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Imidacloprid Persistence, Mobility, and Effect on Ecosystem Function

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Imidacloprid Persistence, Mobility, and Effect on Ecosystem Function

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by

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ABSTRACT

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Imidacloprid is a neonicotinoid pesticide used to protect against biting and sucking insects. Land managers rely on its systemic properties, however long-term studies investigating imidacloprid effects on ecosystem function are limited. This study investigated imidacloprid applications to *Tsuga caroliniana* and *Tsuga canadensis* over time and compared concentrations to measures of ecosystem function including soil respiration, microbial function, and invertebrate density. Results indicate that imidacloprid is persistent ($p<0.001$), mobile ($p<0.05$), and can translocate into non-target plants with a significant monotonic relationship ($p<0.005$). Soil respiration was not significantly different between control and treatment sites ($p>0.5$). Microbial function and invertebrate density were not significantly different between control and treatment locations nor did imidacloprid concentrations correlate with ecosystem functional indicator activity ($p>0.05$). It is evident that imidacloprid does not affect ecosystem function over time, however care should be taken when applying it in sensitive locations where endemic, threatened, and endangered organisms reside.

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CHAPTER 1

INTRODUCTION

Pesticide use involves balancing the requirements for food or commodity production against the cost of agricultural loss and environmental damage caused by native and non-native invertebrate populations. Invertebrates like the brown marmorated stink bug (*Halyomorpha halys*) and hemlock woolly adelgid (*Adelges tsuga*e) have been causing crop and tree damage to the point that pesticide use is necessary either to protect the farmer against economic harm or the tree against probable death. Often, excessive pesticide use creates a cyclical process whereby invertebrates eventually develop resistance and newer pesticides must be developed to keep up with evolution. Consequently, new pesticides are developed including a class of pesticides that can be used prophylactically known as neonicotinoids.

Neonicotinoids are synthetic compounds based on naturally occurring nicotine, which has invertebrateicidal properties. Designed to eliminate pests by targeting invertebrate- specific nicotinic acetylcholine receptors, they result in both acute and chronic invertebrate neurotoxicity and death, depending on the dose received. Of these, imidacloprid was one of the first neonicotinoids developed[1,2] spreading in use because of its systemic mode of transport and efficiency at eliminating agricultural pests.[3,4] Aimed at biting and sucking invertebrates, imidacloprid is distributed in a variety of products including pre-treated seeds, powders, liquids, and topical/oral flea medications.[4] In the United States, imidacloprid has become one of the more popular agricultural pesticide products resulting in increased potential for interactions with environmental matrices, non-target organisms, ecosystem function and human health.

Much of the research surrounding imidacloprid and ecosystem function involves pollinator services with less focus on soils and ecosystem function. Of the research available,

the consensus is that there is potential for imidacloprid to negatively affect ecosystem function, yet there is very little research directly comparing indicators of ecosystem function with imidacloprid application.[5] In 2009 D. Peck observed significant decreases in non-target invertebrate collected in soil cores where imidacloprid was used to control root-feeding scarab larvae. In general he detected little change in abundance of surface dwelling organisms compared to a significant decrease in organisms found below the surface.[6] Capowiez et al. and Chagnon et al. observed behavioral changes in the feeding and foraging behavior of earthworms in soils where imidacloprid was applied. Earthworm species altered their burrowing paths and feeding behaviors resulting in decreased macropore formation and decomposition services in soils.[23,35] In the Southern Appalachians, Knoepp et al.[8] experimented at different elevations with imidacloprid use on hemlock trees. They observed a negative association between imidacloprid concentration and species abundance at higher elevations but detected no significant difference at lower elevation sites.[8] Overall as imidacloprid increased in concentration, species' abundance decreased,[6] and typical behavior (burrowing, feeding, etc.) changed.[23,35] Because of the lack of research specifically focused on imidacloprid use and ecosystem function the following document evaluates imidacloprid literature, investigates imidacloprid use in a Southern Appalachian forest, and assesses potential effects to ecosystem function over time.

Imidacloprid Background

Imidacloprid was first created and patented in 1985, based on the insecticidal properties of nicotine with enhancements made to photostability and water solubility.[2,9] Following structural improvements, imidacloprid was found to work best when applied as a soil drench, via direct injection, or as a seed coating because it performs better when ingested versus when

sprayed as foliar protectant. By 1994 imidacloprid was registered for use in the United States[10] and grew in popularity over the ensuing 25 years because of its systemic properties, mechanism of action, and convenience.

Mechanism of Action

Imidacloprid is most effective when directly ingested, thus many of the products containing the active compound are designed to be consumed rather than sprayed. Structurally, imidacloprid is most similar to a nicotine compound with the exception that a nitromethylene group has been changed to nitroguanidine group, resulting in improved efficacy and stability.[2] Because of its water solubility, imidacloprid is absorbed by root systems and transported throughout plants via xylem transport.[4] This allows multiple parts of a targeted plant to be protected against biting and sucking invertebrates that ingest the active ingredient and die shortly afterwards. Following ingestion, imidacloprid targets post- synaptic nicotinic acetylcholine receptors located in the central nervous system of invertebrates, causing continuous activation and preventing any further signal.[10,11] The resulting neurotoxicity is preceded by intoxication including observable confusion, reduced foraging, reduced homing abilities, and other coordination problems.[12–14]

Environmental Fate

Imidacloprid has low vapor pressure (3×10^{-12} mmHg at 20°C) and Henry's Law Constants (1.7×10^{-10} Pa·m^3/mol), an octanol-water partition coefficient (K_{ow}) of 0.57 at 21°C, is soluble in water at 0.61 g/L at 20°C, and has a soil sorption coefficient (K_{oc}) of 159-960.[10] This indicates that it is unlikely to volatilize and become an inhalation hazard and is also unlikely

to bioaccumulate. It can, however mobilize through porous soils, in run-off or spray drift, and has been detected in water sources near the original point of application.[15] The wide range in soil sorption is due to variability in soil texture with clay and high organic soils providing more binding sites for imidacloprid molecules,[4] decreasing the likelihood of leaching. Soils with higher sand content facilitate imidacloprid movement away from the application site. Because of soil variability, half-lives in soil and persistence over time range from 40 days to 1230 days.[4,10] Degradation in soil and water is primarily via aerobic microbial processes and photolysis. [15] Under alkaline conditions with higher water temperatures, imidacloprid will undergo hydrolysis.[10]

Non-target Organisms

The primary targets of imidacloprid are biting and sucking invertebrates such as aphids, fleas, and ticks, however it is difficult to prevent non-target organisms from being exposed. Non-target organisms known to be affected by imidacloprid include pollinators, birds, decomposers, aquatic species, and amphibians. Of the pollinators, bee species are well researched[5,16–19] and have been found to be acutely and chronically affected. Other pollinators, including butterflies and moths, are less researched, but are anecdotally reported to be adversely affected when comparing organic farming practices to areas with pesticide applications.[5] Pollinators can be exposed through contact with contaminated pollen, dust inhalation, or nectar. When ingested, an individual bee may experience death at higher doses ranging from 3.7 ng/bee to 490 ng/bee or altered behavior at lower doses through reduced homing and foraging capacity.[5] With this information, regulatory agencies in the European Union and the United States initially placed a temporary moratorium and pause on neonicotinoid registrations in 2012.[15,20,21] More recently, the European Union voted to ban all neonicotinoid use and the U.S. Environmental

Protection Agency (US EPA) is anticipating a final ruling on imidacloprid in 2019.

Birds are another non-target organism that are negatively affected by imidacloprid. Routes of exposure include ingestion of contaminated prey, seed, nectar, or indirectly through decrease in prey quality and quantity.[22,23] Birds that are exposed have been observed to experience signs of ataxia[24] and death,[3,24,25] though studies investigating chronic and indirect effects such as rearing young and overall hardiness are lacking.[24] Invertebrates that reside in soil, such as decomposers are also affected by imidacloprid due to exposure through dermal contact and ingestion. For example, earthworms are known to alter their burrowing behaviors in areas treated with imidacloprid[26] and certain parasitic nematodes appear to benefit from its presence.[27]

Recently, the U.S. Geological Survey conducted investigations of surface waters in the mid-western United States and concluded that up to 75% of waters surveyed contained detectable levels of neonicotinoid pesticides (23% contained imidacloprid).[28] In 2016 the US EPA released a preliminary aquatic risk assessment of imidacloprid and noted that the overall concentrations of imidacloprid were below the level considered immediately dangerous to fish or amphibians, though freshwater concentrations were routinely above the toxicity threshold for aquatic invertebrates.[15] Like terrestrial invertebrates, aquatic organisms are exposed through dermal exposure and ingestion. Exposure results in death or behavior modifications including reduced foraging and reproduction.[29]

Ecosystem Function and Imidacloprid

Healthy ecosystems are sustainable and able to maintain overall organization and resilience under stress.[30] Ecosystem functions encompass processes that lead to identifying

services (e.g., pollination, nutrient cycling, and water filtration and holding) that benefit humans and can be economically valuable. Groot et al. specifically defined four areas of ecosystem function including regulatory capacity, habitat provision, food and goods production, and information, which is a qualitative measure for human health benefits such as the positive effect of being in nature.[31] Changes to these processes may alter the overall function of the ecosystem, similar to the way additives in gasoline may affect the functionality of a combustion engine. In modern conservation, much of the focus is on a singular organism with less attention on the ecological network surrounding the target species and how that network is affected.[32] This results in a lack of appreciation about the chronic effects that an anthropogenic component such as organic chemicals can have on an environment. Thus, examining indicators of ecosystem health that contribute to ecosystem function are necessary because of the indirect effects these functions can have on human health.

Identifying specific indicators of ecosystem function that allow for measurable determination of environmental stress can be difficult and expensive. Creamer et al. investigated measures of soil quality and biodiversity including total carbon, nitrogen, soil particle size, soil basal respiration, bacterial community level physiological profiling, and microbial diversity via DNA extraction in order to examine the interconnectedness between indicator density and land use intensity.[32] According to these researchers, the interactions between functional indicators are neglected and thus a network approach is advisable. Similar to what Groot et al. advised with four specific functional areas representing ecosystem wellbeing,[31] Creamer et al. advocated utilizing a broader method that encompasses the functional redundancy of ecosystem indicators and specifically investigates their density and interconnectedness. In short, the more abundant and dense the ecosystem indicators are in an area, the better the ecosystem is functioning. [32]

With regards to environmental stresses such as pesticide use, it is preferential to analyze a variety of indicators that may respond differently to chemical changes in the environment because they may herald future disturbances to ecosystem services. By choosing a balance between specific ecosystem functions and associated indicators, long-term ecosystem-level consequences of pesticide use can be estimated (Tbl. 1).

Sensitive Ecosystems: Appalachia and Imidacloprid

The United States' Southern Appalachians are considered a biological hotspot, home to endemic, threatened, and endangered organisms that rely on specific climatic characteristics unique to this region.[33] Many issues affect the area, but the decimation of native *T. canadensis* and *T. caroliniana* due to *A. tsugae* infestation is one of the more difficult to manage.[12] *A. tsugae* are parthenogenic and actively feed at the base of hemlock needles. This causes the tree to slowly die from the outward branches inward as *A. tsugae* starve it of necessary energy supplies. While *T. canadensis* and *T. caroniana* are not considered threatened, they do act as a "foundation species," providing necessary shade and microclimates for aquatic and terrestrial organisms.[34] Forest managers throughout the southeast determined pesticides containing imidacloprid to be an effective method of control[35] and studies show that the parent compound and metabolites remain present in the tree up to seven years after treatment.[36] While dealing with invasive species is difficult, imidacloprid soil applications may be having an effect beyond controlling *A. tsugae* by unintended leaching and mobility through soils into water systems, sediment, or non-target plants. This in turn may result in additional exposure to non-target organisms and affect ecosystem health.

Problem Statement

Over time, imidacloprid may negatively affect soil and aquatic dwelling organisms with the potential to alter ecosystem functions that support regulatory and habitat functions. Because humans rely indirectly on ecosystem services provided by soil swelling organisms including nitrogen fixation, carbon cycling and sequestration, and nutrient cycling,[32] it is probable that declines to ecosystem function will trickle-down to humans. This is presently becoming of greater interest to scientists and government officials because of observed declines in invertebrate populations across the world due to factors that include the use of pesticides.[37] It is because of such unintended consequences that make gathering temporal evidence of acute and chronic impacts to ecosystem functions (specifically regulatory, habitat, and production functions) a necessary aspect of pesticide research. Consequently, the ensuing body of research sought to examine the persistence and mobility of imidacloprid under field use conditions and the relationship imidacloprid concentration has with ecosystem functional indicators.

