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IMPACTS OF INTRODUCED EARTHWORMS ON SUGAR MAPLE SAP
CHEMISTRY AND THE DISCOURSE OF INVASION ECOLOGY

By

Shelby Nicole Lane-Clark

A THESIS

Submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In Forest Ecology and Management

MICHIGAN TECHNOLOGICAL UNIVERSITY

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This thesis has been approved in partial fulfillment of the requirements for the Degree of MASTER OF SCIENCE in Forest Ecology and Management.

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Author Contribution Statement

This thesis is comprised of two main chapters formatted for different target journals to be published at a later date.

The chapter titled *Shifting the Discourse of 'Invasion' Ecology to 'Introduced Species': the Story of Earthworms in Northeastern North America* is a writing collaboration prepared jointly with myself as the lead author, and the 2nd and 3rd authors being Dr. Valoree S. Gagnon and Dr. Tara L. Bal.

The chapter titled *Exploring potential impacts of introduced earthworms (Annelida: Lumbricidae) on sap chemistry in sugar maple (Acer saccharum) in the Great Lakes region* is a writing collaboration between myself and Dr. Tara L. Bal. I personally completed all associated fieldwork and some chemical analysis. Significant contributions to chemical analysis were made by Sulihat Aloba and Dr. Rebecca G. Ong, and Dr. Carsten Kulheim made significant contributions to statistical analysis.

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Abstract

To delve into further understanding of the relationships between introduced earthworms, forest ecosystems, and the human systems they impact, two approaches were taken. In the first approach, we seek to explore ways we can shift the discourse within and regarding the field of invasion ecology by re-framing how we approach discussion, management, and education regarding introduced species. Language issues surrounding introduced species through the example of earthworms in North America are described and potential solutions are provided, including a repositioned perspective that may facilitate better relationships with the natural world. The goal is to shift the language to be more conscientious and respectful while also promoting inclusivity and diversity of perspectives that will lead to advancements in the field. A theme running through this narrative is approaching the field of invasion ecology through two-eyed seeing, which is an illustrative way to describe equitable utilization of the unique strengths of Western and Traditional Ecological Knowledge systems to create inclusive, holistic, integrative, multi-perspective and multi-disciplinary solutions to ecological issues.

The second approach described here is a pilot study looking into the possible changes that earthworm activity incurs in sugar maple sap. The introduction of earthworms has led to significant ecological impacts in northern hardwood forests, especially on sugar maples, as declines in sugar maple health have been correlated with introduced earthworm activity. Sugar maple sap was collected during the spring of 2023, along with soil sampling, and earthworm population data to explore potential impacts of introduced earthworms on overall sap sugar content using linear regression models. The results highlight a complex, dynamic network of impacts that begin with earthworm activity that induce changes within the soil that subsequently cause shifts in soil biogeochemistry, impacting the overall health of the sugar maples, and leading to the long-term impairment of impacted sugar maples to produce quality sap. Most notably, the presence of anecic earthworms at sites with long-established earthworm communities is negatively correlated with overall sugar content in sap ($R^2 = 0.52$, $p = 0.038$). These findings have important relevance especially to the maple syrup and maple sugar industries.

Overall, this thesis highlights the importance of understanding the relationships introduced organisms are building in their new environments. By taking the time to learn from these organisms, we can create more effective management practices and policies.

1 Introduction

1.1 Overview and Purpose of Thesis

The introduction and subsequent spread of organisms outside of their historical ranges has led to detrimental impacts to ecosystems, economies, and human health around the globe (Reo et al., 2017). These introductions are the result of human activity, whether directly or indirectly, and we do not yet fully understand the repercussions they are having on the environment (Eisenhauer et al., 2014; Larson et al., 2005). Because of this, the field of invasion ecology has become more important than ever as we work to respond to and remediate the impacts introduced species are causing (Lowry et al., 2013). Unfortunately, scientific spaces, especially in crisis disciplines like invasion ecology, can be exclusionary due to the damaging xenophobic language used in discourse regarding introduced species and the way we manage them, which is based in militaristic Western ideology (Cheng et al., 2023; Larson et al., 2005; Reid et al., 2020).

Chapter 2 of this thesis, *Shifting the Discourse of 'Invasion' Ecology to 'Introduced Species': the Story of Earthworms in Northeastern North America*, seeks to explore ways we can shift the discourse within and regarding the field of invasion ecology by re-framing how we approach discussion, management, and education regarding introduced species. The goal of this chapter is to shift the language to be more conscientious and respectful while also promoting inclusivity and diversity of perspectives that will lead to advancements in the field. In this chapter, the running theme is approaching the field of invasion ecology through two-eyed seeing, which is an illustrative way to describe equitable utilization of the unique strengths of Western and Traditional Ecological Knowledge systems to create inclusive, holistic, integrative, multi-perspective and multi-disciplinary solutions to ecological issues (Gibbs et al., 2014; Reid et al., 2020; Shaw et al., 2023).

As humans, we are primarily responsible for the introduction and spread of ecologically problematic species (Larson et al., 2005; Reo & Ogden, 2018). Additionally, as stewards of the natural world, it is our duty to serve the natural world by taking responsibility for our actions and their subsequent repercussions and by working to heal the relationships that have been damaged by our ignorance and negligence (Bach & Larson, 2017). Harmful language and ideologies like those that permeate the field of invasion ecology harm the way we relate to and interact with the natural world, our more-than-human kin, and each other, and this chapter proposes ways in which we can begin to repair these relationships through themes of interconnectedness, reciprocity, and building healthier, more resilient relationships (Gibbs et al., 2014; Larson et al., 2005; Reid et al., 2020; Reo & Ogden, 2018; Shaw et al., 2023).

