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Soil Arthropod Biodiversity: An Investigation in Eastern Hemlocks Following Chemical Treatment

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Abstract

The hemlock woolly adelgid (*Adelges tsugae*) (HWA) is an invasive species that poses a significant threat to eastern hemlocks (*Tsuga canadensis*) and is the main contributor to the declining eastern hemlock populations. The HWA infestations have prompted the use of chemical neonicotinoid pesticide treatments across the eastern US. Previous research has demonstrated that the environmental consequences of these chemicals are a concern for many surrounding animal populations such as birds and invertebrates. This study investigated the impact of dinotefuran and imidacloprid (neonicotinoids) treatments on soil arthropod communities in Sandy Mush Game Land (SMGL), Western North Carolina. For this experiment, we examine the impacts of different combinations of the type of chemical control (imidacloprid, dinotefuran and imidacloprid), and time after application (no treatment, 5 years, and less than a year). Leaf litter samples were collected from five different plots, processed in the laboratory using Berlese Funnels, and observed organisms were classified by order. Based on the results, no significant differences in arthropod biodiversity were observed between plots in this experiment. Differences in abundance varied across groups and could suggest both direct and indirect implications of neonicotinoid use immediately after treatment. These findings suggest that the use of neonicotinoids does not have long term implications for arthropod diversity and abundance and that chemical treatment should proceed to avoid ecological implications of the loss of hemlock stands. Further research should take place with a more extensive experimental area and period of time to continue to investigate direct and indirect impacts of neonicotinoids on the soil organism biodiversity.

Introduction

Eastern hemlocks (*Tsuga canadensis*) are foundational species ranging from Southern Canada to the Southern Appalachians (Ellison, et al. 2005). Their dense, year-round foliage provides deep shade and creates unique microclimates that are integral to the biodiversity of these systems (Ellison, et al. 2005). Many native and endangered species rely on these microclimates as well as the slowly decomposing biomatter that this shade provides.

This plant species uses and stores 50% less water than other deciduous counterparts (Ellison, et al. 2005), enabling them to mediate soil moisture, stabilize stream-base flow, and regulate stream temperatures (Limbu, et al. 2018). This supports a unique assortment of salamanders, freshwater invertebrates, and fish species which are intolerant to seasonal drying (Ellison, et al. 2005). The decline of eastern hemlock stands leads to the loss of the unique microclimates provided by their dense foliage. Increased solar radiation reaching the ground and increased wind effects will cause the drying of leaf litter and decreased moisture content, which is detrimental to the survival of salamander species that rely on these microhabitats (Letheren et al. 2017). These microclimates are also necessary for almost 90 bird species, with two hemlock obligate species, the black-throated green warbler (*Dendroica virens*) and the blue-headed vireo (*Vireo solitarius*) that only live in hemlock habitat (Letheren et al. 2017). Hemlock stands are closely tied to the resilience of biodiversity from New England to the Southern Appalachians.

The millions of hectares of integral eastern hemlock-dominated forests as well as the unique ecosystems they maintain are being threatened by the introduction of the invasive insect pest hemlock woolly adelgid (HWA) (McDonald et al. 2023). HWA (*Adelges tsugae*) is an invasive insect that is native to Japan and was first found in the Eastern United States in 1951, believed to have been introduced through infested seedlings (Havill et al. 2014). It feeds on the stored nutrients in hemlock plants with a stylet that pierces through the base of needles and into the parenchyma cells (Havill et al. 2014). Evidence suggests that the HWA causes a hypertensive effect that compromises water transport in the trees, and the infestations have decimated eastern hemlock populations in the United States (Havill et al. 2014). HWAs are dispersed through wind, birds, and mammals through the transportation of infested hemlock material. Airborne HWA eggs and adelgids have been observed as far as 600 m from the nearest infestations, HWA's were also observed in over 80% of birds captured near infested stands (Limbu et al. 2018). By 2008 infestations had spread into about 40,000 square kilometers of forest and are estimated to spread approximately 15 km per year (Limbu et al. 2018). The impacts of HWA infestations have implications for the surrounding ecosystem due to the extensive loss of hemlock stands. In the Southern Appalachians, northern red oaks (*Quercus rubra*) are a common substitute for these declining stands which fail to perform the same services that hemlocks do such as consuming twice the amount of water, increasing summer water use, decreasing aquatic habitat, and reduce stream flow (Letheren et al. 2017, Birt et al. 2014).

The looming ecological consequences of the extensive decline in eastern hemlock stands have prompted concern. Integrated Pest Management (IPM) has been found to be the most effective strategy for managing HWA populations and preventing further eastern hemlock decline. IPM includes the use of biological control, chemical control, and silvicultural strategies to improve the survivorship of infested stands (Vose et al. 2013). Western and Chinese hemlocks experience HWA predation as a minor pest problem because of a combination of coevolved HWA predators and genetic resistance (Bentz et al. 2007). Biological control has been pursued since the 1990s by releasing predatory insects such as species of lady beetles (*Coccinellidae*), HWA-specific *Laricobius* beetles, and silver flies (*Chamaemyiidae*) from regions of Asia and the Pacific Northwest (Mayfield, et al. 2023). These insects have coevolved with HWA in their relative ranges and are specialized to prey on HWA. Mayfield et al. 2023 report that despite repeated efforts to release multiple species of predatory insects, biological control agents have yielded poor results as populations have struggled to establish. However, according to Mayfield et al. 2023, the *Laricobius nigrinus* beetle has been the most effective biocontrol agent, demonstrating successful establishment and predation of HWA, including in Western North Carolina, though it has not yet been effective enough to reduce mortality. Silvicultural strategies utilize thinning to create more forest gaps, increase canopy light exposure, and improve the survivorship of infested eastern hemlocks (Fajvan et al. 2023).

