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ECOSPHERE

Exploring variation in phyllosphere microbial communities across four hemlock species

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Citation: Rogers, T. J., C. Leppanen, V. Brown, J. A. Fordyce, A. LeBude, T. Ranney, D. Simberloff, and M. A. Cregger. 2018. Exploring variation in phyllosphere microbial communities across four hemlock species. Ecosphere 9(12):e02524. 10.1002/ecs2.2524

Abstract. The hemlock woolly adelgid (HWA) is an insect native to Asia and likely western North America. First reported in eastern North America in 1951, it has devastated eastern hemlock (*Tsuga canadensis*) populations. Loss of hemlock will greatly affect the structure and function of eastern forests. Susceptibility to adelgid infestation varies within eastern hemlocks and across other hemlock species. Our study was conducted to determine whether eastern hemlocks share a similar stem (phyllosphere) microbial community with other co-occurring hemlocks and whether community-level shifts are associated within trees of the same species based on HWA infestation. Surprisingly, we found no difference in microbial community composition or diversity between trees of the same species based on the level of HWA infestation. However, microbial communities varied significantly across the four hemlock trees sampled, native *T. canadensis* and three non-natives: *Tsuga chinensis*, *Tsuga dumosa*, and *Tsuga sieboldii*. Within these tree hosts, microbial communities from *T. dumosa* and *T. chinensis* clustered together, and microbial communities from *T. canadensis* and *T. sieboldii* clustered separately from all other tree species. Additionally, specific indicator taxa were identified for all the tree species sampled. These results indicate that Asian hemlocks might not fill the same niche in eastern forests as the native eastern hemlock. Further work should be conducted to determine how differences in hemlock species and associated microbial communities might scale up to alter organismal interactions involving hemlocks.

Key words: hemlock; hemlock woolly adelgid; microbial community; next-generation sequencing; phyllosphere.

Received 22 May 2018; revised 29 October 2018; accepted 31 October 2018. Corresponding Editor: Charles E. Mitchell.
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INTRODUCTION

The eastern hemlock (*Tsuga canadensis*, Family: Pinaceae) is a critical foundation species within temperate forests in eastern North America (Ellison et al. 2005), providing shade and shelter for many bird, mammal, and arthropod species (Yamasaki et al. 2000, Coots et al. 2012*a*, *b*). Hemlock forests are currently threatened by an invasive insect, the hemlock woolly adelgid, *Adelges tsugae* (Hemiptera: Adelgidae; HWA). Hemlock woolly adelgid is an insect native to Asia and likely western North America (Havill et al. 2006, 2016), introduced to Virginia in the early 20th century with the introduction of ornamental Japanese hemlocks (Havill and Montgomery 2008). Hemlock woolly adelgid does not appear to be detrimental in its native range (McClure 1987). However, after an extended lag, it has spread from Virginia across eastern North America killing large swathes of eastern and Carolina (*T. caroliniana*) hemlocks. Without an adequate

management strategy or a suitable replacement tree species, the loss of these trees could have cascading impacts throughout temperate forests.

Resistance to HWA is apparent in some western North American and Asian hemlocks (Del Tredici and Kitajima 2004, Pontius et al. 2006, Montgomery et al. 2009, Oten et al. 2012, McKenzie et al. 2014), so at the landscape level, replacement of native eastern hemlocks with adelgid-resistant congeners and hybrids is being considered (Evans 2008, Del Tredici 2010, Dampier et al. 2015). To date, attempts to produce replacement hybrids of *T. canadensis* and Asian species have been unsuccessful (Bentz et al. 2008). The most promising candidates are *Tsuga chinensis* and its hybrids with *T. caroliniana* and *Tsuga sieboldii* because they are the most resistant to HWA (Montgomery et al. 2009, Weston and Harper 2009). However, it is unclear whether these hybrid tree replacements would fill a similar ecological niche as a foundation species given the broad range of aboveground interactions between *T. canadensis* and other species in the community.

