Analyzing the Impact of Invasive Species on Tripartite Trophic Networks of Sap Suckers in Point Reyes

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ABSTRACT

Invasive species represent a major worldwide issue, threatening ecosystems all around the world. While invasive species are present globally, it is important to study their impacts on individual locations. The establishment of invasive species can have wide-ranging impacts and effects across trophic levels. This study aims to understand the impact of invasive plant species on arthropods, specifically Hemiptera, and their associated gut microbiota in Point Reyes, California. First, the arthropods DNA and that of their gut microbiota were sequenced, then those results were formed into a dataset, which was processed into graphs and analyzed for relative alpha and beta diversity. It was found that invasive Velvet Grass is permitting the establishment of the invasive arthropod, *Sitobion fragariae* in this ecosystem. Of the four Hemiptera, three were specialist and only one, *Emoscae fabae* was a generalist. *Emoscae fabae* was found to be outcompeted on Velvet Grass by *Sitobion fragariae*, even though it is capable of feeding on it. The specialists each had lower alpha and beta diversity of their gut microbiota than the generalist species. The gut bacterial abundance of the specialist species was lower than that of *Emoscae fabae*. Each specialist had very similar gut microbiota to the other members of their species, while the *Emoscae fabae's* was more dissimilar. Overall, these results indicate that invasive plants in Point Reyes, particularly Velvet Grass, have had a cascading effect across trophic levels on the arthropods and their gut microbiota.

KEYWORDS

Hemiptera, Invasive plants, insect-plant-microbe interaction, microbiome diversity, alpha and beta diversity

INTRODUCTION

Invasive species currently represent a major threat to ecosystems across the globe (Simberloff et al. 2013). For an invasive species to establish itself in an ecosystem, it must overcome multiple barriers and pass through several stages (Blackburn et al. 2011). The stages include being transported, introduced, established, and spreading throughout a new ecosystem (Blackburn et al. 2011). These ecosystem invasions from non-native species are relatively frequent, and if successful, can cause significant damage to a native ecosystem and species (Blackburn et al. 2011). When a species has successfully invaded, it has potential to significantly offset the ecosystem's natural equilibrium through processes such as predation, shifting nutrient cycles or compositions, or removal and addition of physical structures (Simberloff et al. 2013). It frequently becomes exponentially more difficult and expensive to remove an invasive species after it has time to become established in the ecosystem (Simberloff et al. 2013). Some invasions become effectively irreversible and long-term management remains the only option (Simberloff et al. 2013). Invasive species that have established and spread their populations become particularly more difficult to remove from an ecosystem, so it is most effective to detect and remove them as quickly as possible.

The study of arthropods is particularly important within the context of the potential ongoing "insect apocalypse." Currently, insect biomass is dropping rapidly, these understudied insects are integral to almost all ecosystems on the planet (Goulson 2019). Many species of insect carry out roles in nature that are not known because there are far too many species to study (Goulson 2019). While there are many possible factors contributing to the insect decline, one possible cause is invasive species (Goulson 2019).

For this study, I chose to examine arthropods from the order Hemiptera, specifically sap suckers from this order. Sap suckers are a species of insect that feed on tree sap flowing through the phloem of a plant, ingesting it through use of the pressure of sap flowing up the plant (Kuelcher et al. 2012). Due to the high sugar content of plant sap, sap suckers often excrete excess sugary materials in the form of honeydew (Baumann 2005). Another difficulty in feeding on plant sap is that it is often lacking in certain essential amino acids which also cannot be synthesized by animals (Hansen et al. 2014). The method by which sap suckers overcome this boundary is to have symbiotic relationships with microbes that synthesize these essential amino

acids (Hansen et al. 2014). There is also evidence to suggest that these bacteria could be aiding in other tasks such as detoxifying ingested plant material (Hansen et al. 2014). Symbiotic bacteria has been essential for allowing these species to occupy niches where these amino acids are limiting (Hansen et al. 2014). Due to their heavy reliance on microbes to synthesize amino acids, sap suckers have tightly coevolved with their gut microbiota. This close relationship makes them ideal candidates for examining the effects of invasive species on multiple trophic levels.