Today the most effective control method against HWA is chemical control using neonicotinoid pesticides as the primary means of treating infested stands. Current treatment of hemlocks for HWA heavily relies on neonicotinoid pesticides such as imidacloprid and dinotefuran. These pesticides can be applied as a drench or injection in the soil, around the base of the hemlock tree, or by spraying the trees themselves. Benton et al. 2016 reports that these treatments provide lasting, systemic protection from HWA: imidacloprid with 5-7 years of protection and dinotefuran treatments with 1-2 years of protection. Results demonstrate the efficacy of imidacloprid to significantly decrease HWA populations for at least up to three years following treatment (Kung, et al. 2015).

The neonicotinoids are a broad-spectrum pesticide with high toxicity to most arthropods and sublethal impacts on other animals (Goulson. 2013). Additionally, neonicotinoids have been shown to persist and accumulate in soils and are prone to leaching into waterways as they are water-soluble (Goulson. 2013). These chemicals change community structures. Studies have shown that runoff of these chemicals has a negative impact on aquatic and wetland species. Sweeney et al (2020) reported that imidacloprid runoff has sublethal effects on larval wood frogs by slowing reaction time and increasing predation. Impacts on avian species have been observed as well, showing annual rates of decreased biodiversity of up to 5% in bird species in areas treated with neonicotinoids compared to those not treated (Li, et al. 2020).

Soil arthropods are an integral part of soil processes and function as a tool for indicating soil quality (Menta et al. 2020). Soil arthropods represent some of the most important components of soil health by maintaining soil processes such as organic matter translocation, decomposition, nutrient cycling, soil structure formation, and water regulation (Menta et al. 2020). Additionally, since these communities live in the leaf litter, they are directly exposed to neonicotinoids when administered through soil drench or injection. The soil arthropod community is indispensable to soil and other ecological processes. Understanding the impact of neonicotinoids on soil microarthropod communities is critical to understanding the impacts on soil processes and therefore its wider impact on the surrounding ecology.

Previous literature has found conflicting results regarding the impacts of neonicotinoids specifically on soil arthropod biodiversity. Some find negative correlations in soil biodiversity in response to neonicotinoids, while some don't indicate significant changes (Knoepp et al. 2012, Teksum 2021, Peck 2009, Reynolds 2008). Additionally, many of these results vary with soil type, other local environmental characteristics, weather variation, and order studied (Hayasaka et al. 2012). The goal of this project is to investigate the impact of neonicotinoid pesticide use on soil arthropod biodiversity in hemlock stands. This study aims to compare diversity within differing types of neonicotinoid treatments (imidacloprid, imidacloprid and dinotefuran) against each other as well as analyze how these impacts change as time passes following treatment.

2. Methodology

2.1 Site Descriptions

Field collections were conducted in Sandy Mush Game Lands (SMGL), located in Buncombe and Madison counties. SMGL consists of 2,765 acres owned by the state of North Carolina and managed by the North Carolina Wildlife Resources Commission. Forest in SMGL is dominated by oak forests, covering 71% of the game land (North Carolina Wildlife Resources Commission). This study focused only on trees treated with soil drench and at least 3m from the edge of any body of water. Samples were collected from three sites labeled 2018, 2023, and Control. Geographic characteristics differed between the sites in topography and distribution of sample trees.