Hemlocks fill a foundational role in temperate forests because they associate with many avian, mammalian (DeGraaf et al. 1991, DeGraaf and Yamasaki 2001), and arthropod species (Coots et al. 2012*a*, *b*). Differences in arthropod community structure have been linked to differences in plant genetics (Dungey et al. 2000, Johnson and Agrawal 2005, Wimp et al. 2005, Bailey et al. 2006, Shuster et al. 2006). While these aboveground macroorganismal interactions are better characterized, it is unknown what microorganisms interact with these trees aboveground or whether differences in these microbial associations might affect aboveground food web interactions. Hemlock forests provide unique environments for above- and belowground microorganisms owing to their litter chemistry, ability to acidify the soil, and association with belowground mycorrhizal fungi (Jenkins et al. 1999). To date, little work has been conducted examining hemlock–microbe interactions in eastern forests and the implication for host function (Stadler et al. 2005).

Studies examining microbial communities associated with plant leaves and stems—the phyllosphere—suggest that robust microbial communities might help plants resist pathogens and herbivory (Clay 1987, Carroll 1988, Breen 1994, Lindow and Brandl 2003). Communities of epiphytic (surface) and endophytic (interior) microorganisms inhabiting the phyllosphere affect population growth of a number of pathogens (Lindow and Brandl 2003), insect herbivores, and other pests (Berg et al. 2005, Innerebner et al. 2011, Mejia et al. 2014). Such resistance can be conferred by either direct endophytic actions (Herre et al. 2007, Mejia et al. 2008) or indirect actions, for example, by inducing host gene expression that enhances disease resistance (Mejia et al. 2014).

While temperate tree species typically share a core microbiome (Laforest-Lapointe et al. 2016*a*), phyllosphere bacterial associations share significant evolutionary history with their host tree (Kembel et al. 2014, Laforest-Lapointe et al. 2016*a*). In fact, host species identity is often a stronger driver of variation than individual identity (Lambais et al. 2014, Laforest-Lapointe et al. 2016*a*, *b*). Additionally, in many species inter-host species variability of the microbiome exceeds intra-host species variability (Redford et al. 2010, Laforest-Lapointe et al. 2016*b*). Interestingly, there is sometimes little differentiation in bacterial communities of plant hosts belonging to the same species even when separated by thousands of kilometers (Redford et al. 2010).

Given the foundational role of eastern hemlocks in these forests, it is important to determine how HWA alters these microbial associations, and whether different hemlocks support the same microbial communities and interactions before any management practice is implemented. Therefore, our research took advantage of next-generation sequencing to characterize the archaea, bacteria, and fungi inhabiting the hemlock phyllosphere. This work was focused to understand (1) whether microbial community composition varied due to HWA infestation within a tree species and (2) whether phyllosphere microbial communities varied across four hemlock species, native *T. canadensis* and three non-natives: *T. chinensis*, *Tsuga dumosa*, and *T. sieboldii*.

Methods

Site description and sample collection

Samples of hemlock stems for microbial analysis were collected from trees selected from a

0.15-ha plot at North Carolina State University's Mountain Horticultural Crops Research and Extension Center, in Mills River, North Carolina (plot center latitude: 35.420468, longitude: −82.556092, altitude: 643 m) where a variety of hemlock species were planted in 2008 within a mixed-use forested and agricultural (e.g., hops, corn, soybean, mixed ornamentals) landscape.