Taking the time to observe how introduced organisms behave within and interact with their new environment, as well as observing how the environment and other organisms are responding to their introduction, is essential for creating effective response plans for how best to serve impacted ecosystems and mitigate harm (Bach & Larson, 2017; Bhattacharyya & Larson, 2014; Kimmerer, 2021; Reid et al., 2020). This is especially

true for introduced species whose impacts we are still trying to understand, which is true of earthworms that have been introduced to the northern hardwood forests of North America in the past 100-300 years during colonization of the region by European settlers (Gates, 1982). Since their introduction, earthworms have become widely distributed due to their association with human activity, although it didn't become recognized in scientific literature until the 1980's and 90's that they are not endemic to the northern forests of North America (Cameron & Bayne, 2009; Gates, 1982; Holdsworth et al., 2007). Because of this, there is still much that is unknown or unclear about the impacts these earthworms are having in their new environment.

Chapter 3 of this thesis, *Exploring potential impacts of introduced earthworms (Annelida: Lumbricidae) on sap chemistry in sugar maple (Acer saccharum) in the Great Lakes region*, explores a novel aspect of introduced earthworm ecology and acts as a pilot study looking into the possible changes that earthworm activity incurs in sugar maple sap. It has been well documented in previous literature (e.g. Fahey et al., 2013; Ferlian et al., 2020; Frelich et al., 2006; Hale & Host, 2005) that earthworms cause drastic changes in the soil of northern hardwood forests by altering soil texture and composition, disrupting soil microbial communities, and increasing the rate of nutrient cycling.

The texture and nutrient content of the soil is important to the health and vigor of sugar maples and also the chemistry of maple sap, especially the availability of nutrients like Ca, P, Mg, and K, the loss of which is correlated with maple dieback and decline and subsequent changes in maple sap chemistry (West et al., 2023; Wild & Yanai, 2015). Interestingly, introduced earthworm activity has also been found to be correlated with maple dieback and decline (Bal et al., 2017). This potential association between introduced earthworms and changes in maple sap chemistry is the ultimate focus of this chapter, as the maple syrup industry in North America is an important contributor to the economies of Canada and the United States, and the potential impacts earthworms may have on this industry is yet unknown (Farrell & Chabot, 2012; Ramadan et al., 2021).

The results of the study relayed in chapter 3 describe a complex, dynamic network of impacts that begin with earthworm activity that causes changes to nutrient availability within the soil and impairment of the sugar maples' ability to uptake nutrients from the soil due to rooting stress, which subsequently cause declines in the overall health of the sugar maples. This may ultimately culminate in long-term impairment of impacted sugar maples to produce quality sap. There is still much that is unclear about this dynamic, as this study provides only a snapshot in time of the relationship between introduced earthworms and sugar maples. It will be important to further explore this subject in the future, especially by doing long-term observation of how sap chemistry changes over time with earthworm introduction and establishment of multiple species.

Overall, this thesis aims to set an example of how we can change the way we approach the field of invasion ecology by turning the focus to better understanding the relationships introduced organisms are building in their new environments. By taking the time to learn from these organisms and build relationships of understanding, we can better tailor management practices and policy to most effectively mitigate harm.

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2. Shifting the Discourse of ‘Invasion’ Ecology to ‘Introduced Species’: the Story of Earthworms in Northeastern North America

Discourse used to describe introduced species has significant impacts on society's perception, yet there is often a disconnect between public and scientific spheres. Here, we describe language issues surrounding introduced species through the example of earthworms in North America. Communication related to “invasives” is rife with problematic, exclusionary language. We provide potential solutions, including a repositioned perspective that may facilitate better relationships with the natural world by applying the two-eyed seeing framework. Our discussion calls for a paradigm shift for deeper understandings of human and more-than-human relationships. Ultimately, we argue for respectful, considerate, and intentional language and stewardship.

2.1 Introduction

Human activity across our planet has led to the introduction and spread of innumerable species beyond their historical ranges. Both directly and indirectly, introduced species have resulted in significant ecosystem change and devastating ecological (and economic) loss in North America and globally (Reo et al., 2017). As the Anthropocene continues, repercussions become ever more apparent (Larson et al., 2005). Anthropogenic impacts, such as climate change and globalization of trade, are influencing species range shifts in ways not yet fully understood, which makes the study and management of introduced species challenging and uncertain (Eisenhauer et al., 2014). Moreover, there is often a disconnect between scientific and public discourse concerning environmental issues. Effective management of problematic species is dependent on both environmental and social factors (Lakoff, 2010; Schüttler et al., 2011). We assert that time-sensitive issues that require urgent action, such as introduced species and their related discourse, building effective communication is key to engaging the public, creating diverse, multi-scaled resolution options, and motivating effective action (Cameron et al., 2013).

The language used to describe introduced species can have significant impacts on public perception and often does little to bridge the gap between academic and public spheres (Cameron et al., 2013; Cheng et al., 2023; Reid et al., 2020). As in many scientific fields, discussion of introduced species in natural resource management, and the literature used to inform it, is shaped by Western colonial ideology and dominated by aggressive, militaristic, and xenophobic language that implies intent on the part of the organisms (e.g. Carson, 1962; Elton, 1958). This language shifts responsibility away from humans and enforces a negative relationship with these species that become the “enemy” (Reo & Ogden, 2018). This mindset, which is normalized in Western scientific ideology, degrades our relationship with the natural world and our more-than-human kin (Gibbs et al., 2014; Larson et al., 2005). Although out of place, these organisms are themselves living beings. Humans hold primary responsibility for displacing them and it's important to remember that we are environmental stewards and acknowledge their impacts within new environments by being conscientious and informed in creating solutions that are both

respectful and effective in monitoring, mitigating, and minimizing impacts (Bach & Larson, 2017).