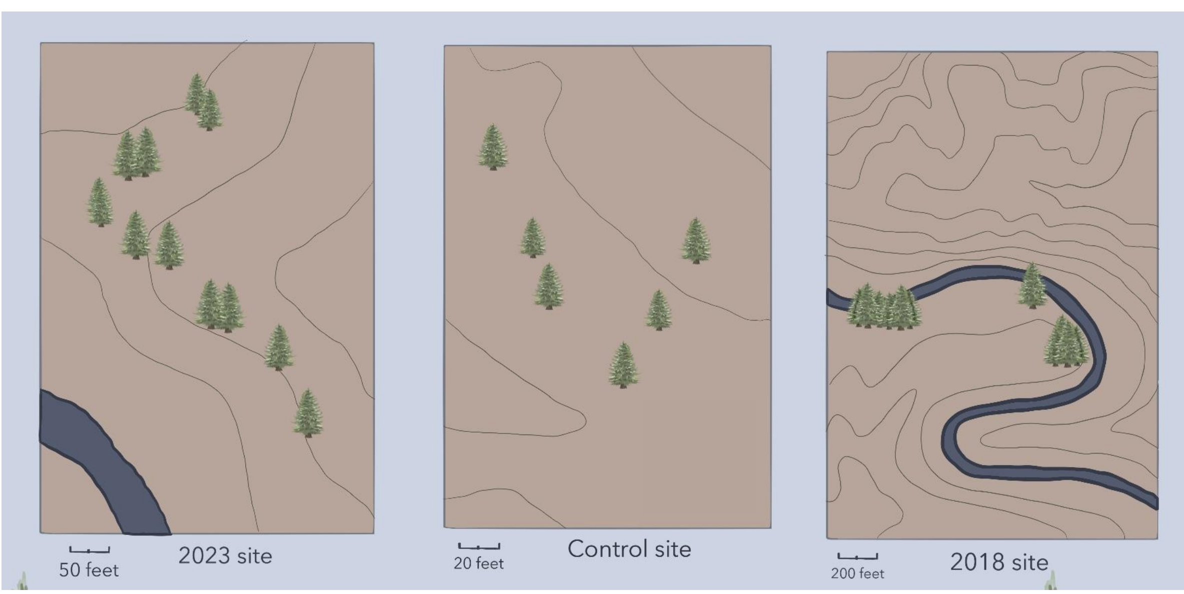

Figure 1. Maps of field sites showing tree distribution and topographic lines. Twelve trees sampled in the 2018 and 2023 plots, six per treatment (imidacloprid, imidacloprid and dinotefuran). Six trees were sampled in the control plot.

Weather, temperature, and humidity varied between collection dates. Conditions were recorded on each collection day to account for changes in temperature and weather in results.

Collection Date	Weather Conditions
May 30 2023	72℉, 4 mph wind, 81% humidity, cloudy
June 26 2023	90℉, UV index-9, 58% humidity, sunny
July 28 2023	88℉, UV index-8, 1 mph wind, 74% humidity, sunny
August 7 2023	87℉, 10mph wind speed, 60% humidity, cloudy and foggy

Table 1. Weather conditions in SMGL on each collection date.

Tree diameters were measured June 26th at breast height (1.37 m above the ground). Diameters were highly variable among individuals within plots (Table 2).

Plot	Tree Diameters (cm)
2018 ID	>60, 51, 58.5, 48.1, 43.3, 34.1
2018 I	43.5, 44.25, 39.75, 46.7, 40.5, 65
2023 ID	33, >60, 42, 46.5, 37.5, 34.5
2023 I	23.7, 42.5, 18.5, 39.5, 37.9, 45.2
Control	32.1, 32.2, 21.7, 24.4, 20.5, 23.7

Table 2. Diameter of trees for each plot. In the plot column, the number corresponds to year treated and the letters correspond to type of treatment (e.g. 2018 ID was treated in 2018 with imidacloprid and dinotefuran).

2.2 Experimental Design

Sites sampled were selected at SMGL based on the year of pesticide application, provided by Hemlock Restoration Initiative records. Within each site treated with pesticides, there were two different types of treatments administered based on observed tree health. Trees treated with imidacloprid had greater than 50% uncompacted live crown ratio (LCR) and medium to high crown density. Trees with between 30%-50% LCR or low crown density were treated with imidacloprid and dinotefuran. These two types of neonicotinoid treatments were only imidacloprid or a combination of imidacloprid and dinotefuran. Five groups were identified: one control and two treated plots (treated in 2018 and 2023), which contained two different types of treatment (imidacloprid, imidacloprid and dinotefuran). The two time frames represent the soil arthropod community 5 years post treatment and 3 months post treatment. Six trees were sampled for each plot. Samples were collected once a month over the course of summer 2023 (May-August). Samples were analyzed at the Natural Enemy Management Applications (NEMA) Lab at the University of North Carolina at Asheville.

2.3 Arthropod Collection Methods

At each tree, two replicates were conducted, one from directly next to the base of the tree and about 1 meter away from the base of the tree. The six trees for an experimental group were split into two groups of three. Leaf litter from all three trees in a group would fill two bags, one for directly next to the base of the tree and one for 1 meter away. Four leaf litter bags per an experimental group were collected in total. Samples were collected by hand into a gallon bag, with collection from one tree filling a third of a bag.

Each sample was prepared with a Berlese Funnel for future evaluation. Leaf litter samples were mixed and placed under heat lamps (40℃) in Berlese Funnels (8.5” x 8.5”) adapted by Pande and Berthet (1973) and Akoijam (2014). Samples were funneled into 25 mL of 70% ethanol. Samples were left in the funnel for 48 hours before test tubes were removed and excess litter was disposed of. Ethanol samples were then examined for microarthropods using a dissecting scope. Arthropods were categorized into groups (Arachnids, Collembola, Insecta, Mollusca, Protura, Myriapoda, Isopoda) and the number of individuals were counted. The data was recorded for statistical analyses. All samples were evaluated at the NEMA Lab at the University of North Carolina Asheville.

2.4. Analysis Methods

Arthropod diversity across plots was visualized using scatter plots with 95% confidence intervals around the mean. The Shannon and Simpson Indices were used to evaluate the diversity of each plot, using measures of abundance and evenness (Shannon 1948, Simpson 1949). Diversity among differing experimental plots was analyzed as a linear model analysis based on the Shannon and Simpson Indices. ANOVA tests were run for both the Simpson and Shannon Indexes. Diversity across collection dates were also evaluated by the Simpson and Shannon Indices and visualized with a linear model analysis. ANOVA tests were conducted for this as well. All significant results were analyzed using a Tukey HSD test to compare specific groups.

Arthropod abundance was evaluated individually for each group identified (Arachnida, Insecta, Collembola, Protura, Mollusca, Myriapoda, and Isopoda). For each group, abundance counts were compared across the plots. Bootstrap tests were performed to construct and visualize mean abundance and 95% confidence intervals in scatterplots. Permutation tests were performed to compare and assess significance of differences across plots for each group.