Invasive species present in an ecosystem can have impacts that range across multiple trophic levels which can highlight varying effects based on location, thus separate ecosystems must often be studied independently. Point Reyes National Seashore is a United States national park located in California, northwest of San Francisco, along the coastline facing the Pacific Ocean. It is home to almost 40 species of mammals, over 12 species of marine mammals, and nearly half of all North American bird species (NPS website). This study focuses on native plants: Coyote Brush (*Baccharis pilularis*) and Coffeeberry (*Rhamnus californica*), and the invasive plants: Velvet Grass (*Holcus lanatus*) and French Bloom (*Genista monspessula*), and with their effects on associated arthropods and their gut microbiota. These invasive plants undoubtedly have effects on the overall plant composition of Point Reyes, but this study looks at a tripartite trophic network: the plant's effects on associated arthropod species, and the effect on arthropod's gut microbiota. It is important to quantify the impact of invasive species across as many different taxa as possible, because ecosystems are interconnected and a shift in one area often can have wide ranging effects. In this case the introduction of invasive plants can affect the arthropods that feed on them, and the gut microbiota of those arthropods. The Central Research Question of this paper is: what is the impact of invasive plant species on arthropod communities in Point Reyes California? I will specifically investigate which species are found on native plants and invasive plants, as well as determine the relative microbial diversities of those insect's microbiota.

METHODS

Site Description

The data for this experiment was previously gathered and sequenced in Point Reyes National Seashore, California by Katherine Roger. It was gathered from two sites in the Olema Valley, where environmental factors such as precipitation are largely constant. One site was at low elevation, and the other was at mid elevation. Replicate plots were selected off the Olema Valley and McCurdy trails, prioritizing shrubland over dense forest and grasses, and set at least 200 meters apart. At each of these 15m radius plots, a plot center was used to estimate percent cover. Arthropods were then gathered using standard beating protocol with three minutes of active beating per plant. After being collected, the arthropods were sorted, and only Hemiptera species that were found on the native Coyote Brush, native Coffeeberry, invasive Velvet Grass, and invasive French Broom were sequenced. Arthropods were stored in 95% ethanol at -80°C until DNA was extracted.

Dataset Production

I obtained a file folder that included site location and the sequences for the arthropods. Bacterial sequences, distinguishing sample site labels, and plants from which the arthropods were collected were in a separate folder. Overall, there were 144 samples of arthropods with associated plant and microbiota information. I processed these folders into a dataset using R's (R Core Team, 2014) DADA 2 package according to the steps outlined in the DADA2 tutorial (Callahan et al. 2016). First, I produced matched separated lists of forward and reversed files for the bacterial and arthropod sequences. Then I visually inspected the quality profiles of the forward and reverse files, and the nucleotides were trimmed to improve reads. I then plotted the error rates to ensure that the estimated error rates fit well with the observed rates. I then applied the core sample inference algorithm to my data and merged the forward and reverse reads. This data was then converted into an amplicon sequence variant (ASV) table, the chimeras were removed, and as a final check, I tracked the number of reads that made it through each step in the pipeline. After that, I assigned taxonomy to the sequences, using the NCBI COI locus database for arthropods, and the SLVA bacterial 16 locus database for gut microbiota (O'Leary et al. 2016 Quast et al., 2013).

Data Filtering

After producing these datasets, I manually filtered them to remove information that was not useful and joined the information together. First, for the arthropod data, there were 5,428 potential arthropod reads for the 144 sample locations. I manually filtered these results, returning the best match based on pident, which means percent of nucleotides in the high scoring pair that match, and base pair length, which was intended to be 418 bp in length. Species with a pident of 97% and above had their species labeled, for 94% - 97% the species was labeled as "Sp.", and for below 94% only the arthropod family was returned. Then r was used to filter out any non-Hemiptera arthropod results, leaving 137 of the 144 samples behind. After this filtering was complete, I joined the three datasets: arthropod, microbes, and plants to make two datasets with all the necessary data to look for alpha and beta diversity. The first dataset was used for alpha diversity and included sample name, sequence, bacterial phylum, bacterial abundance, the plant, plant's invasive status, arthropod family, and arthropod species. The second dataset was used for beta diversity and included the sample names, plants, plant's invasiveness status, arthropod family, arthropod species, and bacterial abundance, however the bacterial abundance was pivoted to allow for the creation of an NMDS plot of bray-curtis dissimilarity.