Because imidacloprid is hydrophilic with varying degrees of persistence in soils, it is proposed that imidacloprid will result in measurable changes to ecosystem functional indicators that include microbial community function, invertebrate density, soil respiration, and non-target plant translocation. Trees treated with imidacloprid are likely to have higher residual concentrations in the soil surrounding their base. Comparing the concentrations of imidacloprid in soil at treated and controlled sampling locations will demonstrate the effects to ecosystem indicators. It is hypothesized that imidacloprid will translocate into non-target plants and be of high enough concentration to effect non-target organisms. It is also proposed that treated trees with higher imidacloprid concentrations in soil will exhibit lower invertebrate densities and reduced microbial activities that will inhibit respiration and community function. Because of the

limited time frame, funding availability, and laboratory apparatus necessary to measure imidacloprid concentrations in the range of parts per million, the primary goal of this project is to compare measurable differences between trees treated in different years compared to a tree without any imidacloprid treatment and examine how these differences relate to ecosystem function.

CHAPTER 2

MATERIALS AND METHODS

Data Collection

Field work was conducted during the spring and summer of 2017 while field scouting and preparation was completed during the spring and fall of 2016. Two-person crews consisted of the author and a field aid. Sample sites were located within the boundaries of Bays Mountain Park in Kingsport, Tennessee. Soil and plant samples were collected based on U.S. EPA and U.S. Department of Agriculture (USDA) methods.[38–40] Microbial community function samples and soil respiration were based on protocol provided by the product manufacturers, BIOLOG, Inc. (Hayward, CA) and Rhizoterra, Inc.(Lolo, MT). Invertebrate density sample collection was based on the *Soil Invertebrate Field Manual* by Ruiz et al.[41] The following sections detail each data collection method used during this project.

Study Location

Bays Mountain Park in Kingsport, Tennessee is a 3500 acre city-owned park engaging in natural resource preservation, environmental education, community involvement, and research.[42] Located in the Southern Appalachians, it is at an epicenter for invasive species management. Since 2012 Bays Mountain has been participating in a state-wide Tennessee Department of Environment and Conservation (TDEC) initiative to control *Adelges tsugae* by drenching the base of select hemlock trees with 34.02 g of imidacloprid if the tree was less than 55 cm in diameter breast height (dbh) and 68.04 g if the tree was greater than 55 cm dbh. Specific trees were arbitrarily chosen by TDEC personnel based on estimated long-term survival potential.

The practice of applying imidacloprid at Bays Mountain Park has continued yearly and is the reason it was chosen for this study. The overall topography of the park includes gradual hills and eroded slopes with numerous small streams that flow into an interior lake or into other receiving water systems. Soils present throughout the park range from Bays silty clay loam to Shelocta silt loam and Wallen gravelly loam[43,44] and indicate the potential for imidacloprid to bind and remain present after application.

Site Layout

Four hemlock trees were chosen for this study based on 1) dbh greater than 55 cm because they received a double-dose of imidacloprid, 2) the year that imidacloprid was applied, and 3) soil profiles with approximate similarity. Three trees were treated with imidacloprid during different years and a fourth tree with similar characteristics but no imidacloprid application was chosen as a control for comparison. Sampling locations were randomly selected within a specified distance zone (0 m, 1 m, 5 m, and 10 m) and labeled via survey flags. Exact sample locations were selected using a grid system and a random number generator. Grids were laid out in the field using flour to mark off grid cells. Figure 1 depicts a typical sampling design around a single tree.

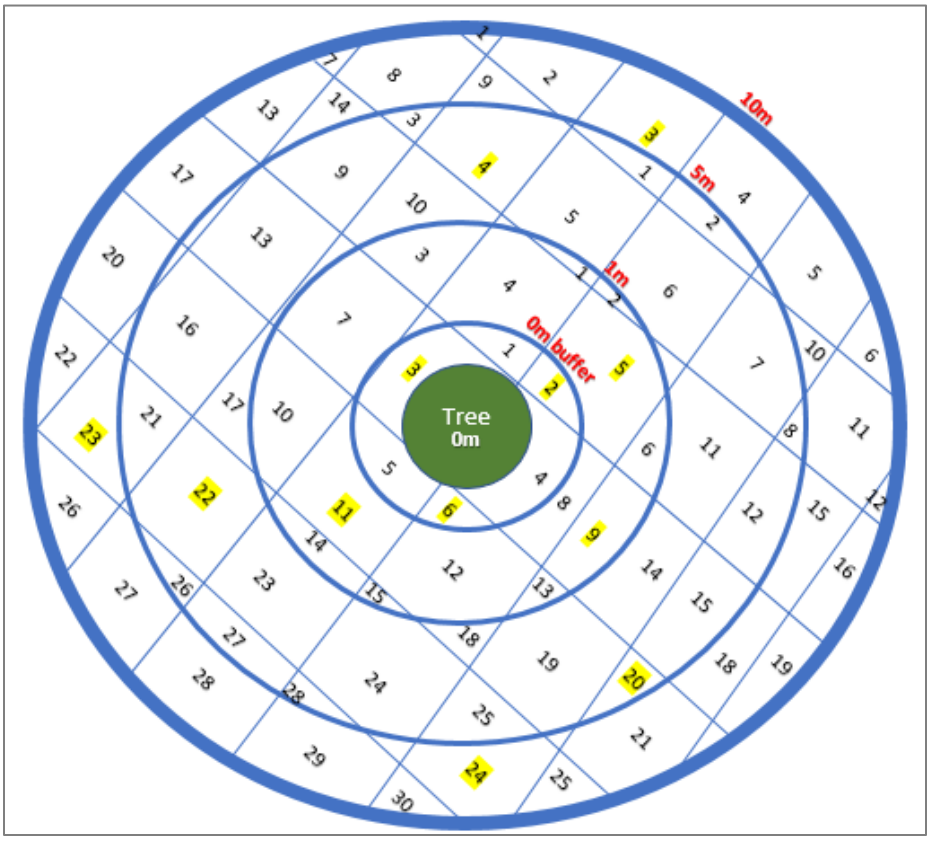

Figure 1. Example of a field sampling grid. Sample locations were randomly selected within each distance zone away from the tree (0 m, 1 m, 5 m, 10 m).

Soil Core Collection

Soil samples were collected using an AMS Inc. soil recovery probe (1 in x 12 in) with open slot to allow easy removal of soil cores and a replaceable bit designed for clay. Depth levels were pre-marked on the soil probe so that sub-samples could be collected based on depth (0-10 cm, 11-21 cm, and 22-32 cm). Three cores per distance zone were collected, sub-sampled, placed in 250 mL amber glass jars with PTFE lids, and held in a cooler with ice packs until return to the ETSU Environmental Sciences Health Lab (EHSL). This resulted in 12 sample locations per tree. Thirty-six samples were collected after sub-sampling by depth. Three duplicates, 3 replicates, 1 field blank, and 1 trip blank were also collected as part of QA/QC procedures described in the QA/QC section. The soil probe was scrubbed with a wire brush, rinsed with tap

water, washed with Citronex® biodegradable soap, rinsed with deionized water, and then rinsed with 10% methanol solution in between new sample locations at each site. All wastewater was collected in a 5-gallon bucket and disposed of at the EHSL in Johnson City, TN or via a private drain with wastewater directed to the Kingsport, TN wastewater system.

Soil Imidacloprid Concentration

Soil samples were collected as described in the soil core collection section, acquiring samples for both depth (0-10 cm, 11-21 cm, and 22-32 cm) and distance zone (0 m, 1 m, 5 m, 10 m). This resulted in 36 total samples per tree plus QA/QC samples.

Biolog Ecoplate™

Soil samples were collected at the same locations as those collected for concentration by distance zone (0 m, 1 m, 5 m, 10 m), resulting in 12 composite samples per tree. Each sample was collected using an AMS soil probe, placed in sterile whirl-pack bags, and stored in a cooler with ice for transportation back to the EHSL.

Soil Respiration

Soil samples were collected at the same locations as those collected for concentration by distance zone (0 m, 1 m, 5 m, 10 m), resulting in 12 composite samples per tree. Each composite was placed directly into Solvita® Field Soil Testing jars.

Plant Collection

Non-target plant samples were collected at the same sample locations as the soil core samples collected for concentration by distance zone (0 m, 1 m, 5 m, 10 m), resulting in 12 composite plant samples per tree. Plants were pulled with root intact, placed into a sterile whirl-

pack bag, and stored in a cooler with ice for transportation back to the EHSL. Plant species collected were identified by the *Manual of Vascular Plants of Northeastern United States and Adjacent Canada* and are found in Table 5.[53] Samples were stored at 4°C until analysis.

Table 5. Plant Species Collected.

Plant Common Name	Genus	Species
American strawberry bush	*Euonymus*	*americanus*
Virginia creeper	*Parthenocissus*	*quinquefolia*
Partridge berry	*Mitchella*	*repens*
Tea berry	*Gaultheria*	*procumbens*
Red maple	*Acer*	*rubrum*
American beech	*Fagus*	*grandifolia*
American holly	*Ilex*	*opaca*
Northern red oak	*Quercus*	*rubra*
White oak	*Quercus*	*alba*
Carolina Hemlock	*Tsuga*	*caroliniana*
Eastern Hemlock	*Tsuga*	*canadensis*

Invertebrate Density

Four transect lines with one sample cup per distance zone away from the tree were set up between 2 PM and 4 PM at each site and left until 10 AM to 11 AM the next morning. Each sampling location included a red solo cup buried to the top lip with isopropyl alcohol in the bottom to kill and preserve specimens. Organisms were collected the following morning in sample jars and returned to the EHSL for identification.

Sample Preparation and Analysis

Two different laboratories were utilized during this project including the ETSU EHSL and an ETSU Quillen College of Pharmacy laboratory managed by Dr. Stacy Brown. Soil and

plant samples were analyzed for imidacloprid concentrations in Dr. Brown's laboratory. Sample preparation and Biolog EcoPlate™ analysis occurred in the EHSL.

Soil Imidacloprid Concentration

Soil samples were initially stored at 4°C until drying and extraction for analysis by LC-MS/MS. Extraction occurred within 14 days of sample collection and LC-MS/MS analysis occurred within 30 days of extraction per U.S. EPA standards.[45] Before extraction, soil samples were spread onto aluminum foil trays and dried at room temperature in a dark cabinet for 1-3 days. After drying, each sample was pressed through a 2 mm sieve to homogenize samples. In between samples the sieve was rinsed with tap water, washed in Citranox® solution, rinsed with tap water, rinsed with deionized water, rinsed with 10% methanol, and dried before sieving another sample.

Extraction was performed using AOAC method 2007.01, also known as QuEChERS (quick, easy, cheap, effective, rugged, and safe), which involves acetonitrile extraction, salting out liquid-liquid extraction with magnesium sulfate ($MgSO_4$), and dispersive-solid-phase extraction (d-SPE) cleanup.[46] The QuEChERS technique was first developed by Anastassiades et al.[47] and further evaluated by researchers investigating pesticide residues in agricultural products, honey, pollen and soil.[48–50] Pre-measured QuEChERS kits were purchased from United Chemical Technologies (UCT). Initial extraction packets contained 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g sodium citrate dihydrate, and 0.75 g sodium citrate sesquihydrate. Clean-up extraction (d-SPE) contained 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18 for soil and 150 mg $MgSO_4$, 50 mg PSA.

Dry, homogenized samples were weighed to 15 g +/- 0.05 g directly in 50 mL centrifuge tubes with 15 mL deionized water. Each centrifuge tube was then shaken by hand and left for 30 minutes to saturate. After the allotted time passed, 15 mL of 1% acetic acid in acetonitrile was added, allowed to mix for 1 minute, and the first QuEChERS extraction packet containing 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g sodium citrate dihydrate, and 0.75 g sodium citrate sesquihydrate was added. Each centrifuge tube was then shaken again vigorously by hand to loosen and mix any clumps and vortexed for 2 minutes. Thoroughly mixed samples were next centrifuged at $\geq$4000 RPM for 5 minutes and 2 mL of the supernatant were removed and placed in 2 mL centrifuge tubes containing the QuEChERS d-SPE clean-up mixture with 150 mg $MgSO_4$, 50 mg PSA, and 50 mg C18. Samples were again vortexed for 1 minute and centrifuged for 2 minutes at $\geq$4000 RPM. Each 2 mL sample was then transferred into Spin-X® centrifuge tube filters containing 0.22 µm cellulose acetate filters, centrifuged for 2 minutes at $\geq$4000 RPM and then transferred into 2 mL amber glass LC/MS vials containing 300 µL conical limited volume inserts and PTFE caps for analysis by LC-MS/MS.