All analysis was conducted in R version 4.3.2 using RStudio (R Core Team). The following packages facilitated the analysis, modeling, and visualization of results: dplyr for data manipulation and summarization (Wickman et al. 2023), vegan to compute biodiversity indices (Oksanen et al. 2023), car to enhance the linear regression analysis (Fox et al. 2022), ggplot2 for plotting and visual representation (Wickman et al. 2016), and multcomp to facilitate post hoc testing (Hothorn et al. 2008).

3. Results

3.1. Soil Arthropod Diversity

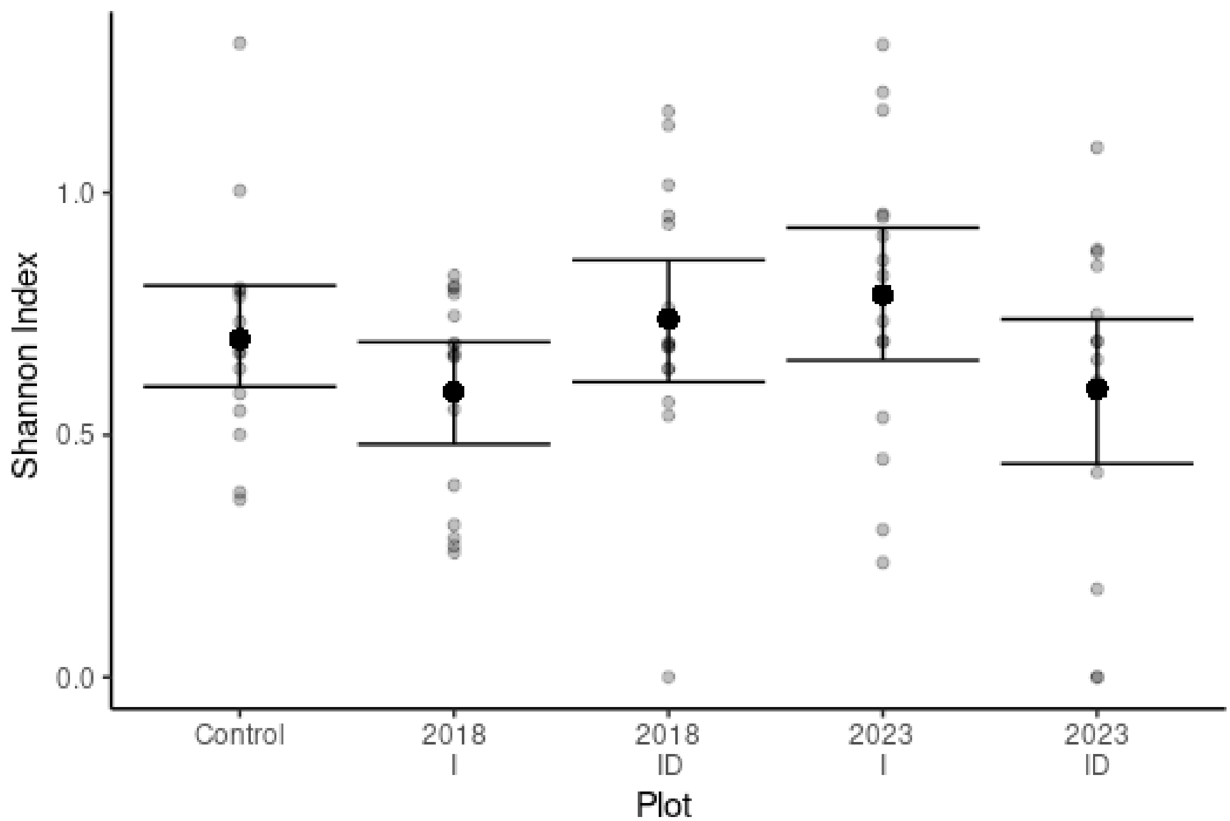

Figure 2. Shannon Index of Diversity across plots. Each plot is designated by the year of treatment application, and the compound (or compounds) with which the plot was treated. Lightly shaded points indicate raw Shannon Index values with higher values indicating higher diversity. Error bars indicate 95% confidence intervals around the mean.

All error bars demonstrating the variability of the mean overlap and point distribution are similar across plots. The highest raw values are in the control and I 2023 plots, of which are over 1.0, compared to the rest of the plots. Additionally, both the 2018 and 2023 plots treated with imidacloprid and dinotefuran contained raw values of zero. Anova tests yielded no statistical difference between any of the plots (F-statistic: 1.73 on 4 and 75 DF, p-value: 0.1523).