To understand whether HWA infestation altered stem microbial communities, samples were collected from *T. canadensis* and *T. sieboldii*. These two hemlock species were chosen because they have variable levels of HWA infestation across individual trees. Uninfested branches were collected from three *T. canadensis* and three *T. sieboldii* with low levels of HWA infestation and three *T. canadensis* and three *T. sieboldii* with high levels of HWA infestation. To examine phyllosphere microbial variation across hemlock species, additional samples were collected from uninfested branches from three *T. dumosa* and three *T. chinensis*. *Tsuga dumosa* and *T. chinensis* lack or have low levels of HWA infestation across all of the individuals at the site. Uninfested branches were chosen to maintain sampling consistency and prevent confounding results with microbial communities associated with HWA. Trees were characterized as having no or minor infestations when HWA were absent or when fewer than 10 HWA ovisacs were detected in 10-min censuses of the entire tree, while trees were characterized as having substantial infestations when HWA were detected on >16 of 20 surveyed branches, five in each cardinal direction from approximately 0.1, 0.5, 1.0, 1.5, and 2.0 m from the ground. All *T. chinensis* and *T. dumosa* lacked sustained HWA infestations, although HWA were found on these two species likely owing to dispersal by birds and overlapping of branches with infested trees. Evidence of historical HWA infestation and close proximity of trees indicates all trees have been exposed to HWA for several years.

From each tree sampled, three uninfested 10-cm terminal branches were collected and combined from northeast, south–southeast, and west–northwest facing foliage at 1.5 m in height. Samples were collected in February of 2017 and frozen at −80°C until sample processing, and DNA extractions were performed in May 2017. To eliminate variability in seasonal phenology that might influence microbial colonization differently in different hemlock species, samples were extracted only from the previous year's growth.

Sample processing and DNA extraction

Because HWA infestation is localized to stems, this tissue was the focal point for microbial community analyses. Needles were therefore removed from samples, and small stems were cut to 1 mm for subsequent DNA extraction. Samples were not washed or sterilized; thus, both the epiphytic and endophytic microbial communities were characterized together. To homogenize the samples and disrupt the plant cell walls, we flash-froze stems in liquid nitrogen, and we homogenized them by bead-beating for 1 min with a small steel bead (diameter = 6 mm). Next, we flash-froze them again for one minute, so samples would not thaw during this homogenization process. This process was repeated three times to ensure sufficient homogenization. DNA was extracted from the homogenized tissue with the MO BIO PowerPlant Pro DNA Isolation kit (Qiagen, Germantown, Maryland, USA) with slight procedural modifications to ensure high-quality, high-concentration DNA yields. These modifications consisted of homogenizing in a Precellys 24 (OMNI International, Kennesaw, Georgia, USA) at 3200 g for three minutes at 30-s intervals of pulse and rest. DNA was eluted in 50 μL of buffer. DNA concentration and quality were measured on a NanoDrop 2000 spectrophotometer (Fisher Scientific, Waltham, Massachusetts, USA).

Sequencing

Extracted DNA was taken to the Genomics Core at the University of Tennessee, Knoxville, Tennessee, USA, for library preparation and sequencing on an Illumina MiSeq (San Diego, California, USA). Libraries were prepped for the 16S rRNA gene by means of a two-step PCR approach with a mixture of custom 515F and 806R primers (Cregger et al. 2018) to characterize archaeal/bacterial communities, and for the ITS2 gene region with a custom mixture of primers to characterize the fungal community present on the stems (Cregger et al. 2018). Custom primer mixes allowed us to detect microbial groups often missed with universal primers across archaea, bacteria, and fungi. Specifically, primers were added to allow better detection of Archaea, Chloroflexi, TM7, Chytridiomycota, Sebacinales,

