. Curr. Microbiol. 53 (4), 270–276. https://doi.org/10.1007/s00284-005-0452-9.

Malathi, V.M., More, R.P., Anandham, R., Gracy, G.R., Mohan, M., Venkatesan, T., Samaddar, S., Jalali, S.K., Sa, T., 2018. Gut bacterial diversity of insecticide-susceptible and -resistant nymphs of the brown planthopper *Nilaparvata lugens* stål (Hemiptera: delphacidae) and elucidation of their putative functional roles. J. Microbiol. Biotechn. 28 (6), 976–986. https://doi.org/10.4014/jmb.1711.11039.

Malone, M., Watson, R., Pritchard, J., 1999. The spittlebug *philaenus spumarius* feeds from mature xylem at the full hydraulic tension of the transpiration stream. New Phytol 143 (2), 261–271. https://doi.org/10.1046/j.1469-8137.1999.00448.x.

Marshall, A.T., 1966. Spittle-production and tube-building by cercopid larvae (Homoptera)-IV. Mucopolysaccharide associated with spittle-production. J. Insect Physiol. 12 (6), 635–644. https://doi.org/10.1016/0022-1910(66)90109-0.

Martinez, Arbizu.P. (2020). pairwiseAdonis: pairwise multilevel comparison using adonis. R Package Version 0.4. https://github.com/pmartinezarbizu/pairwiseAdonis.

Masson, F., Lemaitre, B., 2020. Growing ungrowable bacteria: overview and perspectives on insect symbiont culturability. Microbiol. Mol. Biol. Rev. 84 (e00089–20). https://doi.org/10.1128/mmbr.00089-20.

McIlwaine, D.B., Moore, M., Corrigan, A., Niemaseck, B., Nicoletti, D., 2024. A comparison of the microbial populations in a culture-dependent and a culture-independent analysis of industrial water samples. Appl. Microbiol. 4, 1079–1090.

Mei, Y.H., Li, X., Zhou, J.Y., Kong, F.L., Qi, S.S., Zhu, B., Naz, M., Dai, Z.C., Du, D.L., 2022. Both adaptability and endophytic bacteria are linked to the functional traits in the invasive clonal plant *Wedelia trilobata*. Plants 11 (23). https://doi.org/10.3390/plants11233369.

Mello, M.L.S., Pimentel, E.R., Yamada, A.T., Storopoli-Neto, A., 1987. Composition and structure of the froth of the spittlebug, Deois SP. Insect Biochem. 17 (3), 493–502. https://doi.org/10.1016/0020-1790(87)90009-6.

Moldovan, O.T., Carrell, A.A., Bulzu, P.A., Levei, E., Bucur, R., Sitar, C., Faur, L., Mirea, I. C., Șenilă, M., Cadar, O., Podar, M., 2023. The gut microbiome mediates adaptation to scarce food in Coleoptera. Environ. Microbiome 18 (80). https://doi.org/10.1186/s40793-023-00537-2.

Moran, N.A., Tran, P., Gerardo, N.M., 2005. Symbiosis and insect diversification: an ancient symbiont of sap-feeding insects from the bacterial phylum bacteroidetes. Appl. Environ. Microbiol. 71 (12), 8802–8810. https://doi.org/10.1128/AEM.71.12.8802-8810.2005.

Mujumdar, S., Bhoyar, J., Akkar, A., Hundekar, S., Agnihotri, N., Jaybhay, P., Bhuyan, S., 2023. Chapter 15 - Acinetobacter: a versatile plant growth-promoting rhizobacteria (PGPR). In: Swapnil, P., Meena, M., Marwal, A., Vijayalakshmi, S., Zehra, A. (Eds.), Plant-Microbe Interaction - Recent Advances in Molecular and Biochemical Approaches. Academic Press, pp. 327–362. https://doi.org/10.1016/B978-0-323-91875-6.00009-8.

Muyzer, G., De Waal, E.C., Uitterlinden, A.G., 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59 (3), 695–700.

Nencioni, A., Pastorelli, R., Bigiotti, G., Cucu, M.A., Sacchetti, P., 2023. Diversity of the bacterial community associated with hindgut, malpighian tubules, and foam of nymphs of two spittlebug species (Hemiptera: aphrophoridae). Microorganisms 11 (466). https://doi.org/10.3390/microorganisms11020466.

Nurk, S., Bankevich, A., Antipov, D., et al., 2013. Assembling genomes and mini-metagenomes from highly chimeric reads. In: Deng, M., Jiang, R., Sun, F., Zhang, X. (Eds.), Research in Computational Molecular Biology. RECOMB 2013, Research in Computational Molecular Biology. RECOMB 2013, 7821. LNBI Springer, Berlin, Heidelberg, pp. 158–170. https://doi.org/10.1007/978-3-642-37195-0_13. Vol.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, H.H., Szoecs, E., Wagner, H. (2018). Vegan: community ecology package. R Package Version 2.5-3.

Pathak, P., Rai, V.K., Can, H., Singh, S.K., Kumar, D., Bhardwaj, N., Roychowdhury, R., de Azevedo, L.C.B., Kaushalendra, Verma, H., Kumar, A., 2022. Plant-endophyte interaction during biotic stress management. Plants 11 (17). https://doi.org/10.3390/plants11172203.

Pirttilä, A.M., Brusila, V., Koskimäki, J.J., Wäli, P.R., Ruotsalainen, A.L., Mutanen, M., Markkola, A.M., 2023. Exchange of microbiomes in plant-insect herbivore interactions. MBio 14 (2). https://doi.org/10.1128/mbio.03210-22.