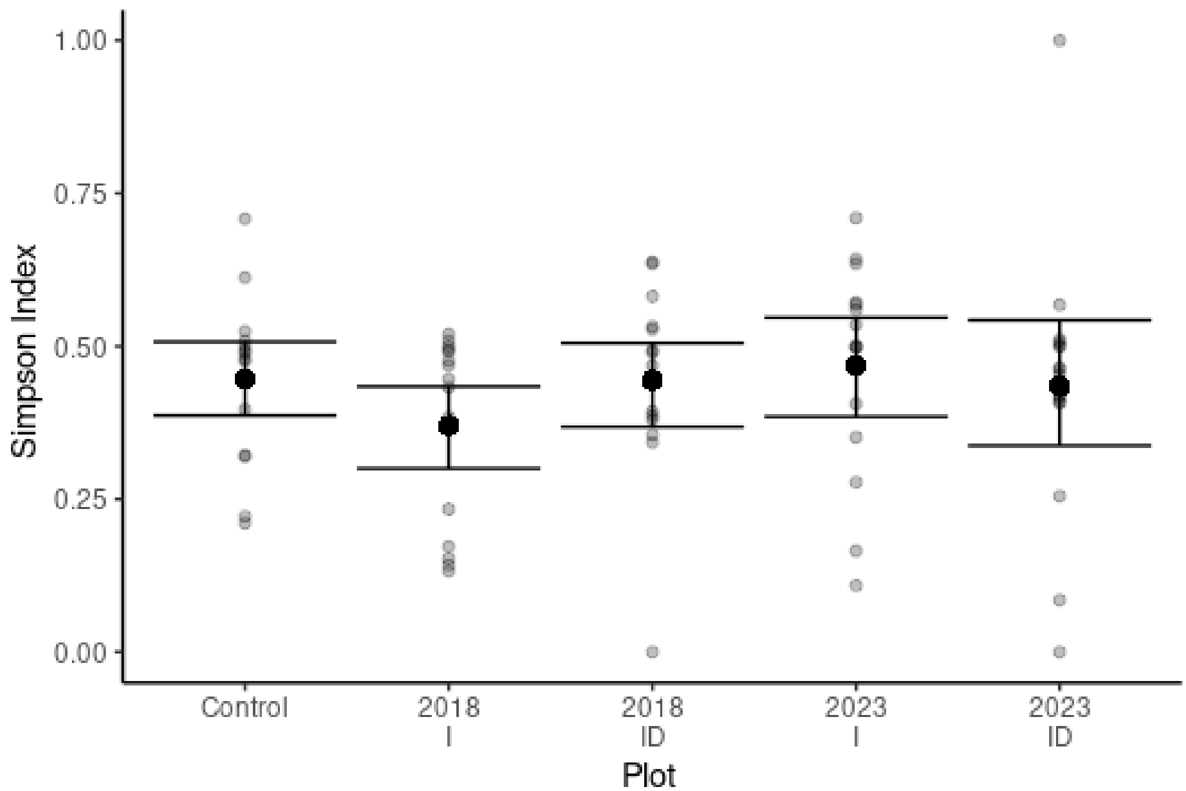

Figure 3. Simpson Index of diversity across plots. Points indicate raw Simpson Index values with lower values meaning higher diversity. The error bars indicate 95% confidence intervals around the mean.

Similar to the Shannon Index, Simpson Index mean variability and raw values are similar across all plots. There are two outliers in the 2023 ID and 2018 ID plots, with the only one containing raw values equaling 1.0. Additionally, 2023 ID and 2018 ID are the only plots containing raw values equaling 0.0. In all plots, raw values are generally centrally located. The ANOVA test yielded not statistically significant differences between different plots (F-statistic: 0.8124 on 4 and 75 DF, p-value: 0.5212).