Glomeromycota, Sordariales, Stramenopila, and Archaearhizomycetes. Primer mixtures, sequences, and appropriate references can be found in Cregger et al. (2018). An adapter sequence was added to each forward and reverse primer to make them compatible with Nextera XT indexes (Illumina). The initial polymerase chain reaction (PCR) consisted of 2× KAPA HiFi HotStart ReadyMix Taq (Roche, Indianapolis, Indiana, USA), 10 μmol/L total for each forward primer combination, and 10 μmol/L total for each reverse primer combination, with approximately 50 ng DNA. 16S rRNA and ITS2 PCRs were performed separately. Both reactions consisted of 3 min at 95°C, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. Successful PCR amplification was confirmed by running 4 μL of PCR product on a 2% agarose gel. The PCR product was then purified by use of AMPure XP beads (Agencourt, Beverly, Massachusetts, USA). Nextera XT indexes were then ligated to the PCR products by use of a second, reduced cycle PCR, such that each sample had a unique combination of forward and reverse indexes. This reduced reaction consisted of three minutes at 95°C, followed by eight cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for five minutes. The products were purified again using AMPure XP beads. Samples were quantified on a NanoDrop spectrophotometer (Fisher Scientific) and pooled into an archaeal/bacterial pool and a fungal pool to approximately equal concentrations within each pool. Final product size and concentration were confirmed on a standard sensitivity Bioanalyzer (Agilent, Santa Clara, California, USA). Both bacterial and fungal samples were diluted to 4 μmol/L, independently combined with 5% of a 4 μmol/L PhiX adapter-ligated library control, and run paired-end on a v2, 500 cycle flow cell of an Illumina MiSeq sequencer.

Data processing and analysis

Raw sequence data were processed with a combined USEARCH/QIIME processing pipeline (Data S1, Metadata S1). Briefly, reads were joined and quality-filtered ($Q > 20$); primer sequences were discarded by use of cutadapt (Martin 2011); chimeric sequences were detected and filtered; and taxonomy was assigned by use of the BLAST algorithm and the GreenGenes database (DeSantis et al. 2006) for archaea/bacteria and the UNITE database for fungi (Kõljalg et al. 2005). Operational taxonomic units (OTUs) were clustered at 97% similarity for both archaea/bacteria and fungi, and OTU tables were generated. Operational taxonomic units present fewer than five times across all samples were discarded. Contaminant plant, chloroplast, and mitochondrial sequences were removed. Prior to any analyses, archaeal/bacterial data were rarefied to 4500 sequences and fungal data were rarefied to 92,000 sequences to control for variation in sequencing depth across samples while sample loss was minimized. After rarefaction, we detected zero archaeal OTUs, 467 total bacterial OTUs, and 1966 total fungal OTUs. The sequence datasets generated during the current study are available at NCBI Sequence Read Archive BioProject ID: PRJNA453158.

Differences in archaeal/bacterial and fungal community composition among tree species were analyzed by means of a distance-based redundancy analysis (db-RDA) on a quantitative Jaccard (Ružička) dissimilarity matrix. Statistical significance was determined based on 99,999 permutations of the data in the R package vegan 2.4-4 (Oksanen 2015). Richness and evenness of archaeal/bacterial and fungal communities were compared by means of Hill numbers (Jost 2006) at orders of $q = 0$, 1, and 2. The parameter q determines the relative weighting of rare species. At $q = 0$, all species are weighted equally (richness); at $q = 1$, species are weighted proportionally to their relative abundance (analogous to Shannon's index); and at $q = 2$, rare species are down-weighted (analogous to Simpson's index). Hill numbers were calculated with the R package vegetarian 1.2 (Charney and Record 2009). We compared α-diversities across tree species with a two-way ANOVA.

Additionally, we tested whether the relative abundance of archaeal/bacterial and fungal phyla present at ≥0.1% relative abundance differed across tree species and between trees with no/minor and substantial HWA infestations using two-way random-effects ANOVA models with tree species and HWA infestation as the random effects (JMP 13; SAS Institute, Cary, North Carolina, USA). This analysis helped us identify whether there were large shifts in microbial communities across tree species and with trees with

varying levels of HWA, or whether most of the changes were at lower taxonomic levels.

Indicator species analysis (De Cáceres and Legendre 2009, De Cáceres et al. 2010) was used to identify microbial OTUs with a high affinity for each tree species. The group-equalized correlation coefficients, r_g, for each tree species were estimated as defined by De Cáceres and Legendre (2009). This metric equalizes the relative abundances of all taxa to allow comparisons between taxa with varying relative abundance levels. Statistical significance was determined from 9999 permutations. Parameter estimates and permutations were conducted in the R package indicspecies 1.7.6 (De Cáceres et al. 2016). We then calculated the relative abundance of these OTUs in relation to each tree.