Data Analysis

Finally, I used the datasets to produce graphs in order to look at alpha and beta diversity. For alpha diversity I produced bar plots using ggplot2 (Wickham, 2016). For beta diversity I extracted all columns that had abundance information and configured it into a matrix. Then I used metaMDS() with distance = "bray" to produce a Bray-Curtis dissimilarity matrix. Then an NMDS graph was produced to plot the Bray-Curtis dissimilarities. I produced an NMDS plot to show Arthropod Family with respect to Plant Invasiveness Status (Figure 1) and Arthropod Species with respect to Plant Invasiveness Status (Figure 2). I also produced NMDS graphs showing Arthropod Species with respect to Plant Species (Figure 3) and Arthropod Family with respect to Plant Species (Figure 4). For Alpha diversity I produced bar graphs showing Microbial Abundance with respect to Arthropod Species (Figure 5), Microbial Abundance with respect to Arthropod Family (Figure 6), and Microbial Abundance with respect to Plant Invasiveness Status (Figure 7).

RESULTS

Arthropods found on which plants

       In my study, some families and species of arthropod were exclusively found on a particular plant species, while others are generalists and were found on many different species. *Aphididae*, most of whom were the species *Sitobion fragariae*, was found exclusively on the invasive plant species velvet grass (Figure 3). *Liviidae* was found exclusively on native plant species Coyote Brush, and *Cicadellidae* was found on both types of plants (Figure 1). However, not all *Cicadellidae* are found on a wide range of plant (Figure 2), *Graphocephala atropunctata*, while only comprising 3 samples, was found only on native species. *Emoscae fabae*, also a member of the family *Cicadellidae* was found on the most different types of plant species.
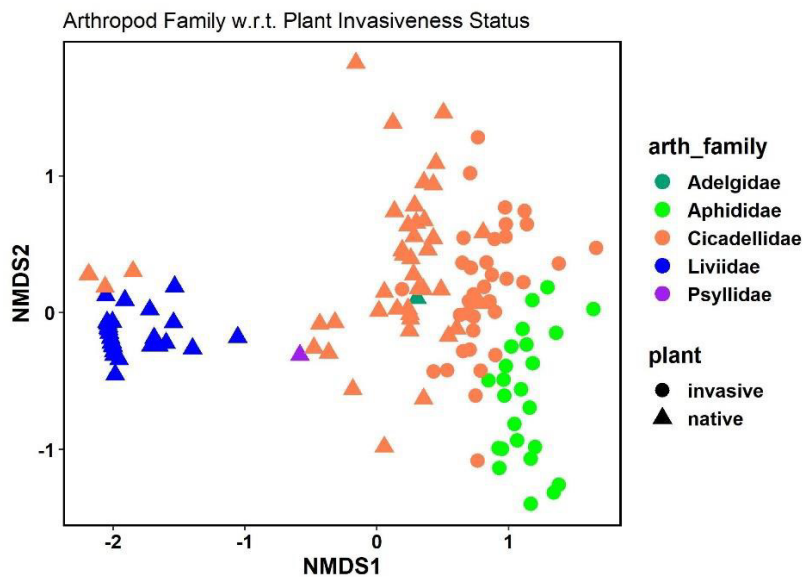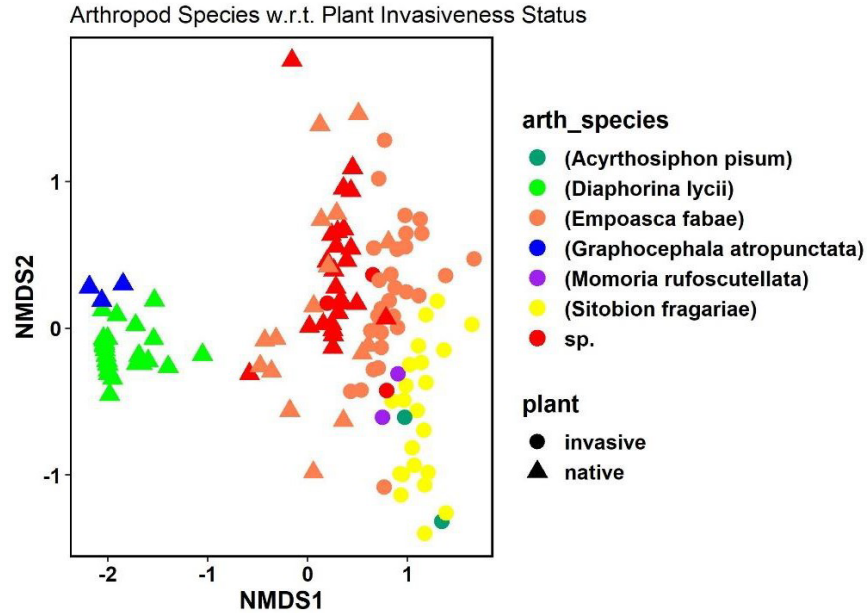