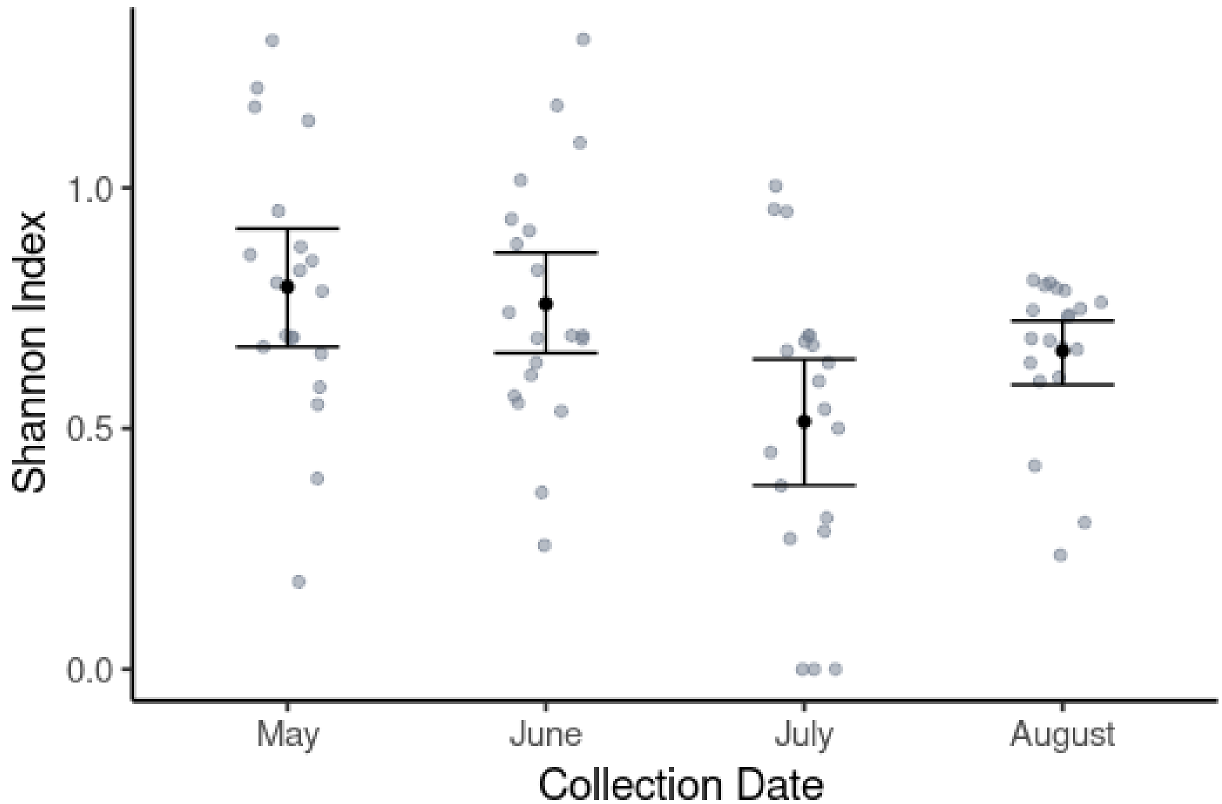

Figure 4. Shannon Index of diversity across collection dates. The gray data points indicate raw Shannon Index values and the error bars represent a 95% confidence interval surrounding the mean Shannon Index values.

The mean variability for May, June, and August are similar to each other. Julys mean variability is lower than the mean variability of the other collection days. Anova tests reported significant difference among the means of the different collection dates (F-statistic: 4.757 on 3 and 76 DF, p-value: 0.004295). The Tukey HSD test reported significant differences between Julys collection with May (p-value: 0.00497) and June (p-value: 0.01798). The difference between Julys and Augusts collection was not significant (p-value: 0.27965). There were no other statistically significant differences among the different collection dates.

3.2. Soil Arthropod Abundance

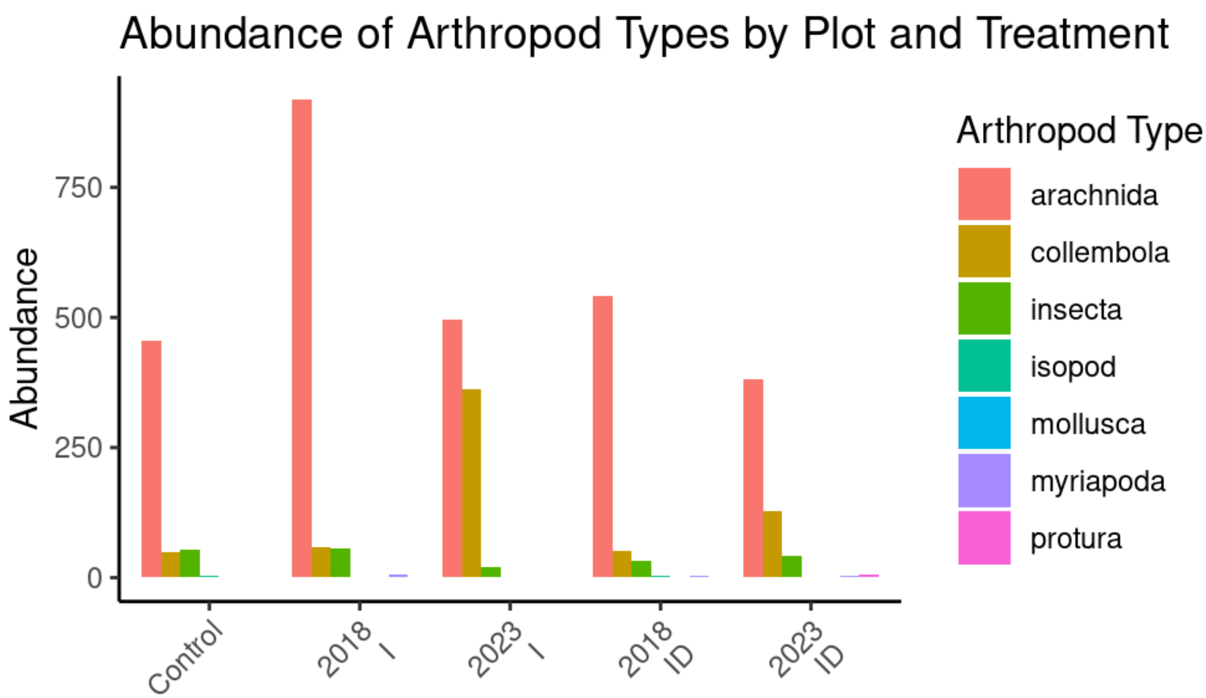

Figure 5. Arthropod abundance across plots. This figure compares the different groups evaluated between the plots. The x axis denotes treatment type with lettering and year treated by the number, and the y axis is raw counts.

The most abundant order identified was Arachnida, with it being 88.8% of our overall findings. A majority of the organisms observed in Arachnida were various mite species. Counts of all other groups were an order of magnitude smaller. The next most abundant group was collembola, constituting 6.9% of our findings. In groups Protura, Myriapoda, Mollusca, and Isopoda only as many as six individuals were counted in a replication.