Results

We were unable to detect any differences in microbial community composition or diversity due to varied HWA infestation levels within *T. canadensis* and *T. sieboldii* ($P > 0.05$ for all α- and β-diversity measurements), but we did detect significant differences in microbial communities across the hemlock species sampled. db-RDA revealed significant differences in bacterial and fungal community composition across the four hemlock tree species sampled (Fig. 1, bacterial $F_{3,14} = 1.88$, $P < 0.01$, and fungal $F_{3,14} = 1.94$, $P < 0.01$). Pairwise comparisons showed that bacterial and fungal community composition differed across all hemlock tree species sampled except between *T. chinensis* and *T. dumosa* (Appendix S1: Table S5). Interestingly, both bacterial and fungal communities were similar on the stems of these two tree species. Surprisingly, though our primers were designed to target archaea as well as bacteria, no archaeal OTU was identified. Specific bacterial and fungal patterns are outlined below.

Bacterial community differences across hemlock tree species

Across hemlock species, 54% of the bacterial community belonged to the Proteobacteria, 21% to the Actinobacteria, 13% to the Bacteroidetes, 5% to the Acidobacteria, 2% to the Planctomycetes, and 2% to the Armatimonadetes, but there was no difference in the relative abundance of bacterial phyla across tree species sampled (Appendix S1: Table S1). The remainder of the phyla each constituted <1% of the OTUs present. Bacterial diversity was greatest on *T. chinensis* and *T. dumosa* and lowest on *T. sieboldii* at $q = 0$ (richness; Fig. 2a, $P = 0.02$). This was even more apparent at $q = 1$ (analogous to Shannon's index, Fig. 2c, $P < 0.01$) and $q = 2$ (analogous to Simpson's index $P < 0.01$). Indicator species analysis, based on r_g, identified specific bacterial OTUs

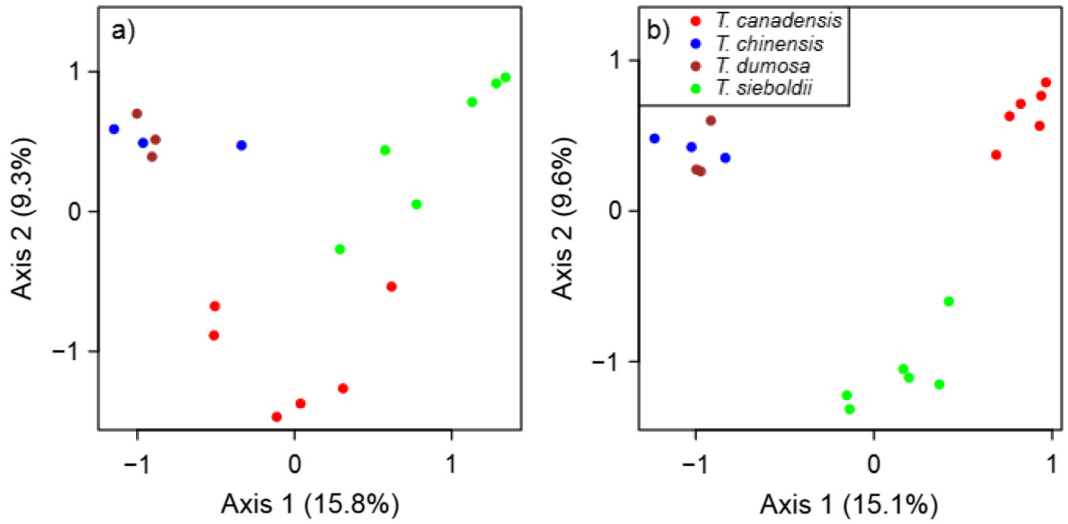


Fig. 1. Distance-based redundancy analysis plots of (a) bacterial and (b) fungal communities across four hemlock species.