Quantification of imidacloprid was conducted on a Shimadzu liquid chromatography mass spectrometry (LC-MS/MS) system with XR upgrade (LCMS-IT-TOF; ion trap-time of flight) using an Agilent Eclipse XDB-C18 (3.5 micron, 4.6 x 150mm) column. HPLC-grade acetonitrile, acetic acid, and 0.1% formic acid in water were purchased from Fisher Scientific. Standards used in each analytical batch were prepared from dry imidacloprid standard (purity $\geq$98%) purchased from Toronto Research Chemicals. Stock standard solutions were prepared in 1% acetic acid in acetonitrile and diluted based on assumed sample concentrations and per-established limits of detection (LOD). Standards ranged from 20 ng/mL to 10,000 ng/mL and were stored at 4°C for up to 6 months.

The LCMS-IT-TOF was run in positive electrospray (+ESI) mode, monitoring mass to charge (m/z) 257 to m/z 183 transition. An isocratic 50/50 method was used with 0.1% formic acid in water in mobile phase A and acetonitrile in mobile phase B. The column oven temperature was set at 50°C with a flow rate of 0.400 mL/min and an injection volume of 10 µL. Total run time was 6 minutes with imidacloprid retention time occurring at 4.15 minutes.

Biolog Ecoplate™

Biolog EcoPlates™ were purchased from Biolog Inc. and contained 31 different substrates plus a blank, in triplicate. Sample preparation procedures were based on research investigating soil bacteria in the Netherlands and Europe.[51,52] Sample processing and analysis were conducted within 24 hours of returning to the EHSL. Samples were manually homogenized in each bag, weighed to 1 g +/- 0.05 g, and placed in 30 mL centrifuge tubes along with 10 mL sterile 0.85% NaCl buffer solution. Each tube was left to hydrate for 60 minutes, shaken and centrifuged at $\geq$ 4000 RPM for 2 minutes. The supernatant was then diluted 1:10 with sterile 0.85% NaCl solution and 100 µL was placed in each well of a 96-well Biolog EcoPlate™. Plates were then placed in a 35°C incubator for up to 72 hours with incremental readings at 24 hours, 48 hours, and 72 hours. Plates were read on a Thermo Electron Corporation Multiskan MCC microplate reader equipped with a 595 nm optical filter.

Soil Respiration

Soil samples from control and treatment locations were placed in Solvita® Field Soil Testing jars to the fill line. A blank soil sample was prepared and placed in the cooler with the field samples using clean soil purchased from Sigma-Aldrich (Milwaukee, IL). Solvita® soil CO_2 probes were then inserted directly into the soil, capped, and placed in a cooler for transport

back to the EHSL. The cooler was left at room temperature in the EHSL overnight and results were read 24 hours after inserting the probe. Readings were qualitatively assessed based on a color wheel included in the sample kit.

Plant Imidacloprid Concentration

Plant samples were frozen prior to sample preparation and homogenized using a Ninja Express Chop® food chopper along with 50 -100 mL DI water. Entire plants were used unless woody stems were too large for the blades to break apart. Excess water was carefully decanted to avoid accidental loss of homogenized plant samples. Each sample was then weighed in 50 mL centrifuge tubes to 15 g +/- 0.05 g and combined with 15 mL 1% acetic acid in acetonitrile. In between samples, both the blades and Ninja Express Chop® container were rinsed with tap water, washed in 10% Citranox® solution, rinsed with tap water, rinsed with deionized (DI) water, rinsed with 10% methanol, and dried before a new sample was processed. Next, the first QuEChERS extraction packet containing 6 g $MgSO_4$, 1.5 g NaCl, 1.5 g sodium citrate dihydrate, and 0.75 g sodium citrate sesquihydrate was added and shaken vigorously by hand to loosen and mix any clumps and vortexed for 2 minutes. Thoroughly mixed samples were then centrifuged at $\geq$4000 RPM for 5 minutes and 2 mL of the supernatant were removed and placed in 2 mL centrifuge tubes containing the QuEChERS d-SPE clean-up mixture with 150 mg $MgSO_4$, 50 mg PSA, and Chlorofiltr. Samples were again vortexed for 1 minute and centrifuged for 2 minutes at $\geq$4000 RPM. Each 2 mL sample was then transferred into Spin-X® centrifuge tube filters containing 0.22 µm cellulose acetate filters, centrifuged for 2 minutes at $\geq$4000 RPM and then transferred into 2 mL amber glass LC/MS vials containing 300 µL conical limited volume inserts

and PTFE caps for analysis by LC-MS/MS. Quantification of imidacloprid in plants by LC-MS/MS followed the same methodology as soil analysis.

Invertebrate Density

Organisms collected in the field were identified within 24 hours of sample collection using *Invertebrate Zoology* by R. Barnes for identification.[54] Sample jars containing multiple invertebrates were poured into sampling trays and observed under dissecting scopes. Organisms collected were identified by order and tallied.

Quality Assurance and Quality Control

Quality assurance and quality control measures were performed throughout this project based on suggestions from the U.S. EPA.[45,55,56] Exact parameters used per analysis are described in Table 2.

Table 2. QA/QC Parameters

Analysis	QA/QC Parameter	Location	Quantity
LC-MS/MS – Soil Soil Respiration	Duplicates	Field	10% of sample batch
LC-MS/MS – Soil	Field Blank	Field	1 per sample event
LC-MS/MS	Field Rinse Blank	Field	1 per sample event
LC-MS/MS – Soil LC-MS/MS – Plants	Instrument Blank	Laboratory	1 per analytical batch
LC-MS/MS – Soil LC-MS/MS – Plants	Lab Rinse Blank	Laboratory	1 per sample event
LC-MS/MS – Soil LC-MS/MS – Plants	Limit of Detection	Laboratory	Once during method development
LC-MS/MS – Soil LC-MS/MS – Plants	Replicates	Laboratory	10% of sample batch
LC-MS/MS – Soil LC-MS/MS – Plants	Spike	Laboratory	5% of sample batch

Analysis	QA/QC Parameter	Location	Quantity
LC-MS/MS – Soil LC-MS/MS – Plants	Standard Addition	Laboratory	5% of sample batch
LC-MS/MS – Soil	Trip Blank	Field	1 per sample event
Biolog EcoPlates™	Triplicates	Laboratory	100% of sample batch

Field blanks, trip blanks, rinse blanks, and instrument blanks were collected and analyzed to examine for contamination during sample collection, preparation, and/or analysis. Duplicates were collected to examine consistency and precision of the field sampling process via the relative percent difference. Replicates were collected to examine accuracy and precision of the extraction process in the laboratory. Standard additions and spiked samples were used to examine for matrix interference and recovery efficiency. Other techniques utilized included representativeness and comparability of samples via random sample location and replication. Instrument sensitivity and limits of detection were determined using prepared standards within the range of expected concentrations. In total, QA/QC samples made up more than 50% of each laboratory analytical batch. QA/QC samples were examined to determine the overall quality of data acquired. The U.S. EPA determines data acceptability for replicates and duplicates to be +/- 20% of each other, spiked recoveries between 70% to 120% of the known concentration, and blanks less than half of the lower limit of detection.[45,55,56]

Statistical Methods

Data were analyzed using a combination of Microsoft Excel and Minitab statistical software. Soil and plant data were initially transformed using log (x), natural log (ln(x)), and square root (x) with 1 added to all variables to account for non-detects or results at the LOD. After attempting data transformations, data were analyzed using non-parametric methods of

analysis. All data, including duplicate and replicate samples, were used in analysis and non-detects or LOD detects (anything less than equal to 20 ng/mL) were kept in the dataset as zero. Specific non-parametric tests used for analysis included Kruskal-Wallis, Spearman-Rho, and Chi-Square using a level of significance of $p < 0.05$. Specific hypotheses tested included:

1. Imidacloprid will translocate into non-target plants and be of high enough concentrations to be toxic to non-target invertebrates.
2. Imidacloprid use will result in measurable changes to ecosystem functional indicators.

CHAPTER 3

RESULTS

Data were collected during the winter and spring of 2017. Attempts to normalize the data for parametric statistical tests were not effective because the large amount of non-detects in the datasets skewing the data (Tbl. 3, Fig. 2). Without the non-detects, the data were somewhat normal, however the non-detects were part of the results and were analyzed as such. Consequently, nonparametric tests were utilized with results presented in the following sections. Specific tables and figures not in the text can be found in Appendix A.

Imidacloprid Residual Concentrations

Soil Concentrations

In total, 205 soil samples were analyzed via LC-MS/MS for residual imidacloprid, including QA/QC samples. Imidacloprid was detected in soil at all treatment locations and at all distances away from the tree (Tbl. 4). In general, concentrations decreased with distance (Fig. 3) and ranged from the LOD (2.0 ppb) to 925.6 ppb (Tbl. 4). The highest residual concentrations were detected at the tree base for each treatment tree with the 2017 tree having the highest max concentration (925.6 ppb) and the 2013 tree having the lowest max concentration (124.4 ppb). There were significant differences when comparing the control tree to the treatment trees at all distances (Fig. 3, $p < 0.05$) and significant differences between treatment trees at 1 m, 5 m, and 10 m (Fig. 4, $p < 0.001$). Like the comparison between control and treatment plots, the treatment imidacloprid concentrations decreased with distance away from the tree.

Non-Target Plant Concentrations

Including QA/QC samples, 44 non-target plant samples were analyzed for imidacloprid from 10 different species (Tbl. 7). In general concentrations detected were lower in plants than soil. Imidacloprid was detected in plants at all treatment locations and at all distances away from the tree (Fig. 5). In general, imidacloprid concentrations detected in plants around each control or treatment tree was lower than residual concentrations in soil. The highest concentration detected was 93.9 ppb at the 2017 tree (Tbl. 8) and overall concentrations decreased with distance away from the tree. Comparing control vs. treatment samples indicated a significant difference at 5 m away from the tree ($p = 0.03$), but not at any other distance. Comparing imidacloprid concentrations in soil and plants resulted in a significant monotonic relationship ($r_s = 0.68$, $p = 0.004$) so that as soil imidacloprid concentrations increased, so did the plant concentrations.

Ecosystem Functional Indicators

Soil Respiration

In total, 49 samples were analyzed for soil basal respiration using the Solvita® respiration kit and color wheel. No sample had less than medium CO_2 utilization after 24 hours. Of 12 control samples, 10 ranked high on CO_2 utilization compared to 34 out of 37 treatment samples of the same ranking. A Chi-square test of independence (Tbl. 9) indicated no significance difference between control and treatment samples, X^2 (1, N = 49) = 0.72, $p = 0.39$.

Microbial Function

Forty-nine samples were analyzed for microbial community function via average well color development (AWCD) with Biolog EcoPlates.™ Initially there was little change in light transmission (nm) after 24 hours of incubation. After 48 hours of incubation there was a slight increase in AWCD, which was repeated after 72 hours of incubation, particularly in soil samples 0 m and 10 m away from the tree. Additional statistical analyses utilized the results of the 72-hour incubation because the greatest change was observed after this incubation period. The AWCD of control samples appeared to increase, decrease, and increase again from 0 m to 10 m distances away from the tree base after 72 hours of incubation (Fig. 6). The AWCD of treatment samples appeared opposite of the control samples, starting out lower at 0 m, increasing at 1 m, and decreasing at 5 m and 10 m from the tree base following the same incubation time. Comparing the control site to the treatment sites yielded no significant difference in carbon substrate utilization ($p = 0.09$). There was no significant difference in the distance away from trees when comparing all distances (Fig. 6). Examining whether there was a correlation between soil imidacloprid concentration and microbial substrate utilization via AWCD showed no significant relationship ($r_s = -0.11$, $p = 0.68$).