A unique example we can use as a lens to explore this topic is the segmented earthworm (Annelida), which is a well-known organism group around the world, yet is introduced and considered to be invasive in much of the northern half of North America (Hale et al., 2005; Hendrix & Bohlen, 2002). Within the public sphere, earthworms are associated with agriculture and fishing, both of which exemplify positive relationships with humans (Blouin et al., 2013; Keller et al., 2007). However, research continues to shed light on the complex, detrimental impacts that earthworms are having outside of their native ranges (Frelich et al., 2006; Frelich et al., 2019). Through the story of the earthworm, we deconstruct the language used in scientific discourse to discuss introduced organisms, particularly those considered ecologically problematic, and examine how this impacts scientific communication, public perception, management practices, and the way we interact with and relate to the natural world. Then, we explore potential solutions that can allow us to build better relationships with and within the ecosystems of which we are a part (Figure 1). Two-eyed seeing is one way of knowing and understanding that we explore to begin to reframe this narrative. This is the conceptual framework through which we will be exploring and proposing alternative ways of approaching invasion ecology discourse. Ultimately, we are not arguing for less research, management, mitigation, or restoration regarding introduced species. Rather, we call for a perspective shift that leads to deeper understanding so humans may act more effectively and use messaging that resonates with a wider audience, with the goal of improving management and the related discourse on introduced species to allow for more respectful, considerate, and intentional stewardship.

2.2 Background: The Ecology of Introduced Earthworms in Northeastern North America

Earthworms are exceptional organisms, well known as ecosystem engineers, and can be found on every continent except Antarctica (Gates, 1982). Humans have had a long-standing beneficial relationship with earthworms; they have acted throughout history as partners in agriculture by enriching the soil used to grow crops that feed us and our livestock. People also use them as bait to catch fish, which is another way earthworms aid in human food systems (Keller et al., 2007). Additionally, earthworms are a food source for many other organisms, both within their endemic range and in areas in which they are introduced (Blouin et al., 2013). This association between earthworms and food systems has reified a positive perception that permeates public spheres. Many people would consider seeing a worm in their garden a sign of good healthy soil, and vermicomposting, widely considered a sustainable practice for both small- and large-scale agriculture, is popular among organic gardeners (Hendrix & Bohlen, 2002).

All this leads to incredulity when those outside of academia learn that earthworms were extirpated from northern regions of North America during glaciations (Cameron et al., 2013; Hale et al., 2005; Hendrix & Bohlen, 2002). Earthworm species endemic to North America still exist in areas south of the glaciation line, and remnants of pre-Pleistocene

earthworm assemblages can be found today in areas spared from glaciation, such as some islands in the Pacific Northwest (Bailey et al., 2002). However, these North American earthworm species have not completely reinhabited previous potential ranges, in part due to their slow rate of travel, which is anywhere between 2-10 meters per year on average depending on species (Cameron et al., 2007; Hale et al., 2005). Soil-dwelling annelids were not naturally reintroduced to previously glaciated areas, such as the Great Lakes region, until multiple species of European earthworms were brought over between 100 and 300 years ago by settlers through transportation of horticultural material and ship ballast during the colonization of North America (Gates, 1982; James, 2004). Today, earthworms continue to be spread by human activity such as constructing infrastructure and roads, forestry operations, global trade, and agriculture (Cameron & Bayne 2009; James & Hendrix, 2004). The fishing and bait trade is also a major contributor to earthworm introductions, particularly near water sources (Keller et al., 2007). Overall, increases in earthworm diversity and abundance are well-documented in association with anthropogenic land use (Cameron et al., 2007; Shartell et al., 2015).

Earthworms can dramatically alter the ecosystems into which they are introduced (either areas with no previous earthworms or where only different types of earthworms were previously), causing significant disturbances to the biogeochemical composition and processes within soil (Frelich et al., 2006). Within their endemic range, their activity is essential for ecosystem building, functioning, and maintenance, giving earthworms an invaluable role to play as a keystone species (Blouin et al., 2013). As a region without endemic earthworms, the northern hardwood forests from around the Great Lakes region to eastern North America evolved over the last 11,000-12,000 years since the glaciers receded in the absence of any earthworm activity (James & Hendrix, 2004). As a result, this has led to a slow cycling, nutrient-limited environment that relies heavily on the activity of macroinvertebrates and microbes like mycorrhizal fungi in the soil for nutrient cycling (Frelich et al., 2006; Hale et al., 2005). Earthworms, once introduced, fill an ecological niche that previously stood unfilled, which leads to greater negative impacts than if they were to be entering an existing niche (Keller et al., 2007). Earthworms shift these systems through a bioturbation process, in which they take material from different soil horizons and mix it up, also changing the overall soil composition, physical conditions, and biodiversity. This leads to an increase in the rate of nutrient cycling and leaching, a decrease in water retention capacity, and an increase in aeration in the soil (Frelich et al., 2006; Hale et al., 2005). The soil is the foundation upon which an ecosystem is built, and the changes earthworms cause have cascading effects that impact all trophic levels within an ecological community (Frelich et al., 2019).

One of the most notable impacts of earthworms in northern hardwood forests is the removal of the organic duff layer by consuming or burying it (Frelich et al., 2006; Hale et al., 2005). The duff makes up the top layer of soil and serves many purposes, such as protecting plant roots from heat and water loss, providing substrate for seed germination, feeding detritivores, and housing macroinvertebrates. Without it, roots are exposed to extreme temperatures, seeds are exposed to desiccation and consumption, and macroinvertebrates begin to disappear due to a lack of food and habitat. This leads to decreases in plant and invertebrate communities that otherwise proliferate the forest floor

(Frellich et al., 2006). Numerous birds, mammals, and amphibians in northern hardwood forests all experience population decreases in the presence of earthworm activity (Frellich et al., 2019). Tree species also face detrimental impacts, and have been shown to experience decreases in seedling regeneration and increasingly stressed crown conditions (Bal et al., 2017). Additionally, the spread of numerous introduced plant species is associated with and facilitated by earthworm activity, and vice versa (Madritch & Lindroth, 2008; Nuzzo et al., 2009).