(Appendix S1: Table S2) common in some hemlock species but lower in relative abundances in others. For example, *T. canadensis* supported higher relative abundances of unidentified bacterial taxa in the families Frankiaceae (3.141%, $r_g = 0.652$) and Microbacteriaceae (2.693%, $r_g = 0.674$) than did the other three hemlock species. On the contrary, we found *T. dumosa* and *T. chinensis* had higher relative abundances of the bacterial taxon *Sphingomonas wittichii* (2.563% and 1.015%, respectively) when compared to *T. canadensis* (0.281%) and *T. sieboldii* (0.237%).

Fungal community differences across hemlock tree species

Across all hemlock species sampled, approximately 85% of the fungal community belonged to the phylum Ascomycota, 7% belonged to the Basidiomycota, and approximately 7% were unidentified. The remaining phyla each accounted for <1% of the relative abundance of fungi identified. The only significant phylum-level difference across tree species involved the fungal phylum Chytridiomycota (Appendix S1: Table S3, $F_{3,14} = 11.25$, $P = 0.01$). Chytridiomycota relative abundance was highest in *T. chinensis* (relative abundance 1.91%) and *T. dumosa* (relative abundance 2.00%), while relative abundance of Chytridiomycota in *T. canadensis* was 0.10% and in *T. sieboldii* was 0.51%. Fungal diversity was similar across all trees examined at $q = 0$ (Fig. 2b, $P = 0.14$), $q = 1$ (Fig. 2d, $P = 0.18$), and $q = 2$ ($P = 0.16$). *Tsuga canadensis* had higher relative abundances of fungal taxa from an

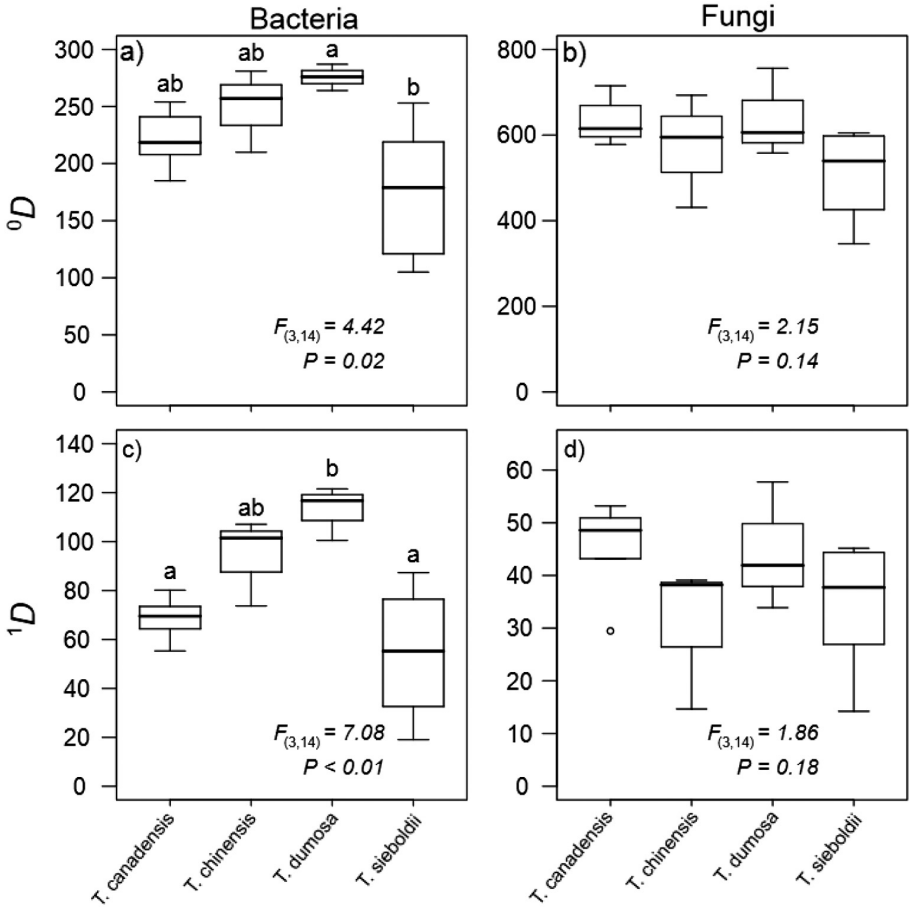


Fig. 2. Varying diversity of bacterial and fungal communities among four hemlock species across orders of $q = 0$ through $q = 1$. As q increases, the importance of rare species is down-weighted. Lower case letters denote significant differences among groups.