Figure 1: This graph shows the gut microbial communities of Arthropod Families with respect to Plant invasiveness status as determined by Bray-Curtis Dissimilarity.

Figure 2: This graph shows the gut microbial communities of Arthropod Species with respect to Plant invasiveness status as determined by Bray-Curtis Dissimilarity. Sp. indicates species for which I did not have high enough sequence confidence to determine their species.

Calculating statistical significance

The microbial alpha diversity of arthropod's gut microbiota is higher in species on native plants than on invasive (Figure 7). Arthropod species and family, plant species and invasive status each had an ANOSIM significance of 0.0001, which is far smaller than 0.05. This indicates that the null hypothesis, that these factors had no significant differences, can be rejected for all of these groupings. In addition, there is a difference in microbial gut bacterial species based on the different groupings, but that difference is not very significant. Arthropod species had an ANOSIM statistic R value of *0.6651*, arthropod family of *0.5935*, plant species of *0.6945*, and plant invasiveness status of *0.6727*. Overall, the microbes had somewhat higher dissimilarity between different microbes based on plant species than based on arthropods, but the relative difference was not significant.
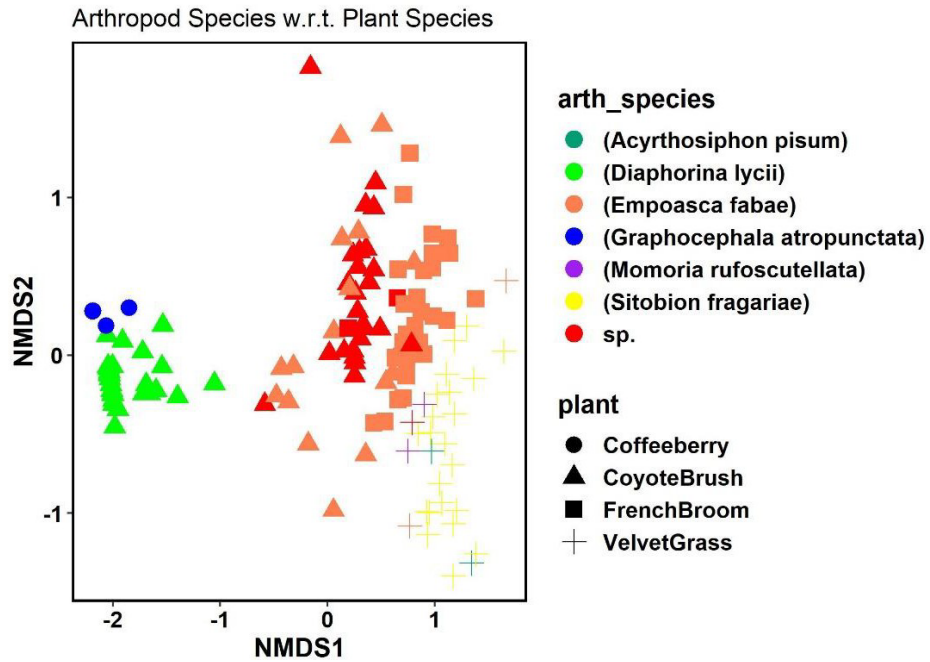
Figure 3: This graph shows the gut microbial communities of Arthropod Species with respect to Plant Species as determined by Bray-Curtis Dissimilarity. Sp. indicates species for which I did not have high enough sequence confidence to determine their species.
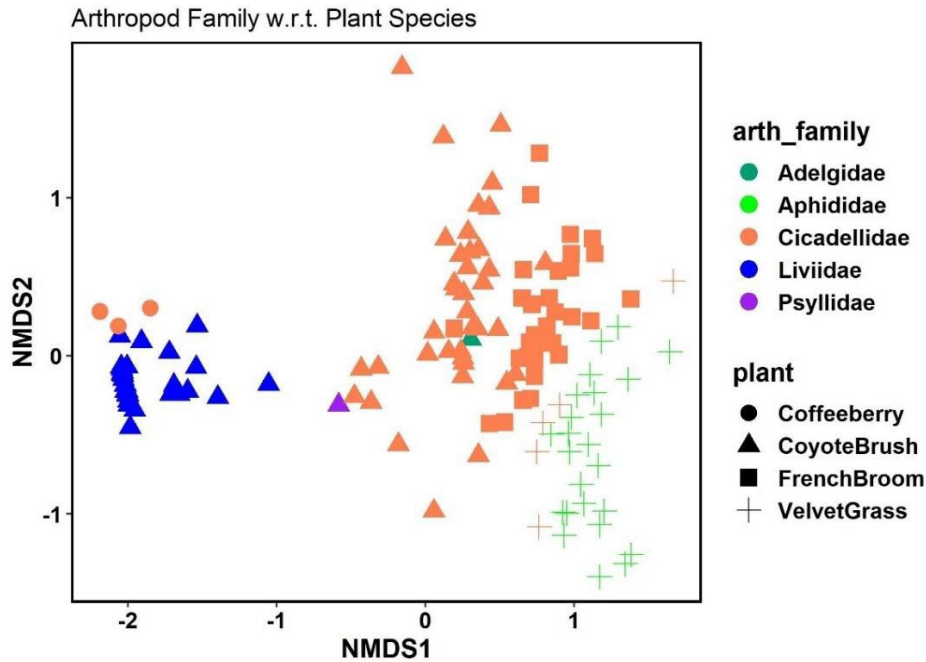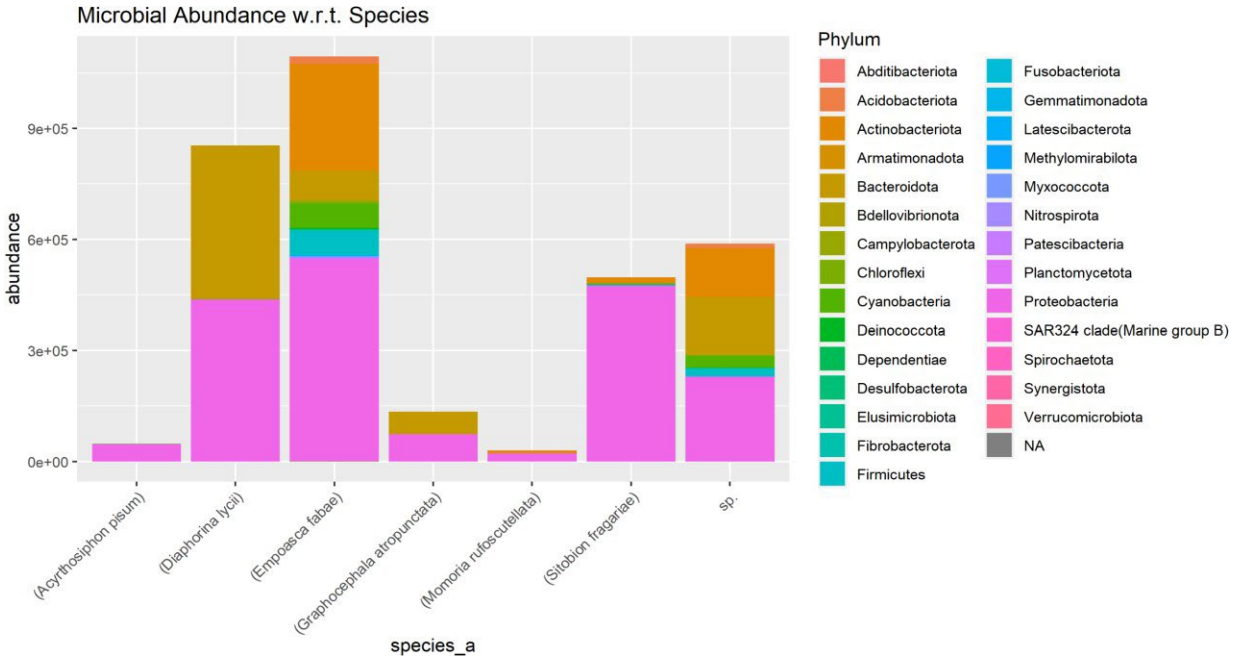