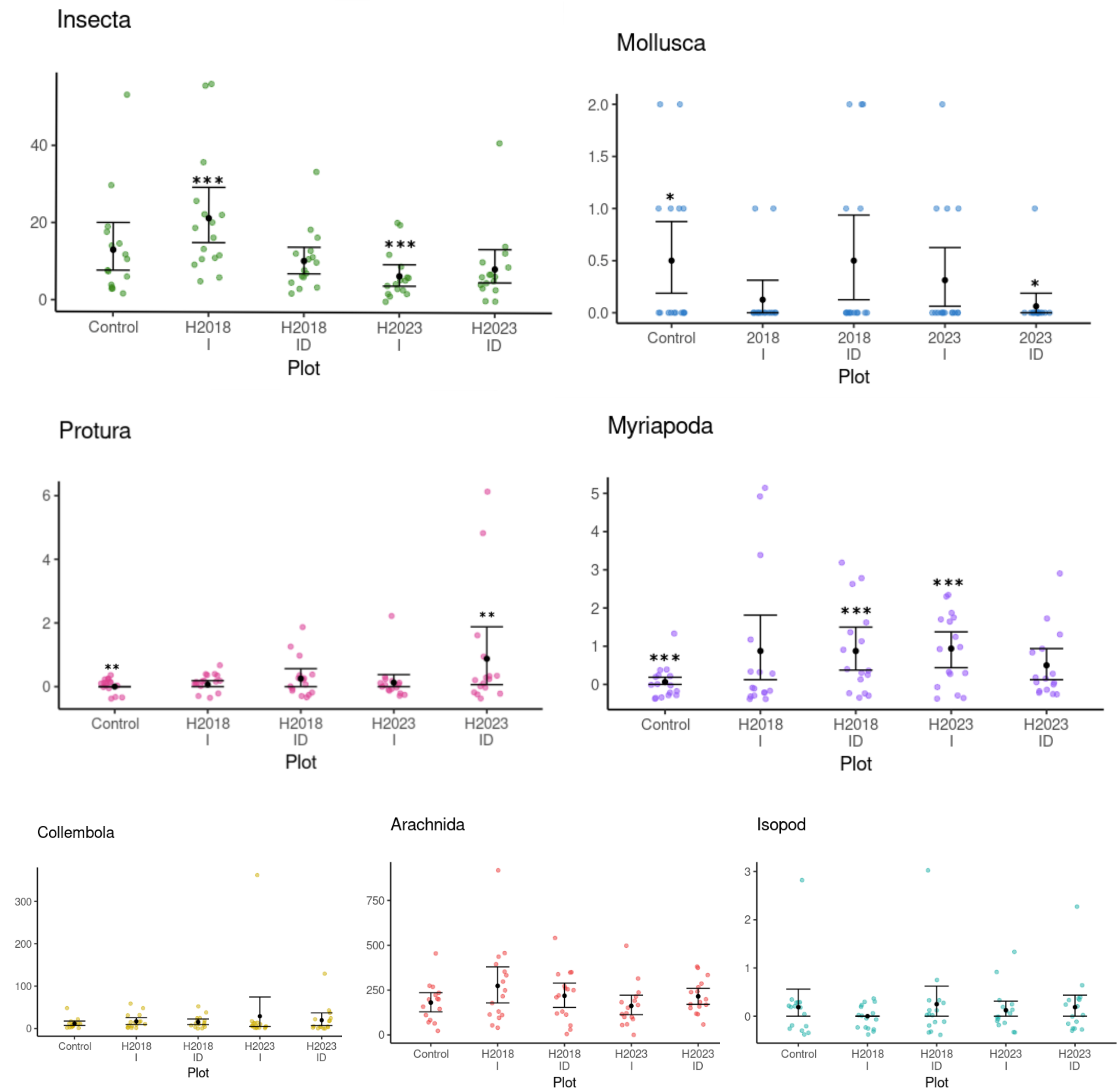

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Figure 6. Individual group arthropod abundance across plots Points indicate raw counts. Error bars represent 95% confidence intervals around the mean. Stars above confidence intervals indicate significant differences.

The mean variability of abundance of Isopoda, Collembola, and Arachnida are similar across all plots. Groups Insecta, Mollusca, Protura, and Myriapoda demonstrated differences in abundance between plots (control, 2018, 2023).

Permutation tests reported significant changes in abundance between plots in four groups identified. Lower abundance in 2023 I plots compared to 2018 I plots was reported in Insecta ($p < 0.001$). Group Mollusca showed lower abundance in the 2023 ID plot compared to the control plot ($p < 0.05$). Protura and Myriapoda both experienced higher abundance in recently treated stands. There is higher abundance of Protura in 2023 I compared to control groups ($p < 0.01$); additionally there is marginally higher abundance in 2018 ID and 2023 I compared to control groups ($p < 0.05$). Myriapoda showed significantly higher abundance in 2018 ID and 2023 I plots compared to control ($p < 0.01$), as well as marginally significant decreases in control compared to 2018 I ($p < 0.05$).

4. Discussion

4.1. Soil Arthropod Biodiversity

Results reported similar diversity across all plots and didn't indicate any significant changes in diversity in response to treatment type used or when it was treated. This suggests that neonicotinoid use on eastern hemlock stands has not impacted soil arthropod diversity. These findings were contrary to expectations, as we hypothesized that diversity would be lower in recently treated plots compared to control and 2018 treated plots.

Results from past literature are varied. Knoepp et al. (2012) indicated that there is a negative correlation between absorbed soil imidacloprid content and soil arthropod populations. In contrast, Kung et al. (2015) found that canopy arthropod communities didn't experience differences between treated and control groups. Teksum (2021) and Reynolds (2008) reported that collembola abundance and richness decreased in response to imidacloprid use while mite abundance and richness were unchanged. Variable results in studies may indicate that soil type, target plant, seasonality, and other environmental conditions may have an impact on the uptake of neonicotinoids.