Invertebrate Density

A total of 255 invertebrates were collected and identified with the highest total counts found at the 2016 treatment tree and control tree (Tbl. 10). In general, there was greater invertebrate diversity and density at control locations vs. treatment locations (Fig. 7), however there was no significant difference between control and treatment plots at any distance away from the tree (Fig.7). Comparing the relationship between soil and plant imidacloprid

concentration to invertebrate density showed a slightly monotonic relationship, but it was not significant. As the concentration in soil and plants increased, the density of invertebrates decreased, however the relationship was not significant (r_s = -0.45, p =0.08). Of the invertebrate orders collected, Orthoptera were the most common followed by Diptera and Coleoptera (Fig. 8).

QA/QC of Field and Laboratory Analyses

Samples analyzed for imidacloprid concentration included four batches of soil samples and one batch of plant samples. Accuracy and precision were measured by percent recovery of spikes and relative percent difference (RPD) of duplicate and replicates. Duplicate and replicate samples were considered precise if the results were within 20% of each other. Spiked samples were considered accurate if the results were within 70-120% based on the known concentration and analytical outcome. Sample results that fell below the limit of detection (LOD) were coded as 2.0 ppb.

Two hundred and ninety-two soil samples were analyzed including 47 lab and field blanks, 35 duplicates, 33 lab replicates, and 16 spiked samples in four analytical batches. Of the field duplicate and lab replicate samples, 51% of the duplicates and 60% of the replicates exceeded 20% RPD. Of the spiked samples, 50% of samples were within the range of acceptability for accuracy. Two percent of lab and field blanks showed contamination (Tbl. 11).

Sixty-two plant samples were analyzed including 11 lab and field blanks, 4 duplicates, 4 replicates, and 2 spiked samples in one analytical batch. All spiked samples exceeded the range of acceptability for accuracy (%R = 167% and 181%). Two of the replicates exceeded the 20% range of acceptability for precision. All duplicates fell within the range of acceptability (Tbl 11).

Soil samples analyzed for respiration were prepared in the field and analyzed further in the lab. These consisted of 57 samples with 4 replicates and 4 duplicates. Replicates were split from the original sample and duplicates were collected in tandem with the original sample. All QA/QC parameters analyzed fell within the range of acceptability (Tbl. 11).

CHAPTER 4

DISCUSSION AND CONCLUSIONS

Introduction

Imidacloprid has been used as a protective form of treatment at Bays Mountain Park since 2012 in efforts to control the spread of the hemlock woolly adelgid (*A. tsugae*) to native hemlock tree species (*T. caroliniana* and *T. canadensis*). Because of its ability to persist in organic or clay-based soils[57,58] and move systemically throughout the tissue of a plant, imidacloprid has the potential to affect organisms that rely on hemlock forests and Appalachian ecosystems. Imidacloprid also has the capacity to affect ecosystem function, which is an area of research that is not well studied, though numerous papers have been written about the effects to pollinators, invertebrates, and birds.[13,22,23,26] Consequently, this study was designed to examine the persistence, mobility, and effect to ecosystem function utilizing indicators of these measures such as invertebrate density, soil respiration, and microbial substrate utilization. Specific hypotheses included the following:

1. Imidacloprid will translocate into non-target plants and be of high enough concentrations to be toxic to non-target invertebrates.
2. Imidacloprid use will result in measurable changes to ecosystem functional indicators.

Major Findings

Residual Imidacloprid Concentration in Soil

Imidacloprid proved persistent and mobile in soils located at Bays Mountain Park in Kingsport, Tennessee (Tbl.4). These soils contained organic matter, sandy loams and clay, and it is unsurprising that imidacloprid was persistent. Other researchers have detected similar trends in imidacloprid persistence owing to the chemical nature of clays and organic matter with their ability to attract polar molecules like imidacloprid. Cox et al. observed that imidacloprid could persist longer in soils containing clay.[57] Bonmatin et al. also determined that imidacloprid was more persistent than originally thought, lasting up to 1000 days in agricultural soils at levels known to be toxic to invertebrates.[4,26] Residual concentrations detected in this study from the tree treated with imidacloprid in 2013 ranged from the LOD to 124.4 ppb (Tbl. 4), which exceeds what Bonmatin et al[4,26] detected by more than 400 days. Results from this study add to the growing evidence that imidacloprid persists, however because this study encompassed only a four-year time frame, additional studies in a similar environment might determine whether residual imidacloprid concentrations exceed the 1,460 days observed in this study.

The mobility of imidacloprid beyond the point of application was not entirely surprising because Jones et al. and Knoepp et al. both observed the horizontal movement of imidacloprid in soil.[8,59] The difference between this research and the previous studies were that this project specifically measured distances beyond the tree dripline. Jones et al. conducted a preliminary study of agricultural fields where imidacloprid was used and found it moved towards the edges of the fields, but the exact distance was not reported.[59] Knoepp et al. observed the horizontal movement of imidacloprid through soils around treated trees, but did not sample beyond the dripline of the tree, which was a max of 3 m from the tree base.[8] This project demonstrated that

imidacloprid is capable of moving at least 10 m away from the original application point, though the concentration generally decreased with distance (Fig. 3 and Fig. 4). The 2017 treatment tree had the highest residual concentrations probably because it was most recently treated and weather patterns at the time may have facilitated movement. Large amounts of precipitation were received during the sample collection time at the 2017 treatment plot. Because imidacloprid is hydrophilic, the movement observed was probably related to the amount of precipitation received. Regardless of the reasons behind imidacloprid movement through soil, the fact that it can move so far indicates greater likelihood for non-target organisms to be exposed.

Comparing the three treatment trees (2013, 2016, and 2017) to each other also proved interesting. All three treatment trees followed a similar pattern of having higher concentrations at the tree base, which decreased with distance (Fig. 5). The 2013 tree was expected to have lower concentrations compared to the 2016 and 2017 trees, however it proved to have higher concentrations than the 2016 treatment tree. This may have been due to the presence of other treated trees in close proximity to the 2013 sample location resulting in an additive affect. Bonmatin et al. observed a similar occurrence in French agricultural fields noting that repeated applications of imidacloprid over time increased its residual concentrations.[4,26,60] The other issue that may have resulted in lower concentrations at the 2016 tree vs. the 2013 tree may be due to laboratory technique. Samples collected from the 2016 were the first to be sampled and analyzed. While the overall technique and procedure had been practiced and finalized, it was still the first analytical batch and may have had operator errors that resulted in over or under estimated residual concentrations.

Residual Imidacloprid Concentration in Non-Target Plants

Imidacloprid was detected in non-target plant samples at all treatment locations and at all distances away from the tree base (Fig. 5). The amount of imidacloprid present in non-target plants was proportional to the amount detected in soil. It is possible that specific plants absorbed more imidacloprid than others, however plant samples for this project were homogenized and not examined by separate species. The ability of imidacloprid to translocate is similar to what Jones et al. noted in the hedges bordering agricultural fields.[59] The concentration levels of imidacloprid detected in plants collected for this study ranged from the LOD to 93.9 ppb. These concentrations are at levels that are considered toxic to invertebrates.[5,61] The significant relationship between imidacloprid soil concentration and imidacloprid plant concentration (r_s= 0.68, p<0.01) supports the initial hypothesis that imidacloprid will translocate into non-target plants and can reach levels that are toxic to non-target organisms.

Soil Respiration

Soil basal respiration was qualitatively ranked medium to high at all sampling locations, indicating both mineralization of organic matter and a microbially active soil ecosystem despite the presence of imidacloprid. This was similar to what Xiao-hua et al. detected with acetamiprid, another neonicotinoid pesticide.[62] In their study conducted over seven days, acetamiprid had no significant effect on soil respiration. While there was no statistical significance in the soil respiration tests conducted during this study (Tbl. 9; X^2 (1, N = 49) = 0.72, p = 0.39), the high observance of carbon dioxide is indicative of healthy ecosystem function.[32] The medium to high respiration rates may also have been indicative of the functional redundancy of ecosystem indicators and the amount of organic matter present around the base of each tree. New or

different bacteria may have moved in to replace any damaged bacteria during the application of imidacloprid and continued the work of decomposition, whereby respiration never changed.

Microbial Function

Biolog EcoPlates™ were used for examining microbial community function via substrate utilization. Rather than examining each substrate utilized, this study investigated the AWCD of each plate and compared the results after 72 hours. This was taken as an indication of microbial community function. The AWCD of control samples compared to treatment samples was not significantly different ($p > 0.05$), nor was it significantly different at specific distances away from the tree. While microbial activity was observed to be generally greater at the control location and appeared to increase with overall distance away from the tree base (Fig. 6), the overall conclusion is that microbial function as analyzed via carbon substrate utilization were unaffected by the presence of imidacloprid and in some cases appeared to increase. This is somewhat similar to observations by Manuel et al., with researchers noting that while imidacloprid appears to inhibit microbial abundance and activity initially, the community seemed to rebound in the long-term.[63] Other researchers noted that in general, cholinesterase inhibitors seem to have little effect on bacterial communities.[25] Based on the observations made during this project and the insignificant differences between control and treatment sites, it is evident that imidacloprid does not significantly alter microbial community function over time.

Invertebrate Density

Eleven different orders of invertebrates were detected across the sites sampled at Bays Mountain Park utilizing transect sampling. Comparing the density collected from the control and

treatment locations indicated no significant difference (Fig. 7, $p>0.05$) and no significant relationship between imidacloprid concentration and invertebrate density ($r_s= 0.46$, $p>0.05$). Despite the lack of significance, invertebrate density was observed to increase with the distance away from the tree. The lack of significance between treatment and control sites using transect sampling is similar to what D. Peck found when investigating surface and subsurface invertebrate populations in areas treated with imidacloprid. There was no difference in surface dwelling organisms collected via transect sampling, though there was a significant difference in beetle grubs collected in the subsurface.[6] Knoepp et al. also found no statistical significance between soil-dwelling microarthropod density and imidacloprid presence.[8] It is possible that the mobility of surface-dwelling organisms affords them a level of protection unavailable to subsurface dwelling organisms. Both Van der Sluijs et al.[37], and de Lima et al.[61] noted that imidacloprid negatively affects non-target invertebrates, though in de Lima et al.'s case the observations were made using controlled conditions where test species could not escape.[61] Based on the observations of this project, it is inconclusive that imidacloprid is affecting surface-dwelling invertebrate densities.

Limitations

Several issues were encountered during the course of this project including QA/QC concerns, cross-contamination between sites, inadequate sampling design, and lack of statistical power between sampling groups. The following sections further detail these issues.

QA/QC and Contamination

Quality assurance and quality control parameters were established at the beginning of this study and included the use of duplicates, replicates, spikes, field blanks, trip blanks, and lab blanks (Tbl. 3). Imidacloprid was detected in approximately 2% of lab blanks run through LCMS (Tbl. 11). Over 50% of spiked and duplicate samples did not result in the expected quantity of imidacloprid (Tbl. 11) and in some instances, the QA/QC sample contained higher quantities of imidacloprid than the original sample. Potential causes of the failed QA/QC samples included:

1. Use of a single sampling probe between sites that may not have been completed de-contaminated,
2. Non-homogeneity of samples resulting in inconsistent sample results,
3. Poor laboratory practices due to inexperience and rushing, and
4. Inadequate experimental design resulting in too few samples collected.

Sample Size

Initially, sample size was determined with the anticipation of using a one-sided t-test or ANOVA and keeping the overall cost of sample preparation and analysis low. Due to the small sample size of this experiment, it was decided to continue with statistical analysis of all samples regardless of the failed percent recovery. Only true samples (not QA/QC) were used for analyses. This did not leave enough room for operator error, contamination, or QA/QC issues resulting in the necessity to keep samples that were part of batches that failed QA/QC measures. The small sample size decreased the overall power of the statistical tests used, which were also lower because of the non-normal distribution of the data. In total, non-parametric statistical

tests, small sample sizes, and failed QA/QC measures may have affected the results, either inflating or deflating the observed outcome.

Additional Concerns

One sample location from 2013 had multiple trees around it that had been treated during the same time-period with imidacloprid. There were no single trees treated in 2013 that were of far enough distance away from other treated trees to use for sample collection and comparison. Consequently, an additive effect may have been observed and cannot be ruled out. Also, the time of year sample collection occurred was generally wet. Because imidacloprid is hydrophilic, this may have sped up the observed movement away from the point of application.