Figure 4: This graph shows the gut microbial communities of Arthropod Families with respect to Plant Species as determined by Bray-Curtis Dissimilarity.
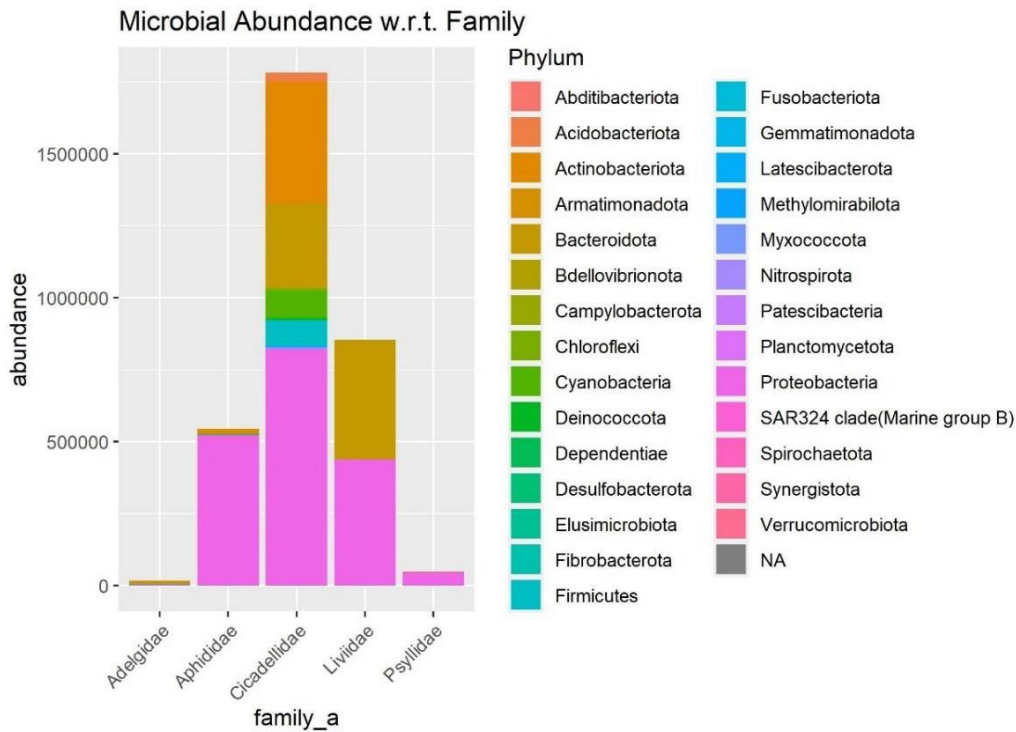
Relative Gut Microbial Diversity of Arthropods

The *Liviidae* family are shown to have very similar gut microbiota to each other. *Liviidae* form a cluster on the left side of the NMDS plot in figure 1, indicating that this family has a low beta diversity. All sampled arthropods of the family *Liviidae* are shown by the NMDS plot in figure 2 to be *Diaphorina lycii*, which further explains their low beta diversity. *D. lycii* also have lower microbial alpha diversity than the more generalist *Cicadellidae*, shown by their lower species richness and abundance (Figure 5, Figure 6). These diversity results, alongside the fact that *D. lycii* is found only on the native Coyote Brush, indicate that they are a specialized species.