There is much we do not know yet about the impacts of introduced earthworms across this region, as the degree to which they are causing damage has only relatively recently come to the attention of the scientific community. It was not understood that earthworms were indeed introduced, at least in the northern regions of North America, until the 1980s (Gates, 1982). As recently as the 1990s, scientific literature still held the perspective that earthworms are positive indicators of biodiversity and good health in forests and often did not recognize they were previously not present (e.g. Coderre et al., 1995). Different species have different impacts, and new species, such as the jumping worm (e.g. *Amyntas* spp), are continuing to be introduced; we know little about the long-term impacts they will have, especially considering additional factors such as climate change (Laushman et al., 2017). Warming conditions may expand earthworms' habitable range or could exacerbate impacts to already vulnerable ecosystems (Eisenhauer et al., 2014). Additionally, there is evidence that earthworm activity in newly established areas is not static, with shifting soil conditions influencing their dynamics over time; long-term studies on the history of land use are needed, as it is often unknown how long earthworm populations have been established (Shartell et al., 2015). Individual species distributions and densities are also not thoroughly documented. Populations can be condensed and localized, meaning that more widespread sampling is needed on a finer scale to track their spread and document new introductions (Keller et al., 2007).

All these considerations suggest there is much to learn to fully understand the ecology and impacts of introduced earthworms and how to properly manage their impacts. Our relationship with earthworms is rapidly evolving as more research is published on their ecological impacts outside of their native range, and as information from the academic sphere is making its way to the public sphere. Earthworms are already well-known and held in a place of admiration in the public mind, so it provides us with an interesting opportunity to explore the field of invasion ecology to discuss and handle introduced species. With this knowledge in mind, we now turn to the existing discourse and the factors that have contributed to its current state.

2.3 The Current Framing of Discourse in the Field of Invasion Ecology

To understand the current state of discourse on invasive species management, we need to acknowledge the existing framing around it, both in public and academic spheres (Larson et al., 2005). When we say the word “invasive” we incite a framework of ideas and concepts that has been constructed over the course of decades. Repeated exposure to language and ideology strengthens associations and framing, causing the dominant

discourse to become reified in our neural systems (Lakoff, 2010). From the very beginning, the field of invasion ecology was established through the use of militaristic language and metaphor (Larson et al., 2005). Use of war metaphors can be traced back to the foundational book on invasion ecology, *The Ecology of Invasions by Animals and Plants* by Charles S. Elton in 1958, which is considered a seminal work in the field and led to invasive species garnering more attention from the scientific community (Elton, 1958; Lowry et al., 2013). The field of invasion ecology has been growing since, experiencing a surge in published literature in the late 1990s and continuing strong today (Lowry et al., 2013). Even Rachel Carson's famous book *Silent Spring* (Carson, 1962), a formative text for many ecologists, uses militaristic language, further cementing this framing of discourse on the topic of environmental issues like introduced species (Larson et al., 2005). This language now permeates all levels of invasive species discourse, from scientific literature and communication, to policy and legislation, to public forums, and so forth. Subsequently, introduced species have become synonymous with negative language (Greenhalgh-Spencer, 2019).

2.3.1 Consequences of harmful, exclusionary language

The words and phrases used in the field of invasion ecology perhaps communicate more about humans than about the organisms we discuss. Language used to discuss introduced species often mirrors language that has been used throughout history in discussions about topics like immigration and racism to refer to people that are considered undesirable, alien, or otherwise other (Anderson, 2017; Cheng et al., 2023; Lancette, 2021). Immigrants and refugees are regularly equated with organisms we consider pests using metaphorical language in an attempt to create fear and animosity; this draws on the Western idea that non-human beings are lesser and must be controlled or even exterminated, reinforcing the narrative that immigrants and refugees should be treated in the same way (e.g., consider the use of pesticides and herbicides, such as Zyklon B and Agent Orange, on both human and non-human beings) (Anderson, 2017; Lancette, 2021; Shinozuka, 2013). Additionally, the xenophobic, militaristic language used in invasion ecology saw a similar exacerbation as the discourse regarding immigrants after 9/11, as invasive species began to be referred to as "terrorists" as well (Druschke et al., 2016; Larson et al., 2005).

The perceived value of a species, or lack thereof, shapes and informs management actions and reflects the biases and expectations of those in charge of making management decisions (Bhattacharyya & Larson, 2014). Economic impact is at the forefront of both arguments, with significant attention given to the threat of financial loss and the rhetoric that immigrants and introduced species both take and use up resources that are considered entitled to the existing occupants of a place (Anderson, 2017). This ideology creates an "us vs. them" mentality, where the lives of "our own" (local or familiar species) are given more value than the lives of the "invaders," thus justifying violence and taking of life to preserve life we deem more valuable (Anderson, 2017; Greenhalgh-Spencer, 2019; Larson et al., 2005). This creates a harmful dichotomy of who belongs and who does not, as opposed to a conversation of how to better understand the complex issues at hand and

work to remedy them in a respectful, equitable, effective way (Cheng et al., 2023; Gibbs et al., 2014).

This is further exemplified in the use of place-based common names when referring to introduced species. While it is true that common names serve an important purpose, especially when it comes to communication and education, associating an organism with a place of origin, especially when the species is considered invasive, incites xenophobic and racist sentiments (Lancette, 2021). Members of marginalized groups, particularly BIPOC individuals, have expressed that the language used in the field of invasion ecology is reminiscent of the “go back to where you came from” mentality with which they themselves have been treated (Cheng et al., 2023). For example, jumping worms in the genus *Amyntas* (specifically the species *Amyntas agrestis*) are commonly referred to as Asian jumping worms. Much like what happened with the discourse surrounding the Japanese beetle, when these worms are regularly associated with the words “invasive,” “threat,” “destructive,” and so on, the moniker of “Asian” draws on xenophobic and racist stereotypes and ideals regarding Asian people that have existed for well over a century within Western society (Lancette, 2021; Shinozuka, 2013). The word “exotic” also poses issues, as it is associated with the dehumanization and commodification of BIPOC communities (Cheng et al., 2023). Ultimately, the process of dehumanization, separation, and othering serves to detach us from the repercussions of injustices, atrocities, and violence committed against living beings, both human and more-than-human (Anderson, 2017; Lancette, 2021).