Conclusions

This project demonstrated that imidacloprid is capable of persisting and mobilizing through soils, which allow it to come in contact with non-target plants. Non-target plants were shown to absorb residual imidacloprid at rates that are proportional to the concentrations in soil. The concentration range detected in plants and soils are high enough to affect non-target organisms such as soil invertebrates but did not appear to affect the density of surface-dwelling invertebrates. Imidacloprid presence did not lead to significant differences in microbial activity as represented by soil respiration and microbial community function, which may be due to the natural redundancy of the ecosystem indicators. Consequently, this project supports the hypothesis that imidacloprid can mobilize, persist, and translocate at toxic concentrations, but it does not support the hypothesis that imidacloprid will alter the activities of functional indicators. Imidacloprid is beneficial in protecting native trees from invasive invertebrate populations, but

care should be taken during application to minimize the potential for mobility and translocation. Changing from the current application method of soil drench to tree injection may reduce the horizontal mobility and decrease the risk of unintended exposures.

REFERENCES

1. Jeschke P, Nauen R. Neonicotinoids - From zero to hero in insecticide chemistry. Pest Management Science. 2008;64(11):1084–1098.
2. Tomizawa M, Casida JE. Neonicotinoid insecticide toxicology: mechanisms of selective action. Annual review of pharmacology and toxicology. 2005;45:247–268.
3. Gibbons D, Morrissey C, Mineau P. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental Science and Pollution Research. 2014:103–118.
4. Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell E a D, et al. Environmental fate and exposure; neonicotinoids and fipronil. Environmental Science and Pollution Research. 2014:35–67.
5. Pisa LW, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Downs C a., Goulson D, Kreutzweiser DP, Krupke C, Liess M, McField M, et al. Effects of neonicotinoids and fipronil on non-target invertebrates. Environmental Science and Pollution Research. 2014;22(1):68–102.
6. Peck DC. Long-term effects of imidacloprid on the abundance of surface- and soil-active nontarget fauna in turf. Agricultural and Forest Entomology. 2009;11(4):405–419.
7. Van Dijk TC, Van Staalduinen M a., Van der Sluijs JP. Macro-invertebrate decline in surface water polluted with imidacloprid. PLoS ONE. 2013;8(5):e62374.
8. Knoepp JD, Vose JM, Michael JL, Reynolds BC. Imidacloprid movement in soils and impacts on soil microarthropods in southern Appalachian eastern hemlock stands. Journal of environmental quality. 2012;41(2):469–78.
9. MATSUDA K, SHIMOMURA M, IHARA M, AKAMATSU M, SATTELLE DB. Neonicotinoids Show Selective and Diverse Actions on Their Nicotinic Receptor Targets: Electrophysiology, Molecular Biology, and Receptor Modeling Studies. Bioscience, Biotechnology, and Biochemistry. 2005;69(8):1442–1452.
10. National Pesticide Information Center. Imidacloprid Technical Fact Sheet. Active Indgredient Fact Sheets. 2011.
11. Gibbons D, Morrissey C, Mineau P. A review of the direct and indirect effects of neonicotinoids and fipronil on vertebrate wildlife. Environmental science and pollution research international. 2015;22(1):103–118.
12. Van der Sluijs JP, Simon-Delso N, Goulson D, Maxim L, Bonmatin J-M, Belzunces LP. Neonicotinoids, bee disorders and the sustainability of pollinator services. Current Opinion in Environmental Sustainability. 2013;5(3–4):293–305.
13. Suchail S, Guez D, Belzunces LP. Characteristics of Imidacloprid Toxicity in Two Apis Mellifera Subspecies. Environmental Toxicology and Chemistry. 2000;19(7):1901–1905.

14. Decourtye A, Devillers J, Cluzeau S, Charreton M, Pham-Delègue MH. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicology and Environmental Safety. 2004;57(3):410–419.

15. U.S. Environmental Protection Agency. Preliminary Aquatic Risk Assessment to Support the Registration Review of Imidacloprid. 2016.

16. Dively GP, Embrey MS, Kamel A, Hawthorne DJ, Pettis JS. Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. PloS one. 2015;10(3):e0118748.

17. Li Y, Kelley R a., Anderson TD, Lydy MJ. Development and comparison of two multi-residue methods for the analysis of select pesticides in honey bees, pollen, and wax by gas chromatography–quadrupole mass spectrometry. Talanta. 2015;140:81–87.

18. Thompson H, Harrington P, Wilkins S, Pietravalle S, Sweet D, Jones A. Effects of neonicotinoids seed treatments on bumble bee colonies under field conditions. FERA (UK Food and Environment Research Agency). 2013;(March):76.

19. Whitehorn PR, O'Connor S, Wackers FL, Goulson D. Neonicotinoid Pesticide Reduces Bumble Bee Colony Growth and Queen Production. Science. 2012;336(6079):351–352.

20. USEPA. April 2015 Letter to Registrants Announcing New Process for Handling New Registrations of Neonicotinoids. 2015.

21. Gross M. Systemic pesticide concerns extend beyond the bees. Current Biology. 2014;24(16):R717–R720.

22. Goulson D. An overview of the environmental risks posed by neonicotinoid insecticides. Journal of Applied Ecology. 2013;50(4):977–987.

23. Hallmann C a, Foppen RPB, Turnhout C a M Van, Kroon H De, Jongejans E. Declines in insectivorous birds are associated with high neonicotinoid concentrations. Nature. 2014;511(7509):341–343.

24. Mineau, Pierre, Palmer C. The Impact of the Nation's Most Widely Used Insecticides on Birds. 2013.

25. Sánchez-Bayo F, Tennekes H a, Goka K. Impact of Systemic Insecticides on Organisms and Ecosystems. Insecticides - Development of Safer and More Effective Technologies. 2013:367–416.

26. Chagnon M, Kreutzweiser D, Mitchell E a D, Morrissey C a., Noome D a., Van der Sluijs JP. Risks of large-scale use of systemic insecticides to ecosystem functioning and services. Environmental Science and Pollution Research. 2014:119–134.

27. Koppenhöfer AM, Cowles RS, Cowles EA, Fuzy EM, Kaya HK. Effect of neonicotinoid synergists on entomopathogenic nematode fitness. Entomologia Experimentalis et Applicata. 2003;106(1):7–18.

28. Hladik ML, Kolpin DW, Kuivila KM. Widespread occurrence of neonicotinoid insecticides in streams in a high corn and soybean producing region, USA. Environmental Pollution. 2014;193:189–196.

29. Morrissey C a., Mineau P, Devries JH, Sanchez-Bayo F, Liess M, Cavallaro MC, Liber K. Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review. Environment International. 2015;74:291–303.

30. Rapport DJ, Costanza R, McMichael AJ. Assessing ecosystem health. Trends in Ecology & Evolution. 1998;13:397–402.

31. Groot RS De, Wilson M a, Boumans RMJ. A typology for the classification, description, and valuation of ecosystem, functions, goods and services. 2002;41(May):1–20.

32. Creamer RE, Hannula SE, Leeuwen JP Van, Stone D, Rutgers M, Schmelz RM, Ruiter PC de, Hendriksen NB, Bolger T, Bouffaud ML, et al. Ecological network analysis reveals the inter-connection between soil biodiversity and ecosystem function as affected by land use across Europe. Applied Soil Ecology. 2015;97:112–124.

33. Simon SA, Collins TK, Kauffman GL, Mcnab WH, Ulrey CJ. Ecological Zones in the Southern Appalachians: First Approximation. USFS Southern Research Station. 2005.

34. Austin DA, van de Gevel SL, Soulé PT. Forest dynamics and climate sensitivity of an endangered Carolina hemlock community in the southern Appalachian Mountains, USA. Botany. 2016;94(4):301–309.

35. USDA Forest Service. Imidacloprid - Human Health and Ecological Risk Assessment – Final Report. 2005. 283 p.

36. Benton E, Grant JF, Cowles R, Webster J, Nichols R, Lagalante A, Coots C. Forest Ecology and Management Assessing relationships between tree diameter and long-term persistence of imidacloprid and olefin to optimize imidacloprid treatments on eastern hemlock. Forest Ecology and Management. 2016;370:12–21.

37. Van der Sluijs JP, Amaral-Rogers V, Belzunces LP, Bijleveld van Lexmond MFIJ, Bonmatin JM, Chagnon M, Downs C a, Furlan L, Gibbons DW, Giorio C, et al. Conclusions of the Worldwide Integrated Assessment on the risks of neonicotinoids and fipronil to biodiversity and ecosystem functioning. Environmental Science Pollution Resources. 2014:148–154.

38. Burt R. Soil Survey Field and Laboratory Methods Manual. 2014;(51).

39. Mason BJ. EPA Preparation of Soil Sampling Protocol. 1983.

40. Sava R (California E. Guide to Sampling Air, Water, Soil, and Vegetation for Chemical Analysis. 1994.

41. Ruiz N, Lavelle P, Jiménez J. Soil macrofauna field manual . 2008:1–113.

42. Bays Mountain Park & Planetarium. About Us. 2017 [accessed 2017 Jan 1]. http://www.baysmountain.com

43. Davis HC, Hartgrove NC. Soil Survey of Sullivan County, Tennessee.

44. Morrell A, Dell PO, Priest J, Spencer CN, Baker G. Custom Soil Resource Report for Sullivan County, Tennessee. 2015.

45. Barth DS, Mason BJ, Starks TH, Brown KW. Soil Sampling Quality Assurance User’s

Guide. 1989:279.

46. AOAC International. AOAC Official Method 2007.01 Pesticide Residues in Foods by Acetonitrile Extraction and Partitioning with Magnesium Sulfate. Official Methods of Analysis of AOAC International. 2011;90(2):17–26.

47. Anastassiades M, Lehotay S, Stajnbaher D, Schenck F. Fast and Easy Multiresidue Method Employing Acetonitrile Extraction/Partitioning and "Dispersive Solid-Phase Extraction" for the Determination of Pesticide Residues in Produce. Journal of AOAC Int.AOAC Int. 2003;86:412–431.

48. Vera J, Correia-Sá L, Paíga P, Bragança I, Fernandes VC, Domingues VF, Delerue-Matos C. QuEChERS and soil analysis. An Overview. Sample Preparation. 2013;1:54–77.

49. Souza D, E S, Borges E. Determination of Pesticides in Grape Juices by QuEChERS and Liquid Chromatography-Tandem Mass Spectrometry. Journal of Brazilian Chemical Society. 2016;27(9):1626–1635.

50. UCT. Analysis of Neonicotinoids in Honey by QuEChERS and UHPLC-MS / MS. 2015.

51. Rutgers M, Wouterse M, Drost SM, Breure AM, Mulder C, Stone D, Creamer RE, Winding A, Bloem J. Monitoring soil bacteria with community-level physiological profiles using Biolog^{TM} ECO-plates in the Netherlands and Europe. Applied Soil Ecology. 2016;97:23–35.

52. Gryta A, Frac M, Oszust K. The Application of the Biolog EcoPlate Approach in Ecotoxicological Evaluation of Dairy Sewage Sludge. Applied Biochemistry and Biotechnology. 2014;174(4):1434–1443.

53. Gleason H, Cronquist A. Manual of Vascular Plants of Northeastern United States and ADjacent Canada. New York: D. Van Nostrand Company; 1963.

54. Barnes R. Invertebrate Zoology. Philadelphia: W.B. Saunders Company; 1974.

55. US EPA. Quality Assurance/Quality Control (QA/QC) for 301h Monitoring Programs: Guidance on Field and Laboratory Methods. 1987.

56. US EPA. Description and Sampling of Contaminated Soils. 1991.

57. Cox L, Koskinen WC, Celis R, Hermosin MC, Cornejo J, Yen PY. Sorption of Imidacloprid on Soil Clay Mineral and Organic Components. Soil Science Society of America Journal. 1998;(62):911–915.

58. Papiernik SK, Koskinen WC, Cox L, Rice PJ, Clay SA, Werdin-Pfisterer NR, Norberg KA. Sorption-desorption of imidacloprid and its metabolites in soil and vadose zone materials. Journal of Agricultural and Food Chemistry. 2006;54(21):8163–8170.

59. Jones A, Harrington P, Turnbull G. Neonicotinoid concentrations in arable soils after seed treatment applications in preceding years. Pest Management Science. 2014;70(12):1780–1784.