unidentified family of Chaetothyriales (3.631%, $r_g = 0.783$), an unidentified class of the Ascomycota (4.071%, $r_g = 0.685$), and an unidentified order of the Eurotiomycetes (1.444%, $r_g = 0.530$). *Tsuga chinensis* further supported a significantly higher relative abundance of an unidentified family of the Pleosporales (19.868%, $r_g = 0.651$) than the other three hemlock species. *Tsuga dumosa* was the only other tree species to have a relative abundance >0.1% of this Pleosporales family at 2.639%.

Discussion

Overall, our results show that microbial communities vary within the phyllosphere of four different hemlock species. This variability suggests that Asian hemlocks interact with different microorganisms than the native eastern hemlock. We hypothesize this mismatch in microorganismal associations could scale up to alter higher trophic interactions; therefore, further testing should be done to examine these aboveground interactions. Surprisingly, we were unable to detect differences in microbial community composition or diversity due to varying levels of HWA infestation within *T. canadensis* and *T. sieboldii*. This might be explained by our small sample size ($n = 3$) within each hemlock group, or by our sampling design. We targeted branches with limited adelgid infestation to maintain consistency across species and to avoid confounding our results with microbial communities associated with HWA. While these communities appear to diverge, the divergence was not statistically significant; thus, further testing is necessary to verify this pattern, for example, surveys conducted across seasons and with increased sample numbers. Interestingly, we did not detect any archaeal OTUs within our samples. Previous studies have shown low abundances of these organisms within the phyllosphere of a variety of trees (Finkel et al. 2011, Ma et al. 2013, Watanabe et al. 2016, Cregger et al. 2018).

In all hemlock species, the fungal community was dominated by two phyla Ascomycota and Basidiomycota. This is consistent with studies that characterize the plant phyllosphere (Jumpponen and Jones 2009, Vořišková and Baldrian 2013, Kembel and Mueller 2014, Perazzolli et al. 2014, Menkis et al. 2015, Wei et al. 2016). The dominant bacterial taxa were Proteobacteria (54%) and Actinobacteria (24%). Previous studies have demonstrated that Proteobacteria were the dominant group found within the phyllosphere, with α- and γ-Proteobacteria the most common groups (Whipps et al. 2008, Laforest-Lapointe et al. 2016*b*). While Actinobacteria have been found in low abundances within the tree phyllosphere (Whipps et al. 2008), our results show they are quite common in the hemlock phyllosphere. This is consistent with patterns seen in other conifer species like white spruce, *Picea glauca* (Laforest-Lapointe et al. 2016*b*). Actinobacteria are widely spread through soil systems, where they play a role in the decomposition of plant debris (Goodfellow and Williams 1983, Ventura et al. 2007). Further, *Streptomyces* is a genus of Actinobacteria that has been shown to inhibit growth of potential fungal pathogens in root systems (Goodfellow and Williams 1983); thus, Actinobacteria might be serving a similar function in the phyllosphere of these hemlocks.

Across the phyla measured, only Chytridiomycota differed between the hemlock species sampled. *Tsuga chinensis* and *T. dumosa* had approximately two times higher relative Chytridiomycota abundances than either *T. canadensis* or *T. sieboldii*. Few studies have found Chytridiomycota on conifers, but one study found a representative of this group on the needles of a single white pine (Bullington and Larkin 2015). Chytridiomycota are known to parasitize pollen grains of conifers that have fallen from the parent tree (Goldstein 1960). Chytridiomycota might arrive on this plot with windblown pollen, preferentially colonizing *T. chinensis* and *T. dumosa*. Further research is needed to determine whether Chytridiomycota influence interactions in the phyllosphere of these hemlock trees.