The *Aphididae* family were also shown to have similar gut microbiota to each other. They are shown to be less clustered than *Liviidae* on the NMDS plot in figure 1, but there is still an obvious relationship between individual *Aphididae's* gut microbiota. While the *Aphididae* family is composed of a few different species, figure 2 shows that most of the arthropods are *Sitobion fragariae*. This cluster of *Aphididae* on the NMDS plot indicates low beta diversity because it indicates that the gut microbiota have relatively low dissimilarity. Though the beta diversity of *Aphididae* does not appear to be as low as for *Liviidae*. *Aphididae* have the lowest alpha diversity out of the arthropod families and species that were sequenced more than three times (Figure 5, Figure 6). Like *Liviidae*, these alpha and beta diversity results indicate that *S. fragariae*, and likely all *aphididae* family members are specialists.
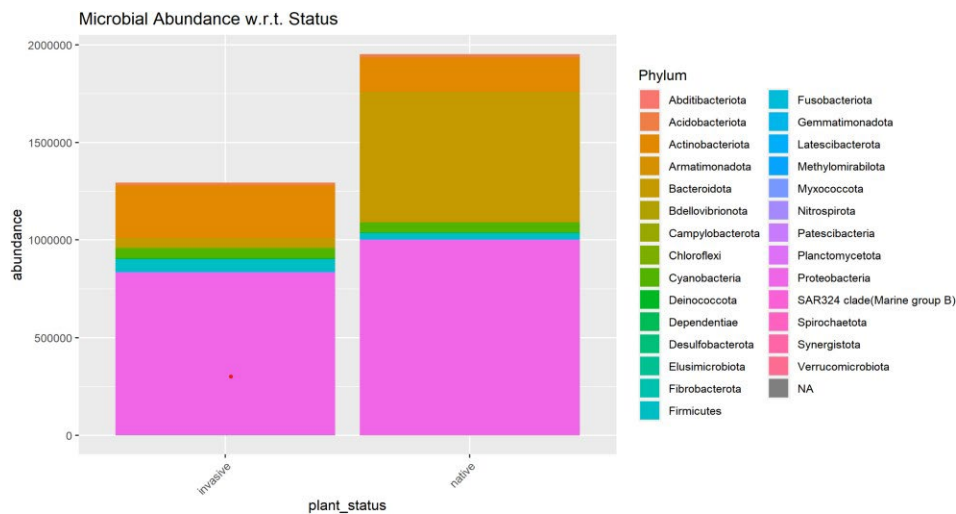
Figure 5: This graph shows the relative Microbial Abundance and richness with respect to the Arthropod Species.

Figure 6: This graph shows the relative Microbial Abundance and richness with respect to the Arthropod Families.

The *Cicadellidae* family had multiple species, one with high alpha and beta diversity of microbiota and the other with very low values for both. The family *Cicadellidae* has a very wide range of microbiota that are very dissimilar to each other (Figure 1). *Cicadellidae* also includes all of the microbiota sequences with low confidence that were returned as Sp. (Figure 2, Figure 5). This wide range suggests that the *Cicadellidae* all have a high beta diversity in gut microbiota. However, *Graphocephala atropunctata*, a member of the *Cicadellidae* family, do in fact have very low beta diversity of gut microbiota (Figure 2). There were only three arthropods of this species sequenced, but they are clustered on the NMDS plots in a way that indicated have low species richness (Figure 5). While they also have low abundance, this data could be skewed by the lack of *G. atropunctata* that were sequenced, there were only three *G. atropunctata* samples compared to at least twenty samples for other species. The other member of the family, *Empoascae fabae* has a wide distribution on the NMDS plot (Figure 2), indicating high beta diversity. *E fabae* were also present on all species of plants collected besides Coffeeberry (Figure 3) *E. fabae* has the highest alpha diversity of any species sampled, with a higher abundance and richness of microbial gut species.

Figure 7: This graph shows the relative Microbial Abundance and richness with respect to the Plant's Invasiveness Status.

DISCUSSION

This study examined the impact of invasive plant species on arthropod communities and their associated gut microbiota in Point Reyes National Park, California. Invasive species such as velvet grass and French Broom have been introduced into the environment and have potentially had cascading effects on the ecosystem. To determine the effect of these invasive plants, my study looked at what arthropod species were found on which plant species. I also examined the relative microbial diversities of those arthropod's gut bacteria. The invasive plant species had an impact on the diversity of arthropods and their microbiota. Particularly the introduction of Velvet Grass appears to have enabled the establishment of invasive *Aphididae* that are outcompeting other native arthropods. The gut microbiomes of the arthropods differed in both alpha and beta diversity. Both plant species/family and arthropod species/family had significant impacts on the beta diversity of gut microbiota. However, interestingly, the greatest dissimilarity was caused by plant species, which differs from the results of similar studies.