60. Bonmatin JM, Moineau I, Charvet R, Colin ME, Fleche C, Bengsch ER. Behaviour of Imidacloprid in Fields. Toxicity for Honey Bees. In: Lichtfouse E, J S, Robert D, editors. Environmental Chemistry. Berlin: Springer; 2005. p. 483–494.

61. Lima C De, Brennan N, Brouwer JM, Commandeur D, Verweij RA, Gestel CAM Van. Comparative toxicity of imidacloprid and thiacloprid to different species of soil invertebrates. Ecotoxicology. 2017:555–564.

62. Yao X, Min H, Lü Z, Yuan H. Influence of acetamiprid on soil enzymatic activities and respiration. 2006;42:120–126.

63. Manuel J, Diaz C, Martin-laurent F, Beguet J, Nogales R, Romero E. Science of the Total Environment Fate and effect of imidacloprid on vermicompost-amended soils under dissimilar conditions : Risk for soil functions , structure , and bacterial abundance. Science of the Total Environment, The. 2017;579:1111–1119.

64. Stone D, Ritz K, Griffiths BG, Orgiazzi A, Creamer RE. Selection of biological indicators appropriate for European soil monitoring. Applied Soil Ecology. 2016;97:12–22.

65. De Bello F, Lavorel S, Diaz S, Harrington R, Cornelissen JHC, Bardgett RD, Berg MP, Cipriotti P, Feld CK, Hering D, et al. Towards an assessment of multiple ecosystem processes and services via functional traits. Biodiversity and Conservation. 2010;19(10):2873–2893.

66. Van Bruggen AHC, Semenov AM. In search of biological indicators for soil health and disease suppression. Applied Soil Ecology. 2000;15(1):13–24.

APPENDIX

Tables and Figures

Tables

Table 1. Indicators of Ecosystem Function.

Indicators of Ecosystem Function	Ecosystem Function (regulatory, habitat, production, information)	Source
Microbial Diversity	Regulatory Habitat Production	Stone et al. (2016)[64] Creamer et al. (2015)[32]
Invertebrate and Mesofauna Diversity and Abundance (Identified by molecular methods or morphology) • Earthworms, • Enchytraeids, • Mites, • Collembola, • Nematodes, • Protista	Regulatory Habitat Production	Stone et al. (2016)[64] Creamer et al. (2015)[32]
Microfauna Diversity • Pyrosequencing of soil DNA	Habitat Production	Stone et al. (2016)[64] Creamer et al. (2015)[32]
Functional Indicators/Genes • Antibiotic Producers, • Extracellular Enzyme Assays (EEA), • Community Level Physiological Profiling (CLPP) • Nitrifiers, • Denitrifiers	Regulatory Habitat Production	Stone et al. (2016)[64] Creamer et al. (2015)[32]
Functional Traits • Decomposition/Mineralization • Nutrient/Sediment Retention • Fodder Productivity • Evapotranspiration • Herbivory • Carbon Sequestration • Soil Formation • Superficial Water Flow Control • Soil Erosion • Pollination • Invasion Resistance • Fire Risk Control • Pest Regulation	Regulatory Habitat Production Information	de Bello et al. (2010)[65]

Indicators of Ecosystem Function	Ecosystem Function (regulatory, habitat, production, information)	Source
• Fiber Production • Soil Water Flux Control • Heat Exchange • Primary Production • Livestock Consumption/Health • Fishery for Recreation • Sense of Place • Hurricane/Wind Risk Control • Permafrost Insulation • Seed Dispersal • Allergenic Control • Habitat Provision		
Respiration • Basal, • SIR-Glucose, • Multiple Substrate Induced, • BIOLOG	Regulatory	Stone et al. (2016)[64] Creamer et al. (2015)[32]
Molecular Microbial Biomass	Regulatory Production	Stone et al. (2016)[64]
Nitrification Potential	Regulatory Production	Stone et al. (2016)[64]
Multiple Enzyme Assay	Regulatory Production	Stone et al. (2016)[64]
Litter Bags	Habitat Production	Stone et al. (2016)[64]
Plant/Animal Disease Outbreak	Habitat Production Information	van Bruggen et al. (2000)[66]
Bacterial Succession Analysis • Index of Microbial Succession Stage • Ratio of Copiotrophic bacteria to Oligotrophic • Ratio of Respiration to Microbial Biomass	Regulatory Habitat Production	van Bruggen et al. (2000)[66]

Table 3. Data Transformations and Normality Testing of Soil and Plant Data

Statistic	Soil Samples				Plant Samples			
	Raw Data	*Log (x+1)*	*Ln (x+1)*	*SqRt (x+1)*	*Raw Data*	*Log (x+1)*	*Ln (x+1)*	*SqRt (x+1)*
Mean	46.39	1.04	2.41	5.03	8.85	0.59	1.37	2.49
Standard Error	6.99	0.06	0.13	0.33	2.61	0.09	0.20	0.29
Median	15.36	1.21	2.79	4.05	2.54	0.55	1.26	1.88
Mode	0	0.00	0.00	1.00	0.00	0.00	0.00	1.00
Standard Deviation	100.14	0.82	1.89	4.71	17.30	0.57	1.31	1.93
Sample Variance	10027.37	0.67	3.57	22.16	299.40	0.32	1.71	3.74
Kurtosis	33.05	-1.19	-1.19	5.60	14.33	-0.58	-0.58	4.12
Skewness	4.98	-0.01	-0.01	1.99	3.50	0.60	0.60	1.91
Range	925.62	2.97	6.83	29.44	93.90	1.98	4.55	8.74
Minimum	0	0.00	0.00	1.00	0.00	0.00	0.00	1.00
Maximum	925.62	2.97	6.83	30.44	93.90	1.98	4.55	9.74
Sum	9509.55	214.16	493.11	1031.88	389.45	26.14	60.20	109.54
Count	205	205.00	205.00	205.00	44.00	44.00	44.00	44.00
Confidence Level (95.0%)	13.79	0.11	0.26	0.65	5.26	0.17	0.40	0.59

Table 4. Descriptive Statistics for Imidacloprid Concentrations in Soil (ppb)

Tree Description	N	Min	Q1	Median	Q3	Max
2013 Tree	36	2.00	3.03	23.0	44.3	124.4
2016 Tree	35	2.0	2.0	3.1	29.4	407.9
2017 Tree	39	10.7	27.3	49.4	86.5	925.6
Control Tree	36	2.00	2.00	2.00	3.63	19.0

Table 6. Descriptive Statistics for Imidacloprid Concentrations in Non-Target Plants (ppb)

Tree Description	Distance	Median	Average	Standard Deviation
Control	0 m	2.68	2.46	0.40
Control	1 m	2.47	2.47	0.67
Control	5 m	2.00	2.00	0
Control	10 m	2.00	2.00	0
2013	0 m	11.8	11.8	13.9
2013	1 m	2.00	2.00	0
2013	5 m	3.67	3.67	1.91
2013	10 m	2.17	2.17	25.1
2016	0 m	31.2	31.2	38.5
2016	1 m	2.00	2.00	0
2016	5 m	2.28	2.28	0.40
2016	10 m	3.06	3.06	1.50
2017	0 m	49.6	49.6	62.7
2017	1 m	20.8	20.8	12.9
2017	5 m	14.6	13.9	7.88
2017	10 m	4.76	10.9	15.0

Table 7. Chi-Square Contingency Table for Soil Respiration

	Soil Respiration Between Control and Treatment Plots		
	High	**Medium**	
Control	10 *10.78* (0.06)	2 *1.22* (0.49)	**12**
Treatment	34 *33.22* (0.02)	3 *3.78* (0.16)	**37**
	44	**5**	**49**

X^2 (1, N = 49) = 0.72, p = 0.39

Table 8. Descriptive Statistics for Invertebrate Density

Tree Description	Distance	Total Count	Median	Average	Standard Deviation
Control	0 m	18	2	3.00	2.68
Control	1 m	10	2	2.00	1.22
Control	5 m	26	2	3.71	3.45
Control	10 m	26	4	5.20	5.02
2013	0 m	10	1	2.00	1.41
2013	1 m	12	1	2.00	1.57
2013	5 m	5	1	1.25	0.50
2013	10 m	8	2	2.00	0.82
2016	0 m	16	1.5	2.00	1.20
2016	1 m	20	2	2.86	2.27
2016	5 m	15	1	1.88	1.25
2016	10 m	34	2	4.86	4.78
2017	0 m	7	1.5	1.75	0.96
2017	1 m	13	1.5	1.63	0.74
2017	5 m	14	1	1.27	0.47
2017	10 m	21	2	3.00	2.65

Table 9. QA/QC Results

Sample Matrix	Analysis	N	Rep. (n)	Dup. (n)	Spike (n)	Rep. >20% RPD	Dup. >20% RPD	Spike %R <70%, >120%	>20% RPD Range	Spike %R Range
Soil	LCMS	292	33	35	16	20	18	8	31.19-192.97	2.0-62.2; 156.9-195.4
Plants	LCMS	62	4	4	2	2	0	2	30.62-66.24	167.75-181.62
Soil	Solvita Soil Respiration Kit	57	4	4	0	0	0	n/a	n/a	n/a

Figures

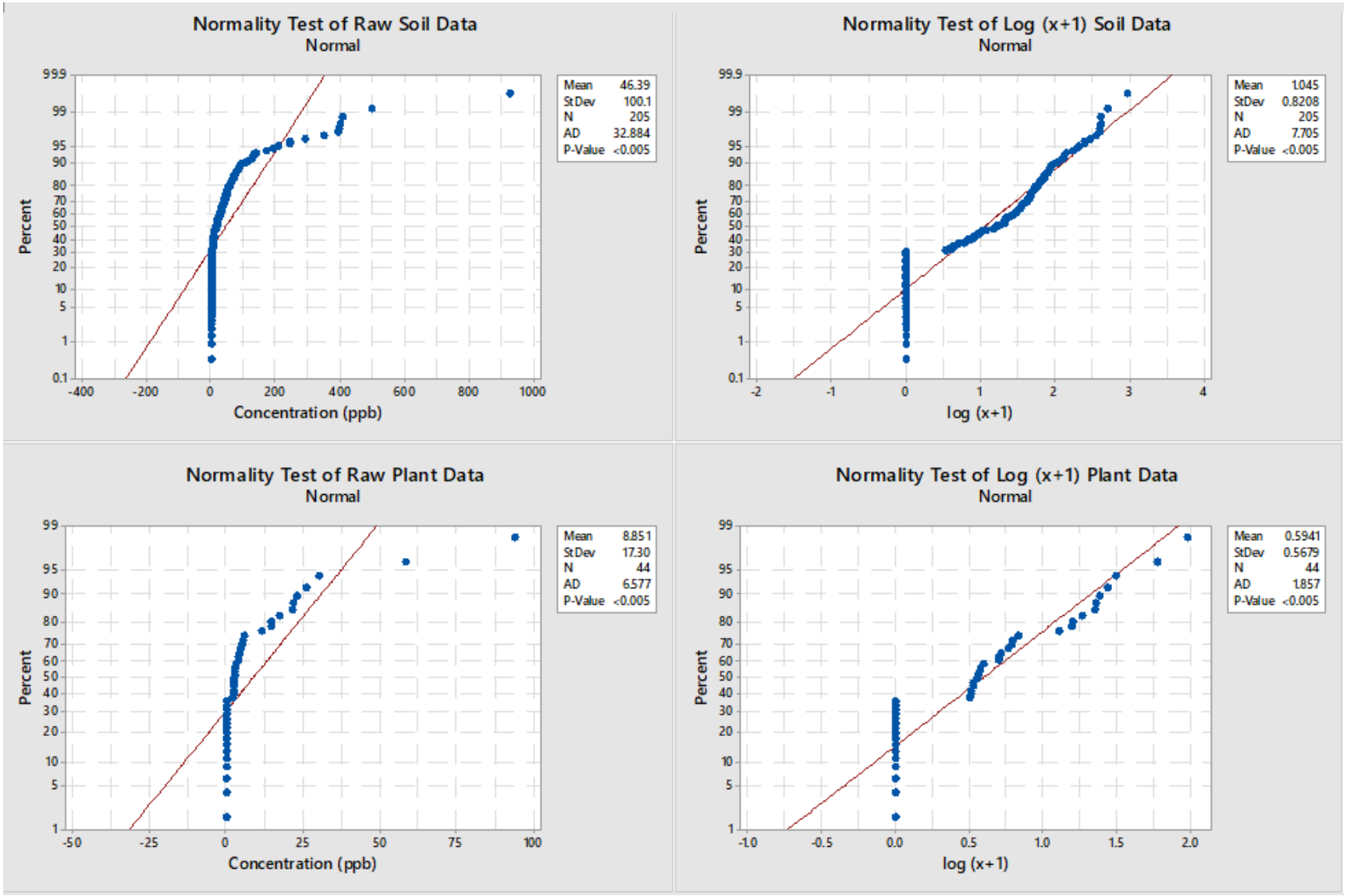

Figure 2. Normality Tests of Soil and Plant Data. Includes raw data and log (x+1) transformations.