Within each species, we identified specific microbial OTUs with high relative abundances that served as indicators of that tree species (Appendix S1: Table S4). Replacing *T. canadensis* with another species of hemlock could thus result in alterations of bacterial and fungal associations. For example, within *T. canadensis,* the indicator OTU from an unidentified genus of the bacterial family Propionibacteriaceae had a relative abundance of 2.659%; but across the other trees sampled, this indicator OTU comprised <0.400% of the abundance. Interestingly, the

presence of Propionibacteriaceae has been documented in domestic pollen grains of Kiwifruit (Kim et al. 2018) and the phyllosphere of *Magnolia grandiflora* (Jackson and Denney 2011). BLAST characterization of the sequence revealed a 99% identity match with *Friedmanniella endophytica*, which was originally isolated from the bark of the mangrove tree, *Kandelia candel* (Tuo et al. 2016). An unidentified genus from the family Frankiaceae had a relative abundance of 3.141% in *T. canadensis* and only 0.822% in *T. chinensis*, 0.719% in *T. dumosa*, and 0.681% in *T. sieboldii*. Similar patterns were seen in 10 unidentified fungal classes of Ascomycota, an unidentified family of Chaetothyriales, *Cyphellophora sessilis*, and 33 other indicator fungal taxa within *T. canadensis*. Replacing *T. canadensis* with any of the other three hemlock species could result in significant decreases in the abundances of these OTUs. These results demonstrate that, at a microbial level, the different hemlock species examined have significantly different stem microbial communities. These differences might affect food web associations.

Eastern hemlocks associate with many aboveground arthropods, so changes in the stem microbial community might have cascading consequences. For example, 44 species of Psocoptera (Coots et al. 2012*b*), 24 species of mycetophilids (Coots et al. 2012*a*), and 47 predatory arthropod families (Hakeem 2008) have been found associated with eastern hemlock. Psocoptera graze on microepiphytes (fungi, algae, and lichens) and are eaten by predators (Thornton 1985), while mycetophilids in their larval stage use fleshy fungi (Coots et al. 2012*a*). While the significance of these associations is not well understood, these groups constitute an essential component of the food web within the eastern hemlock system. An understanding of these associations is vital for assessing conservation-based management strategies that involve replacing eastern hemlock with another species (Coots et al. 2012*a*, *b*).

While our results do not indicate an association between HWA infestations and aboveground microbial community structure, we did find that microbial communities vary significantly across hemlock species. Occupying approximately 4×10^8 km^2, the phyllosphere is one of the largest biological surfaces on earth (Lindow and Leveau 2002); thus, associations with the phyllosphere likely contribute significantly to ecosystem interactions. It is unclear how alterations in aboveground communities scale to alter ecosystem processes in these eastern forests. Future studies are needed to address these complex interactions across the range of eastern hemlock. Understanding of these interactions could aid in mitigation strategies for maintaining and restoring these important ecosystems.

Acknowledgments

This research was supported by the Laboratory Directed Research and Development Program of Oak Ridge National Laboratory, managed by UT-Battelle, LLC, for the U.S. Department of Energy through a Liane Russell Fellowship awarded to M.A. Cregger, the Eppley Foundation for Research, the endowment of the Nancy Gore Hunger Professorship in Environmental Studies at the University of Tennessee, and the National Science Foundation (DEB 1638922) to J.A. Fordyce. This research was also sponsored by the Genomic Science Program, U.S. Department of Energy, Office of Science, Biological and Environmental Research, as part of the Plant Microbe Interfaces Scientific Focus Area at ORNL (http://pmi.ornl.gov). Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DEAC05-00OR22725. This manuscript has been authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The US Government retains, and the publisher, by accepting the article for publication, acknowledges that the US Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for US Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

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