Plant-Arthropod Relationship

In my study, the invasive velvet grass appears to be permitting the establishment of the invasive *aphididae, Sitobion fragariae*. The low alpha diversity of *Sitobion fragariae* would also suggest that this species is a specialist. *Sitobion fragariae* are a species native to Europe, first recorded in the United States under that name in 1966 (Hales et al. 2010 and Lambers, 1966). The specialist nature of this species suggests that the *Aphididae* match the invasion syndrome hypothesis that it is a species whose phenological niche is separated from the native species and can thus establish itself (Perkins et al. 2013). Further, this would suggest that velvet grass itself is supporting the establishment of this invasive species. While *Aphididae* are not exclusive in their ability to inhabit Velvet Grass, they dominate it, and *Cicadellidae* inhabits only a few Velvet Grass. A study on *Acatia longifolia's* effects on insect biodiversity found that it led to a reduction in richness, abundance, and biomass of insects (Núñez et al. 2017). This effect cascaded and affected the parasitoids of those insects, and even appeared to affect the herbivores that were generalists (Núñez et al. 2017). These effects are similar to those found as a result of Velvet Grass. Similarly to *Aphididae*, *Graphocephala atropunctata* and *Diophorina lycii* were

all found on the native Coyote Brush, indicating that they are native specialists. The only generalist identified was the species *Emposcae fabae*, which was found on all plants, invasive and native. Though *Emposcae fabae* also appears to have been outcompeted by *Aphididae* on velvet grass.

Relative Gut Microbiota Diversity of Arthropods

I found the lowest ANOSIM statistic R for arthropod families and species, which suggests that both plant and arthropod species and family contributed to Bray-Curtis dissimilarity. The ANOSIM statistic R value for plant invasiveness was the highest, followed by plant species, then arthropod species, then family. These results suggest that the gut microbiota, when compared for plant invasiveness status are the most dissimilar, and when grouped by arthropod species and family, microbiota had the least dissimilarity. These results are somewhat consistent with results of other studies finding for aphids, that arthropod species was an important contributor to microbial composition, but plant species was not (Qin et al. 2021). Similar results for the importance of aphid species for symbiotic microbiota were found by McLean et al (2019). Among aphids it was found by McLean et al. that the communities of different species of aphids that were feeding on the same host plant were not more similar than those of aphids feeding on different host plants (McLean et al. 2019). This is different from my results, which found plant species to be a significant contributor to the beta diversity of arthropod's gut microbiota. The difference between these results and mine could occur as a result of the fact that Qin et al. and McLean et al. were studying only aphid species, which may have had less specialization among those species.

Overall, my research found that the more specialized arthropods had less alpha and beta diversity than generalists, as well as that the plant and arthropod species had an effect on insect gut microbiota. Some Hemiptera were highly specialized, only feeding on one plant species, and usually having very similar gut microbiota. On the other hand, some Hemiptera were very generalist and had very different microbiota while feeding on many different plant species. While the difference was not statistically significant for whether arthropod species, arthropod family, plant species, or plant family caused the most dissimilarity, it is interesting that plant species was significant.

Limitations and Future Directions

This experiment was limited by the number of species collected, and the accuracy of some of the samples. There were some species of hemiptera that only had a few species collected, such as *Graphocephala atropunctata, Momoria rufoscutellata, and Acyrthosipon pisum*. It could have been interesting to have more samples of these species to determine a more complete picture of their alpha and beta diversity. This experiment also experienced hurdles as a result of the poor sequence matches for many of the Cicadellidae family results. The low pidents of many of these results led to inaccurate species determination and thus, the species dataset was incomplete.

A potential future direction is to compare the microbiomes of invasive species in Point Reyes with their microbiomes in their natural habitats. In Rothman et al.'s (2021) study, invasive wasps had different microbiomes based on geographic locations, indicating that the environment had an effect on their microbiota. Other studies have found that Hemiptera tend to inherit their microbiota (Baumann, 2005). However, it would be interesting to test whether this is the case for *Emoscae fabae*.

Conclusion

In conclusion, the gut microbiota of arthropods should be further studied as an understanding of the effects of certain gut microbiota could help control their effects on ecosystems that they invade. It could also potentially open further understanding of what is causing the ongoing insect apocalypse. My study showed that invasive species of plants cause invasive arthropods to move in and feed on them, and potentially outcompete native arthropods on the invasive plants. Many arthropods, Hemiptera in particular, have close relationships with their gut microbiota, and any change in the environment or their bacteria could have cascading effects across the ecosystem. These systems are complicated, but the more that these bacteria, arthropod, and plant species are studied, the better our understanding of their relationships.

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