The systemic nature of imidacloprid and dinotefuran may localize their impact and reduce exposure to non-target organisms in the soil. The dissipation time in soil for dinotefuran was found to be 75 days and anywhere from 450 to 1200 days for imidacloprid, depending on soil type (Goulson 2013). Knoepp et al. (2012) suggest that absorption of imidacloprid in southern Appalachian soils may restrict its movement and minimize the impact on soil microarthropods. This literature may shed some light on our results, as neonicotinoids may have already moved into the root system and tree tissues, and only left trace amounts of neonicotinoids in the soil.

4.2. Soil Arthropod Abundance

There was no significant change in abundance between plots in Arachnida, Isopoda, and Collembola. This finding suggests that these orders are less sensitive to neonicotinoid use and is consistent with literature for Arachnida. Many studies have found that exposure to imidacloprid increases fecundity in mites, of which indirect impacts on predatory species have been proposed (James et al. 2002, Szczepaniec and Raupp 2012). This consistency in abundance in Arachnida could be attributed to increased mite fecundity. Teksum (2021), Reynolds (2008), and Peck (2009) all reported decreased Collembola abundance and richness in treated stands. However El-Naggar and Zidan (2013) reported higher Collembola abundance in treated plots, which they proposed was due to a decrease in predators. Our findings in comparison with other studies may suggest that impact to abundance in response to pesticides is variable based on environmental factors and local community dynamics.

Myriapods had higher abundance in 2018 I, 2018 ID, and 2023 I sites compared to the control site. Proturans also demonstrated higher abundance in 2018 ID, 2023 I, and 2023 ID compared to the control site. This suggests that recent neonicotinoid application in 2023 increased the abundance of these two orders. There was no significant changes correlated with the type of chemical treatment used. These differences are consistent with previous literature that report these orders are less sensitive to neonicotinoids through direct and indirect mechanisms. Reynolds (2008) reports that Myriapoda are less susceptible to pesticide use; Atwood et al. (2018) also found increased Myriapoda abundance in treated areas compared to non-treated, for which they suggested tolerance to neonicotinoids as well as ecological mechanisms reducing competition or predation as causes. Additionally, Peck (2009) reported that Protura abundance was unchanged by imidacloprid use. Atwood et al (2018) and Reynolds (2008) suggest that higher abundance could be attributed to indirect impacts, such as emptied niches, reduced competition for resources, and loss of predator species.

Abundance in 2023 treated stands was lower than abundance in treated and control sites in groups Insecta and Mollusca. In Insecta, higher abundance was observed in 2018 I compared to 2023 I. Mollusca abundance is higher in the control plot compared to the 2023 I plot. Similar to Protura and Myriapoda, no significant changes were observed that correlated with differing treatment type. This response was expected in insects. Neonicotinoids are targeted towards insects in particular, and they are more susceptible to neonicotinoid toxicity (Botias et al. 2016). The changes in Mollusca abundance were unexpected as they're generally known to be more resistant to neonicotinoid use (Mortl et al. 2020). However, Mortl et al. 2020 also reported observed toxicity in mollusks. These findings suggest that insects and molluscs are more sensitive to imidacloprid and dinotefuran use than other identified orders.

The population abundance response to neonicotinoid use was not uniform among all the identified orders. The varied responses among different groups are corroborated by some studies that have shown variable tolerance for neonicotinoids between different arthropod orders (Teksum 2021) (Reynolds 2008). These findings are also consistent with many studies that report community dynamics and interactions determine how neonicotinoid consequences will present themselves (James et al. 2002, Szczepaniec and Raupp 2012, El-Naggar and Zidan 2013, Atwood et al. 2018). There was no significant impact of the type of treatment (imidacloprid or imidacloprid and dinotefuran). This may be attributed to the quick dissipation time of dinotefuran in soil (Goulson 2013).

4.3. Environmental Factors

Seasonality (collection date) appeared to have an impact on the yield of sampling. Our third collection reported significantly lower diversity than the other collection dates. No specific weather conditions differed between this collection and the others, however sites were particularly overgrown during this collection compared to others. Additionally, cross-contamination of treatment types could have skewed results. Trees were treated individually based on the technician's assessment of the trees' health. Trees treated with imidacloprid were in close proximity to those treated with a dual application of imidacloprid and dinotefuran. Cross-contamination cannot be ruled out because of this. Additionally, the control site was directly adjacent to wheat and corn crops grown to attract deer for hunters. Pesticides could have been utilized to support these crops, which could've created cross-contamination in the control site as well.

5. Conclusion

Results report no significant changes in biodiversity of soil microarthropod communities in response to differing pesticide treatments over time. Observed impacts of treatment year on abundance differed between groups. The reduced abundance in Insecta and Mollusca following 2023 treatment suggest an immediate, direct impact of neonicotinoid application on these groups, and may suggest more susceptibility to neonicotinoid use. According to previous literature, higher abundance reported in recently treated sites for Protura and Myriapoda may suggest indirect impacts on these groups due to alterations in community structure and ecological interactions. We recommend long term future research with segmented treatment to further investigate the impact of neonicotinoids on soil arthropod communities. The use of neonicotinoids within the eastern hemlock IPM strategy is a balancing act between environmental consequences. The loss of eastern hemlock stands will perpetuate ecological consequences long term. Though neonicotinoids are harmful to non-target organisms, treatments by way of soil injection or drench have been reported to localize the spread and minimize outside impacts (Goulson, et al. 2013). Neonicotinoid treatments are necessary for the survivorship of the eastern hemlock and their associated ecosystems and should continue to be applied to preserve their species.

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