Poorniammal, R., Sundaram, S.P., Kumutha, K., 2009. *In vitro* biocontrol activity of *methylobacterium extorquens* against fungal pathogens. Int. J. Plant Prot. 2 (1), 59–60. https://doi.org/10.13140/2.1.3086.0163.

Provorov, N.A., Onishchuk, O.P., 2018. Microbial symbionts of insects: genetic organization, adaptive role, and evolution. Microbiology 87 (2), 151–163. https://doi.org/10.1134/S002626171802011X.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glöckner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41 (D1), 590–596. https://doi.org/10.1093/nar/gks1219.

R Core Team, 2022. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Roma, J.S., D'Souza, S., Somers, P.J., Cabo, L.F., Farsin, R., Aksoy, S., Runyen-Janecky, L.J., Weiss, B.L., 2019. Thermal stress responses of *Sodalis glossinidius*, an indigenous bacterial symbiont of hematophagous tsetse flies. PLOS Negl. Trop. Dis. 13 (11), 1–20. https://doi.org/10.1371/journal.pntd.0007464.

Saeed, Q., Xiukang, W., Haider, F.U., Kučerik, J., Mumtaz, M.Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M., Mustafa, A., 2021. Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: a comprehensive review of effects and mechanisms. Int. J. Mol. Sci. 22 (19). https://doi.org/10.3390/ijms221910529.

Sah, S., Krishnani, S., Singh, R., 2021. *Pseudomonas* mediated nutritional and growth promotional activities for sustainable food security. Curr. Res. Microb. Sci. 2. https://doi.org/10.1016/j.crmicr.2021.100084.

Saponari, M., Loconsole, G., Cornara, D., Yokomi, R.K., De Stradis, A., Boscia, D., Bosco, D., Martelli, G.P., Krugner, R., Porcelli, F., 2014. Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: aphrophoridae) in Apulia, Italy. J. Econ. Entomol. 107 (4), 1316–1319. https://doi.org/10.1603/EC14142.

Taerum, S.J., Duong, T.A., de Beer, Z.W., Gillette, N., Sun, J.H., Owen, D.R., Wingfield, M.J., 2013. Large shift in symbiont assemblage in the invasive red turpentine beetle. PLoS ONE 8 (10). https://doi.org/10.1371/journal.pone.0078126.

Teoh, M.C., Furusawa, G., Veera Singham, G., 2021. Multifaceted interactions between the pseudomonads and insects: mechanisms and prospects. Arch. Microbiol. 203 (5), 1891–1915. https://doi.org/10.1007/s00203-021-02230-9.

Tonelli, M., Cotta, S.R., Rigotto, A., Dias, A.C.F., Andreote, F.D., Bento, J.M.S., 2020. The composition of the bacterial community in the foam produced by *Mahanarva fimbriolata* is distinct from those at gut and soil. Braz. J. Microbiol. 51 (3), 1151–1157. https://doi.org/10.1007/s42770-019-00211-1.

Tonelli, M., Gomes, G., Silva, W.D., Magri, N.T.C., Vieira, D.M., Aguiar, C.L., Bento, J.M. S., 2018. Spittlebugs produce foam as a thermoregulatory adaptation. Sci. Rep. 8, 4729. https://doi.org/10.1038/s41598-018-23031-z.

Viana, A.T., Caetano, T., Covas, C., Santos, T., Mendo, S., 2018. Environmental superbugs: the case study of *pedobacter* spp. Environ. Pollut 241, 1048–1055. https://doi.org/10.1016/j.envpol.2018.06.047.

Villa, M., Rodrigues, I., Baptista, P., Fereres, A., Pereira, J.A., 2020. Populations and host/non-host plants of spittlebugs nymphs in olive orchards from Northeastern Portugal. Insects 11 (720). https://doi.org/10.3390/insects11100720.

Wang, M., Noor, S., Huan, R., Liu, C., Li, J., Shi, Q., Zhang, Y.J., Wu, C., He, H., 2020. Comparison of the diversity of cultured and total bacterial communities in marine sediment using culture-dependent and sequencing methods. PeerJ 8, e10060. https://doi.org/10.7717/peerj.10060.

Yamamoto, K., Toya, S., Sabidi, S., Hoshiko, Y., Maeda, T., 2021. Diluted Luria-Bertani medium vs. sewage sludge as growth media: comparison of community structure and diversity in the culturable bacteria. Appl. Microbiol. Biotechnol. 105, 3787–3798. https://doi.org/10.1007/s00253-021-11248-4.

Yin, C., Casa Vargas, J.M., Schlatter, D.C., Hagerty, C.H., Hulbert, S.H., Paulitz, T.C, 2021. Rhizosphere community selection reveals bacteria associated with reduced root disease. Microbiome 9 (86). https://doi.org/10.1186/s40168-020-00997-5.

Yurtsever, S., 2000. On the polymorphic meadow spittlebug, *Philaenus spumarius* (L.) (Homoptera: cercopidae). Turk. J. Zoo. 24 (4), 447–459. https://doi.org/10.1080/03014223.2000.9517632.

Zaim, S., Bekkar, A.A., 2023. Advances in research on the use of *Brevundimonas* spp. To improve crop and soil fertility and for soil bioremediation. Alger. J. Biosciences 4 (1), 045–051. https://doi.org/10.57056/ajb.v4i1.109.

Zhang, Y., Ji, J., Guan, C.F., Jin, C., Li, Q., Yan, B., Wang, G., Wang, Y.R., 2018. Screening of straw-degrading strains and their enzyme-producing conditions. J Cellul. Sci. Technol. 26, 28–38.