2.3.2 Implications for education and outreach communication

Scientists tend to fall into old habits of delegating information, which is not an effective mode of communication (Fischer et al., 2014). Education and outreach are essential when it comes to crisis disciplines like climate change and the management of introduced species, as humans are at the core of the issue and changes in our behavior are the basis of the solution (Bach & Larson, 2017; Verbrugge et al., 2021). In a study by Cameron et al. (2013) where they attempted to increase local anglers’ awareness of the impacts of introduced earthworms with the goal of changing bait handling habits and reducing the rate of bait dumping, they saw little to no difference before and after the campaign, despite significant time investment and multiple media types being utilized. While it is true that it is more difficult to garner public attention and support for the management of species considered less charismatic, such as earthworms, these shortcomings in outreach are present throughout the field of invasion ecology (Cameron et al., 2013; Verbrugge et al., 2021).

When it comes to education and outreach, the language of invasion ecology is also not conducive to creating an inclusive environment for marginalized groups, and actually may ostracize them by using xenophobic and racist language that they may have experienced used against them (Cheng et al., 2023). This can act as a deterrent to diversity, restricting who has access to scientific spaces and excluding differing perspectives and ideas (Lancette, 2021; Schüttler et al., 2011). Terminology used within the field is also messy; many terms have vague and ambiguous definitions and may

incorrectly be used interchangeably, which leads to unclear, confusing messaging (Cheng et al., 2023; Iannone et al., 2020). Some terms are specific to legislation, such as the term “noxious weed,” while others are frequently used in outreach but are incorrect or misleading, such as “native invasive” (Iannone et al., 2020). There is also often a lack of coordination between educators, academics, policy-makers, and industry professionals on what terminology to use (Verbrugge et al., 2021). Overall, education and outreach have fallen short in effectively reaching the public sphere, which has contributed to the importance of media in filling that role.

Mass media plays a crucial role in bridging the gap between academic and public spheres when it comes to communicating scientific issues (Cameron et al., 2013; Larson et al., 2005). This comes at the cost of neutrality, however, as there is pressure for media industries to garner attention through views, clicks, and engagement that leads to the messages being sensationalized for attention (Anderson, 2017). This is especially evident when it comes to reporting on introduced species. Using emotional, fear-inducing language is an effective way to grab viewers’ attention (Larson et al., 2005). This is also true on the part of the scientists and organizations that are communicating information to the public, as those that act as mouthpieces in a scientific story need to provide newsworthy material that will benefit media partners (Bach & Larson, 2017). To get public stakeholders involved in management, it is necessary to not only have their attention and interest, but also to spur action (Iannone et al., 2020; Verbrugge et al., 2021). While education-based communication would be the most effective long-term and highly beneficial for building an environmentally conscious society, using sensationalism and fearmongering is often the most effective way to motivate immediate action (Druschke et al., 2016). Ultimately, the language used by those within the scientific sphere shapes and informs policy, management, future research, and scientific communication, which in turn shapes public opinion, creating a self-perpetuating feedback loop (Druschke et al., 2016; Larson et al., 2005).

2.3.3 Implications for science, policy, and management

Reflecting the mindset that humans are above and separate from the rest of the natural world, people are often excluded from the narrative of invasion ecology, ignoring or even omitting human involvement in the movement of organisms, despite being at the core of the process (Gibbs et al., 2014; Reid et al., 2020). We see this in the case of the earthworm, which have been introduced in some areas for longer than many people’s memory and are so ubiquitous, especially in association with human agriculture, that many assume they have always been here and could not be anything but beneficial (Cameron et al., 2013; Schüttler et al., 2011). Subsequently, the current state of invasive species discourse in Western science prevents us from seeing beyond the immediate negative impacts of introduced species, particularly when we only focus on the implications for human interests (e.g. economy, human health, etc.) (Anderson, 2017; Gibbs et al., 2014). This disconnect between humans and the environment can stifle the responsibility to care for and live in cooperation with the natural world. It can also lead to the idea that people have power over the environment and can, or should, manage it for

our benefit, contributing to “command and control” management regimes (Lakoff, 2010; Reo & Ogden, 2018).

These attitudes impact management and policy and can be seen in the way we perceive and manage introduced species like earthworms. People often place responsibility on the organism, implying malicious intent on their part, despite being the force that acted to displace them (Bach & Larson, 2017; Gibbs et al., 2014). Additionally, Western science often views nature as fragile and static, espousing an impression of helpless victimhood, and subsequently treats and manages it as such, holding onto a notion of “untouched nature” and the “wild” (Larson et al., 2005; Reid et al., 2020). This idea that people are detached saviors that must maintain nature as a pre-human condition, while well-intentioned, is unrealistic and problematic. This also relates back to the idea of ecosystems that we view as “our own” being under attack from “foreign invaders” discussed earlier (Anderson, 2017). As a result, this leads to invasive species management regimes focused on eradication, evoking the mindset of “waging war” that pervades invasion ecology (Larson et al., 2005). Novel ecosystems, and the transitional processes within them, have come to represent human failure, where the perception is that native ecosystems have been lost due to the inability of humans to save them (Gibbs et al., 2014). There is an inherent sense of futility and failure when “fighting a losing battle” against an introduced species (Bach & Larson, 2017). Unfortunately, invasive species management is resource-intensive and expensive, and funding typically hinges on the establishment of an economic impact; i.e., resources are only allotted to management of introduced species when there is a proven economic benefit, or a threat of significant loss. Because of this, the focus of education and outreach in the field is communicating with stakeholders (Iannone et al., 2020).