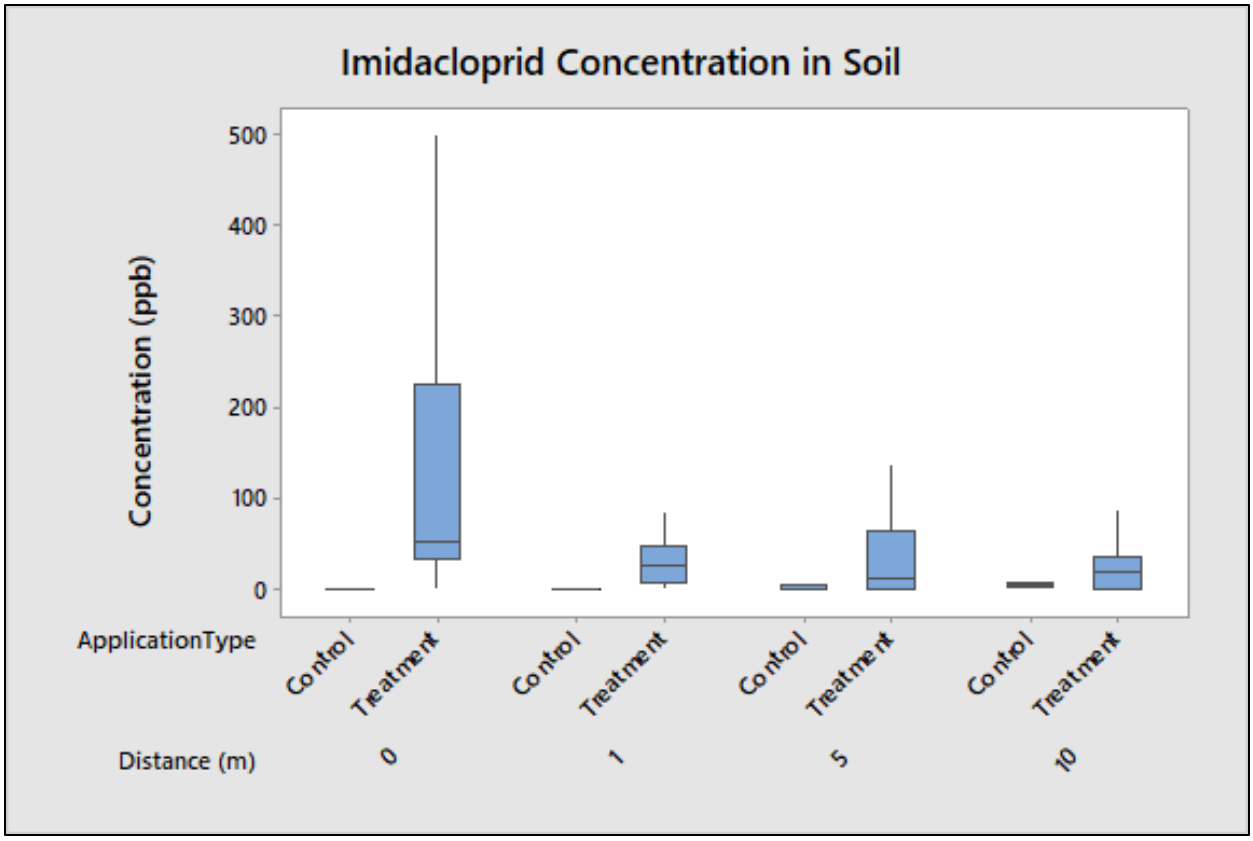

Figure 3. Imidacloprid Concentrations in Soil. Analysis by Kruskal-Wallis. Significant differences between control and treatment medians at all distances. Distance 0 m, Control plot (n = 10) IQR = 0.59 ppb, Treatment plot (n = 41) IQR = 193.0 ppb (p = 2.6E-06); Distance 1 m, Control plot (n = 9) IQR = 0 ppb, Treatment plot (n = 39) IQR = 38.8 ppb (p = 8.8E-05); Distance 5 m, Control plot (n = 15) IQR = 5.24 ppb, Treatment plot (n = 42) IQR = 62.5 ppb (p = 0.01); Distance 10 m, Control plot (n = 18) IQR = 4.79, Treatment plot (n = 31) IQR = 35.2 (p = 0.04).

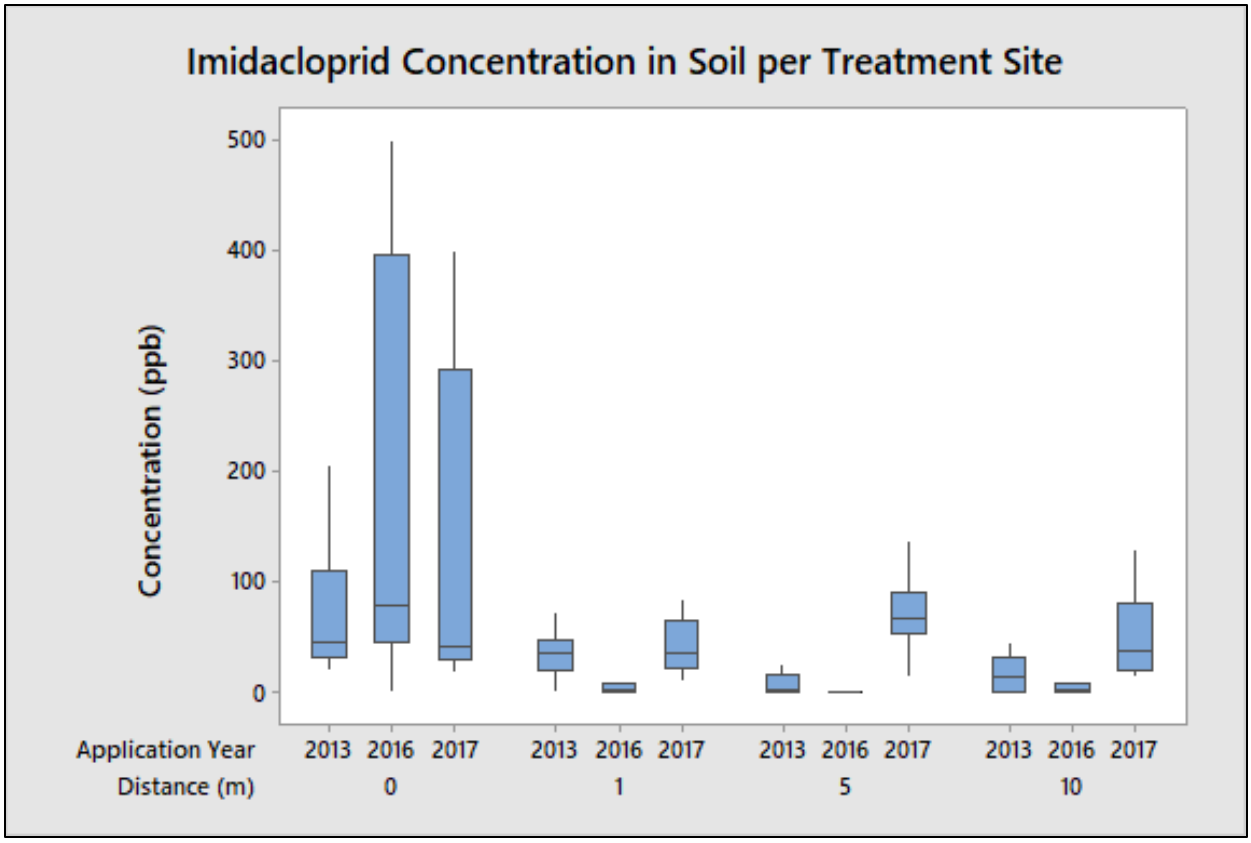

Figure 4. Imidacloprid Concentration in Soil per Treatment Site. Analysis by Kruskal Wallis. Significant differences between treatment plot medians were found at 1 m, 5 m, and 10 m. Distance 0 m, 2013 Tree (n = 14) IQR = 77.9 ppb, 2016 Tree (n = 12) IQR = 351.5 ppb, 2017 Tree (n = 15) IQR = 262.7 ppb (p = 0.25); Distance 1 m, 2013 Tree (n = 15) IQR = 26.2 ppb, 2016 Tree (n = 9) IQR = 7.23 ppb, 2017 Tree (n = 15) IQR = 44.1 ppb (p = 0.001); Distance 5 m, 2013 Tree (n = 15) IQR = 15.3 ppb, 2016 Tree (n = 12) IQR = 0 ppb, 2017 Tree (n = 15) IQR = 38.4 ppb (p = 1.1E-05); Distance 10 m, 2013 Tree (n = 9) IQR = 30.8 ppb, 2016 Tree (n = 10) IQR = 7.42 ppb, 2017 Tree (n = 12) IQR = 60.4 ppb (p = 0.0004).

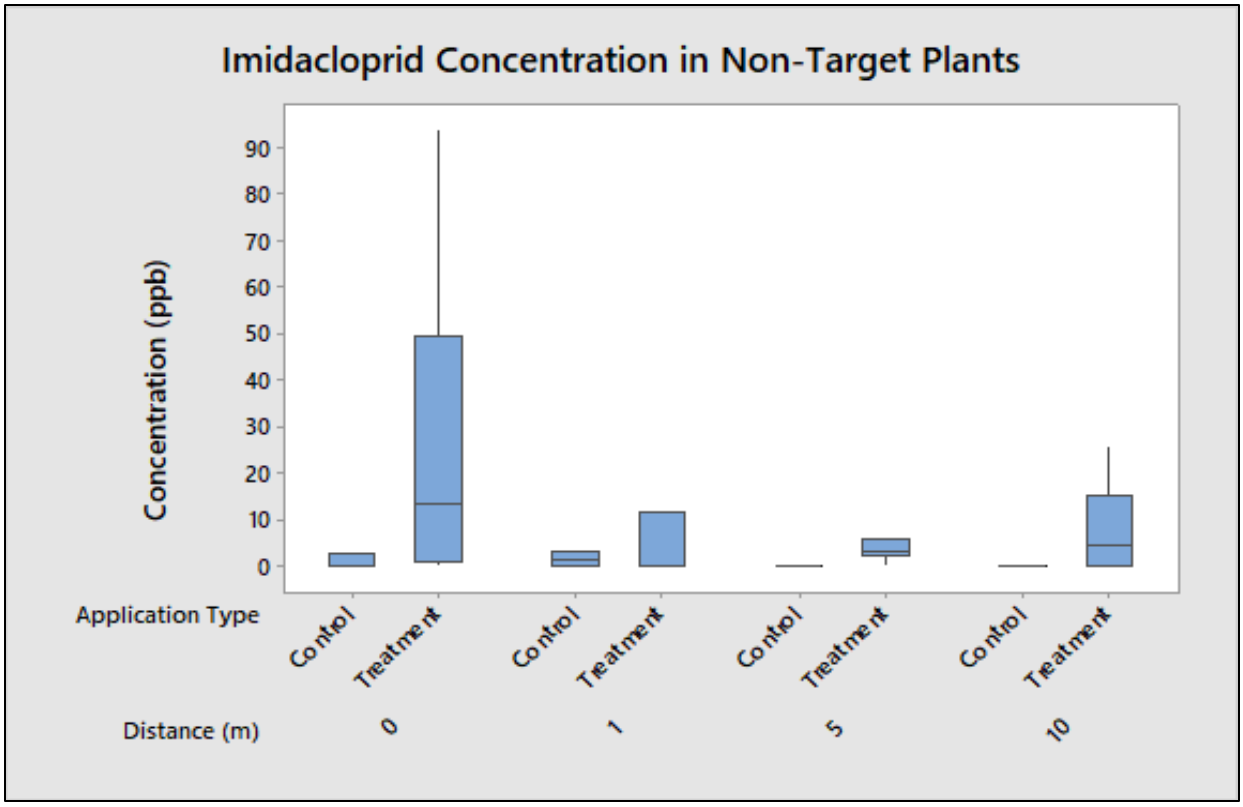

Figure 5. Imidacloprid Concentration in Non-Target Plants. Analysis by Kruskal-Wallis. Significant differences between control and treatment medians at 5 m, but no significant difference between medians at other distances. Distance 0 m, Control plot (n = 3) IQR= 2.69, Treatment plot (n = 8), IQR= 48.6 (p = 0.15); Distance 1m, Control plot (n = 2) IQR = 2.94, Treatment plot (n = 7) IQR = 11.7 (p = 0.88); Distance 5 m, Control plot (n = 3) IQR = 0, Treatment plot (n = 8) IQR = 3.16 (p = 0.03); Distance 10 m, Control plot (n = 3) IQR = 0, Treatment plot (n = 10) IQR = 15.3 (p = 0.08).