These factors contribute to the degradation of human relationships with each other and with the natural world. The ideology that humans are separate from and above all other beings in the natural world has become normalized and embedded in the way Western society thinks and behaves, and it is difficult for many to consider that alternative ways of relating to nature even exist (Greenhalgh-Spencer, 2019; Reo & Ogden, 2018). Challenging the dominant dialogue and attempting to deconstruct and reconstruct the framing surrounding a topic is not an easy task, especially when presenting new information and new issues, but it is not impossible (Lakoff, 2010), a fact often overlooked. In the final section, we will explore new ways to approach the discussion and management of introduced species like earthworms using more respectful, considerate, and intentional practices.

2.4 Reframing Discourse on Introduced Species Through Two-eyed Seeing

To reframe discourse associated with introduced species, we suggest transitioning to multi-perspective approaches for communication and management that also seek to build better relationships with the natural world (Gibbs et al., 2014). Changes in the way we perceive and discuss introduced species requires a shift to a holistic, integrative mindset that prioritizes collaboration and communication between disciplines, knowledge

systems, and communities. We believe such changes have potential to contribute to the creation of novel policies that result in improved management strategies (Druschke et al., 2016). In what follows, our discussion is framed through the two-eyed seeing approach, with the goal of proposing an alternative framework for the study, teaching, communication, and management of introduced species that will ultimately facilitate discourse that is inclusive of diverse ways of knowing and communities, as well as support beneficial ecosystem management across disciplines (Reid et al., 2020).

Importantly, a multi-perspective shift would promote equitable additions of traditional Indigenous knowledge systems alongside Western scientific knowledge, to address longstanding power dynamics that prioritize Western science above alternative bodies of knowledge (Bartlett et al., 2012). We acknowledge the plurality of knowing and understanding the world and recognize that a practice of knowledge coexistence and complementarity supersedes one of integration or incorporation (Reid et al., 2020). Indigenous worldviews are not monolithic and should be respected with individual integrity (e.g. Kimmerer 2019; Waasegiizhig Price, 2023). There are, however, shared common traits, values, and processes that occur widely across Indigenous knowledge systems, such as interconnectedness, reciprocity, and relationship-building (Reid et al., 2020; Shaw et al., 2022). Thus, we propose two-eyed seeing as a good framework to benefit the study, management, and discourse associated with introduced species.

Two-eyed seeing is an illustrative way to describe an approach that metaphorically uses one eye to view the world through an Indigenous knowledge system approach while using the other eye to view through the Western knowledge system (Reid et al., 2020). In the case of introduced species, Western and Indigenous sciences are not mutually exclusive. Both sciences are valuable, neither are superior, and each has unique strengths to contribute to the other (Shaw et al., 2022). In partnership, each supports and strengthens a holistic approach so that collaborative, novel solutions can be applied to contemporary ecological challenges (Reo et al., 2017). For Indigenous communities, traditional knowledge systems include ways of knowing and being as everyday relations and seasonal practices with lands, waters, foods, medicines, and much more (Salmon, 2000). For example, the giant earthworm (*Rhinodrilus alatus*) is a vital part of the diet and medicinal practices of many Indigenous peoples in Brazil (Drumond et al., 2015). In contrast to Western ideals, Indigenous ways of knowing acknowledge human positionality as part of the natural world. As part of natural systems, humans seek to sustain honorable relationships, live in kinship with the more-than-human beings with which we share environs, and work in partnership with others to maintain healthy ecosystems for future generations (Bhattacharyya & Larson, 2014; Horn et al., 2021; Reid et al., 2020). Two-eyed seeing implores action, inspiring applications of new knowledge in ways that value diversity and equity while upholding connection and relationship (Reid et al., 2020). Such values prioritize respectful, inclusive language to inform enhanced practices in education, communication, and management of ecosystems where introduced species reside.

2.4.1 Transitioning to respectful, inclusive language

Criticism of the language used for introduced species in the field of invasion ecology is not new, tracing origins to the 1990s. Early arguments challenged the use of negative language that assigned xenophobic, war-like attributes to these organisms (Druschke et al., 2016; Larson et al., 2005). Although acknowledged as exclusionary and discriminatory, little progress has been made on the transition to unbiased language across disciplines in the sciences (Cheng et al., 2023). In the ecological sciences, positive efforts are being made. For example, The Better Common Names Project, initiated in 2021 through the Entomological Society of America (ESA), aims to transform the field of entomology to be more respectful by changing common names that may cause harm due to their derogatory or dehumanizing nature (Cheng et al., 2023; Lancette, 2021). Notably, the campaign recently changed the common name of the moth *Lymantria dispar* from one that contained a racial slur to spongy moth (Lancette, 2021). Additionally, the Just Language in Ecology Education initiative works to challenge xenophobic, warlike terms, and shift to neutral language that does not assign negative values to organisms (Cheng et al., 2023). Some groups are currently compiling resources to make them more accessible to educators and communicators, such as The Ecology and Evolutionary Biology (EEB) Language Project, with the goal of making systemic change more effective and successful (Cheng et al., 2023).

Although genuine change begins with an individual, interdisciplinary collaboration will be necessary to create the collective change needed for real impact (Cheng et al., 2023; Lancette, 2021). An important first step is to stay current with the ongoing changes to common names. Up-to-date educators can make conscientious choices to use updated nomenclature in new outreach materials, as well as update existing resources (Lancette, 2021). Applying such changes to earthworms means avoiding the use of location-specific or racially associated descriptors for common names (i.e., refer to Asian jumping worms as jumping worms or African nightcrawlers by their scientific name *Eudrilus* instead). These terms incite a xenophobic mindset of “otherness.” Transitioning to alternative terminology may include “introduced,” “novel,” “neophyte,” and “peregrine” which maintains descriptiveness and denotes the species’ range has been impacted by human-activity without specifically assigning negative values to particular organisms (Anderson, 2017; Bhattacharyya & Larson, 2014).

To support the use of respectful, inclusive language, we encourage using and/or creating common names that assign value and define organisms based on what an organism is, in contrast to what the organism is not (Gagnon et al., 2022). Transitioning away from using the prefix “non” in descriptive names better draws attention away from how organisms are lacking or that the difference is a negative attribute (Gagnon et al., 2022). Terms such as “adventative,” “naturalized,” or “short-term residents” are good alternatives, in addition to others. In Ojibwe Anishinaabemowin, the phrase “bakaan ingoji ga-ondaadag” was coined by Ojibwe author Lee Obizaan Staples to refer to introduced species, and roughly translates to “that which comes from somewhere else and now resides here” (Tribal Adaptation Menu Team, 2019). Similarly, the Kimberly Aboriginal people of Australia use “kartiya,” which denotes that a being is from or associated with a

place other than where it is presently (Bach & Larson, 2017; Waasegiizhig Price, 2023). Shifts in introduced species language are taking place, but challenging the dominant discourse will require a continuous dialogue on neutral naming. Neutral naming is not only respectful to species but can also enhance the inclusivity and accessibility of the study of introduced species, which in turn has positive synergies for education and communication, and thus the next generation of policy and management practitioners (Cheng et al., 2023; Lancette, 2021). Using respectful language does not diminish the effectiveness of management actions that take place to mitigate impacts from introduced species.

2.4.2 Improving outcomes for education and outreach communication

It is essential to begin unbiased language transitions in early education, building base knowledge and constructing the framework needed for lifelong awareness and more holistic understandings of social and environmental issues (Lakoff, 2010; Verbrugge et al., 2021). Investing the time and effort needed to build educational foundations is invaluable though rarely the approach taken, particularly for pressing ecological issues (Fischer et al., 2014; Verbrugge et al., 2021). These frames also inform the foundation for beliefs, and subsequently attitudes and behaviors, and therefore become deeply ingrained in one's neural network; once intact, it is difficult to alter, even when new and accurate information is provided, and the typical response is to take the path of least resistance, relying on the dominant discourse already in place (Bhattacharyya & Larson, 2014; Fischer et al., 2014; Lakoff, 2010). This is exemplified in the case of introduced earthworms, where the predominant reaction is to reject ideas that earthworms could be anything but beneficial (Cameron et al., 2013). Moreover, public audiences may not possess the necessary background knowledge about issues within the field of invasion ecology (hence why we provide a background about earthworm ecology). Thus, one's reliance on frames and beliefs, accurate or not, will be the rational default.

Building on foundations provided in education, outreach communication efforts can strengthen more accurate, holistic framings. Effectively engaging the public is essential in addressing contemporary environmental issues, but often there is a lack of coordination or inconsistency in messaging between educational organizations that contributes to ineffective communication (Iannone et al., 2020; Verbrugge et al., 2021). Adjusting outreach approaches and adapting specific content, delivery, and messaging are vital steps when applying ongoing scientific research and other expertise within a diverse public sphere (Verbrugge et al., 2021). For example, involving trusted local community leaders as ambassadors has been shown to have positive outcomes in outreach efforts (Cameron et al., 2013). Additionally, voices of local knowledge-holders need to be uplifted and empowered, as they retain extensive knowledge and expertise gained through on the ground experience instead of formal education (Fischer et al., 2014). With growing acceptance, local Indigenous knowledge-holders are receiving timely recognition for valuable contributions, and we are seeing potential benefits for public relations, policy, and management (Drumond et al., 2015). In outreach education, communication efforts are increasing emphasis on the benefits of interdisciplinary

collaboration; for example, partnering with psychologists to create more effective materials (Cameron et al., 2013; Verbrugge et al., 2021). Consistently using unbiased language across and between communication efforts creates clear, unified messages on introduced species, their impacts, and how to respond, which will enhance overall communication efficacy more broadly (Cheng et al., 2023; Iannone et al., 2020). In short, regardless of age, knowledge level, ethnicity, etc., outreach must communicate accessible, adaptable information from trusted sources, including local knowledge-holders.

2.4.3 Improving outcomes for science, policy and management

Two-eyed seeing is an important, powerful method for enacting discourse transitions in science, policy, and management (Stirling et al., 2023). Indigenous peoples and their existing work on introduced species can provide culturally informed, integrative management strategies, as they approach environmental issues in adaptive ways that consider community and society holistically in management (Bhattacharyya & Larson, 2014; Reo et al., 2017; Shaw et al., 2022). Long-held records of traditional and cultural practices and knowledge provide invaluable information and act as a resource that allow Indigenous nations to better understand ecological relationships and how they change over time, which helps inform decisions about how to build relationship and interact with the environment (Bach & Larson, 2017; Drumond et al., 2015; Shaw et al., 2022). Western scientific practices are not designed to build intimate, long-term relationships with individual organisms, and may be unable to parse out fine details about ecology that traditional scientific practices can (Drumond et al., 2015). Local knowledge systems can help fill gaps in Western scientific knowledge, adding new perspectives that can strengthen and fortify policy and management (Bach & Larson, 2017; Drumond et al., 2015; Schüttler et al., 2011).

In the case of introduced species, especially those that are cryptic and more difficult to study such as earthworms, knowledge holders within the endemic range can be a resource for others. Importantly, existing traditional and cultural relations with these organisms have the potential to provide insights and inform socio-ecological relationships elsewhere. Understanding long-term organism characteristics, behavior, and interactions within their endemic range has the potential to support management efforts, including forecasting outcomes associated with their introduction elsewhere (Reo & Ogden, 2018; Shaw et al., 2022; Tribal Adaptation Menu Team, 2019). For example, the Minhocuçu Project in Brazil sought to improve giant earthworm (*Rhinodrilus alatus*) management, and until this study, Western scientific methods had proven unsuccessful in producing meaningful results (Drumond et al., 2015). There was a cultural and economic need to preserve and maintain local uses and practices involving the giant earthworm, thus the Project elected to partner with local knowledge holders to improve the study and monitoring of this otherwise cryptic organism. As a result, the Minhocuçu Project developed an adaptive management plan that considers the life cycle and nuanced ecology of the giant earthworm and allowed them to better understand an appropriate scale at which to implement management activities and related policies to support good practice. Overall, the Minhocuçu collaboration, drawing on Indigenous and Western

knowledge systems, exemplifies a two-eyed seeing approach and its potential effectiveness for others (Drumond et al., 2015; Reid et al., 2020).