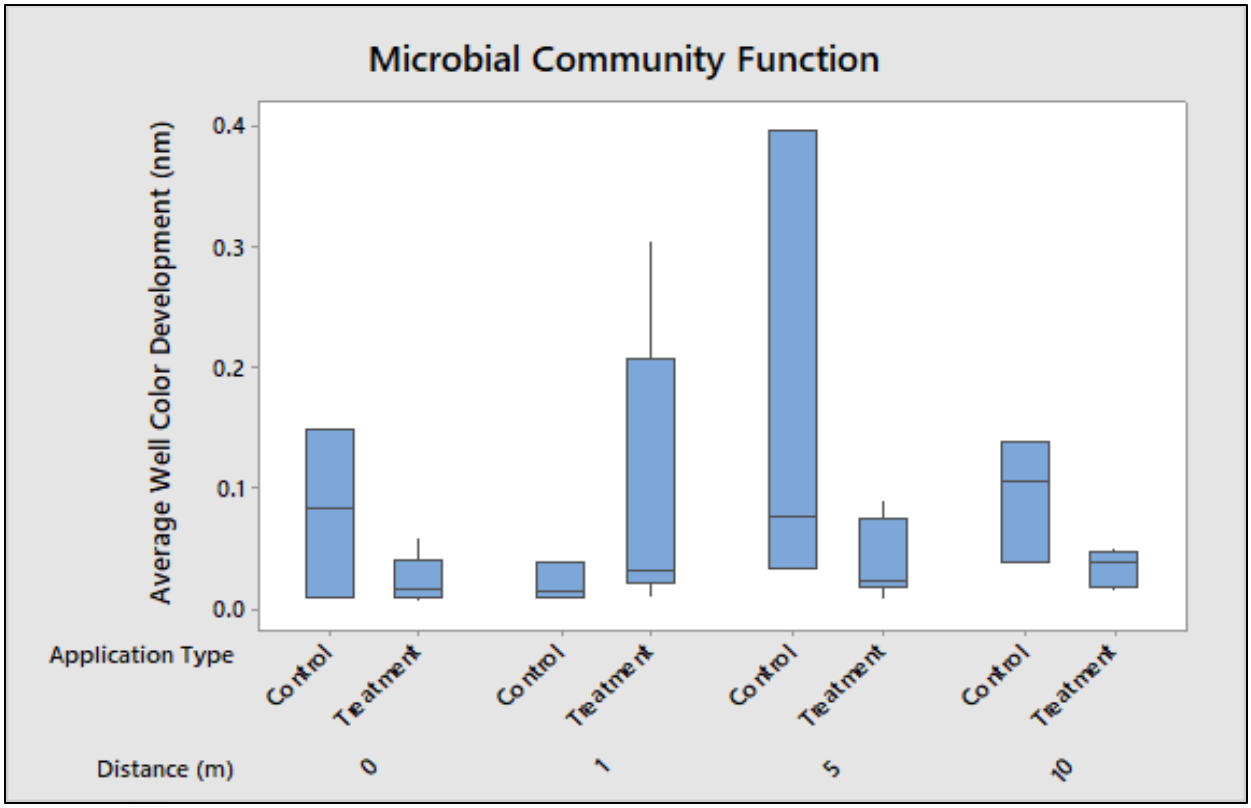

Figure 6. Microbial Community Function. Boxplot of microbial function via Biolog EcoPlate™ analysis 72 hours after incubation. Analysis by Kruskal-Wallis. No significant difference in medians at any distance. Distance 0 m, Control plot (n=3) IQR = 0.14 nm, Treatment plot (n=9) IQR =0.03 nm (p = 0.23); Distance 1 m, Control plot (n=3) IQR = 0.03 nm, Treatment plot (n=9) IQR= 0.19 nm (p = 0.23); Distance 5 m, Control plot (n=3) IQR = 0.36 nm, Treatment plot (n=10) IQR = 0.06 nm (p = 0.13); Distance 10 m, Control plot (n=3) IQR = 0.10 nm, Treatment plot (n=9) IQR = 0.03 nm (p = 0.08).

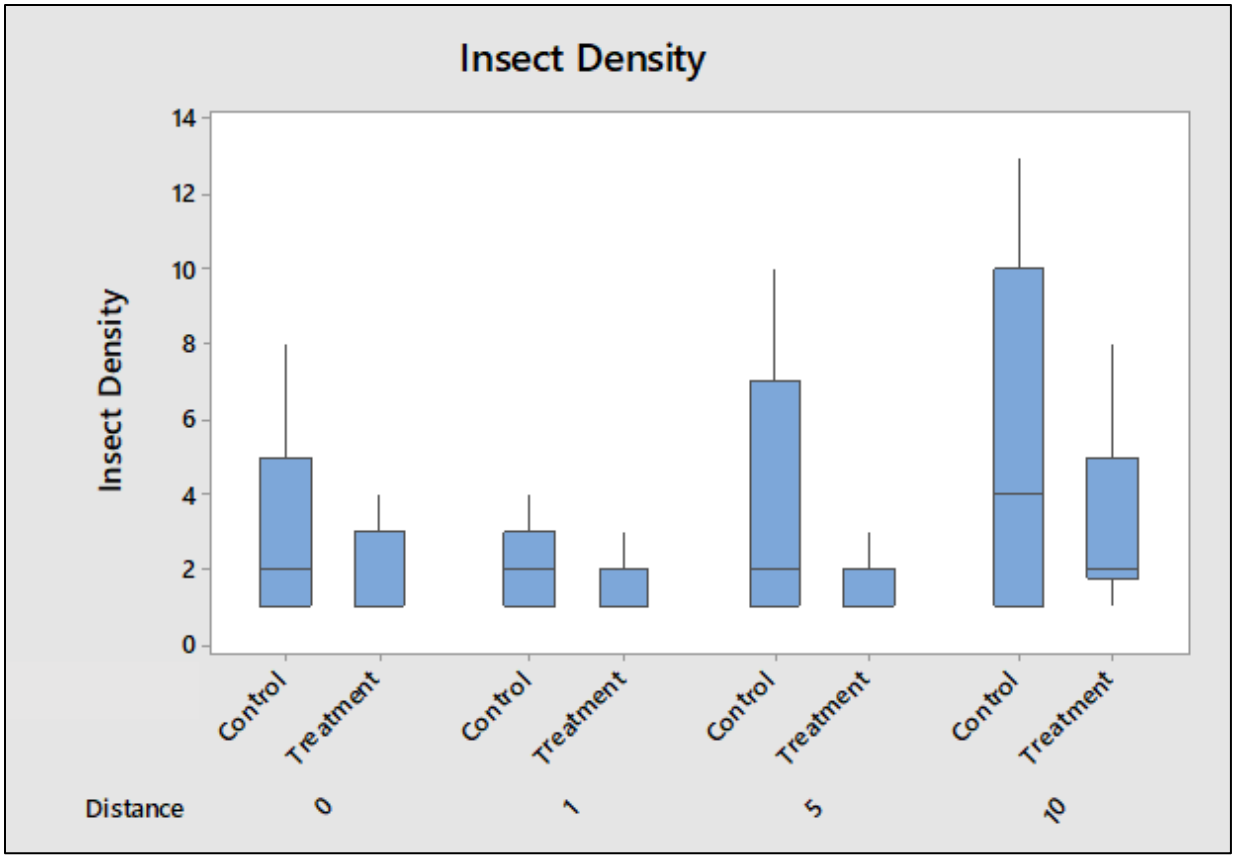

Figure 7. Invertebrate Density. Boxplot of invertebrate density from control and treatment plots. Analysis by Kruskal-Wallis. Distance 0 m, Control plot (n=6) IQR = 4, Treatment plot (n =17) IQR = 2 (p = 0.44); Distance 1 m, Control plot (n=5) IQR = 2, Treatment plot (n=21) IQR= 1 (p = 0.87); Distance 5 m, Control plot (n=7) IQR = 6, Treatment plot (n=23) IQR = 1 (p = 0.06); Distance 10 m, Control plot (n=5) IQR = 9, Treatment plot (n=18) IQR = 3.25 (p = 0.71).

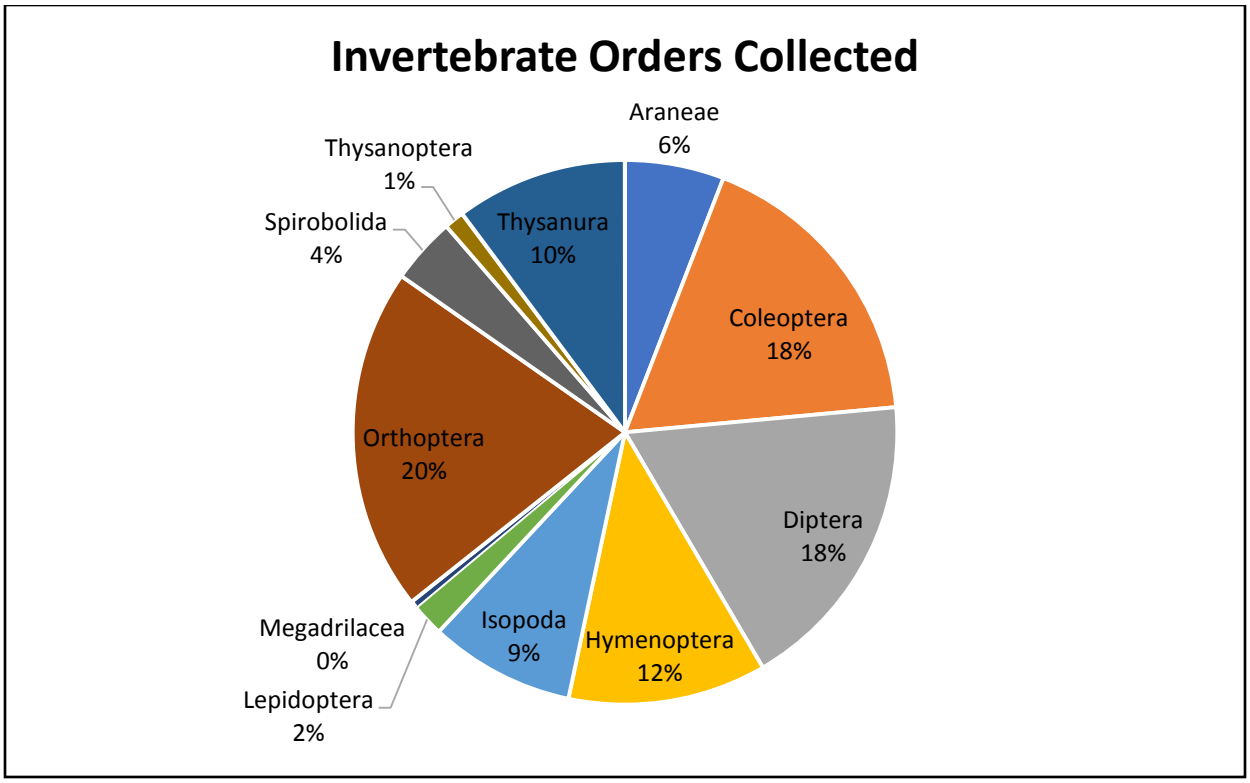

Figure 8. Invertebrate Orders Collected. Percentage of invertebrate orders collected across all sampling locations.

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