In many Indigenous knowledge systems, all living beings (introduced or otherwise) are considered to be persons living in kinship with all others and all have a right to exist (Horn et al., 2021; Waasegiizhig Price, 2023). As persons, we all live to share our gifts with others, which may be interpreted as serving a specific purpose within a specific ecosystem (Horn et al., 2021; Reo & Ogden, 2018). It has been articulated that the human duty is to “learn from,” in contrast to solely “learning about,” more-than-human beings (Kimmerer 2021). As such, the human obligation is to learn why a particular more-than-human species has migrated to another region, as well as how we, as humans, can develop new relations (Kimmerer 2021; Reo & Ogden, 2018; Waasegiizhig Price, 2023). Earthworms are a relatively recent arrival to North America, and it is even more recent that humans have become aware of the impacts earthworms have on forested ecosystems, so there is still much work yet to be done in establishing a relationship. We, as humans, do not yet know what purpose they may be here to serve or what we have yet to learn from them, so it is our responsibility as stewards to build that relationship and gain greater understanding.

Humans are responsible for relocating innumerable species worldwide, and subsequently, are responsible for acknowledging fault and taking accountability for the myriad of unforeseen impacts (Waasegiizhig Price, 2023). It is becoming more necessary to shift our perception of ourselves as human from being apart from and above nature to being a part of and within nature (Bhattacharyya & Larson, 2014). As a result, humans are obligated to create informed solutions that are both respectful and effective for life more broadly (Bach & Larson, 2017). Instead of a crisis-management or eradication approach to invasive species management, Indigenous land managers take time to observe and learn before proceeding to management decisions and actions (Bach & Larson, 2017; Tribal Adaptation Menu Team, 2019). The emphasis is understanding how the environment is responding and how we can act in partnership with our more-than-human kin to enact management strategies that reflect ecological relationships and maintain healthy systems for generations to come (Bhattacharyya & Larson, 2014; Reid et al., 2020).

While it is our responsibility as stewards to monitor, minimize, and mitigate the harm that we cause, it is more beneficial to redefine success by focusing on the positive outcomes of a healthier, more resilient ecosystem (Bach & Larson, 2017). Managing ecosystems so they are resilient will make it so they are less susceptible to the detrimental impacts of introduced species (Tribal Adaptation Menu Team, 2019). Bach & Larson (2017) provide an example of how this change in perspective on introduced species can change attitudes, perceptions, and behaviors in teams of Aboriginal weed management rangers in Australia. When the rangers focused and relied on language taught to them in professional training, their outlook was narrow, negative, and made them feel like their work was futile. However, when they switched to using language traditionally used by Aboriginal elders, they were able to think more broadly about the implications and

purpose of their work and how it was helping to improve the overall health of the land, finding more success in their work.

Dynamic pragmatism can be valuable to the education, communication, and institutional practices concerning introduced species (Drummond et al., 2015; Druschke et al., 2016; Gibbs et al., 2014). For established introduced species, such as earthworms, it would be wise to move beyond a sole focus on negative impacts (Cameron et al., 2013). Instead, it may be more productive to accept altered ecosystems and the particular set of gifts provided in expanded ranges. In contrast to abrasive management, scientists, educators, and practitioners may desire to provide information on ways introduced species can be managed to minimize harm and mitigate impacts to others (Druschke et al., 2016). By drawing on positive effects and interactions, management regimes could better work towards repairing ecological relationships (Bach & Larson, 2017). Conducted in a thoughtful, careful manner, reframed discourse can discourage the spread of problematic species while also avoiding the promotion of an apathy for land stewardship. Acknowledging positive and negative consequences can support better human relationships within ecosystems and with more-than-humans (Verbrugge et al., 2021).

Species impacts are not homogenous or isolated, and managing them under that assumption leads to ineffective practices and poor results (Bhattacharyya & Larson 2014). Effective management should look at the big picture, considering the network of interactions and relationships an organism, introduced or not, has within its environment (Bach & Larson, 2017; Gibbs et al., 2014; Shaw et al., 2022). In some cases, managers are opting to shift away from trying to totally eradicate problematic species when it becomes clear that eradication is not feasible, as is the case with introduced earthworms (Cameron et al., 2013). Instead of funneling resources and effort into trying to remove these species entirely, the focus in cases like these should be shifted to helping ecosystems adapt and become more resilient (Druschke et al., 2016). Since many introduced species, like earthworms, are associated with human activity, the best thing we can do is to promote realistic, accessible ways that the public, organizations, and corporations can get involved and help (for earthworms, this may look like not dumping bait, better practices for vermicomposting, brushing boots, regulating travel into non-impacted areas, washing vehicles and equipment, and conducting studies to understand their impacts and relationships, etc.) (Hendrix & Bohlen, 2002; Tribal Adaptation Menu Team, 2019).

Discourse transitions serve to improve human relationships with each other and the natural world, and especially, the more-than-human beings with which we share environs. By approaching introduced species with two-eyed seeing, we seek to gain an enhanced understanding of ecosystem relations (Figure 1). Further, this knowledge can be applied to intentionally engage with diverse knowledge systems, and transition to be more respectful and inclusive in science, policy, and management discourse. As a result, discourse transitions have the potential to simultaneously facilitate the general public's framing and everyday discourse. Reframing for the transition to unbiased discourse on introductory species has the potential for synergistic efforts across scientific

communication, education, outreach, policy, and management practices and relations more broadly.

2.5 Conclusion

Introduced species are a concern and have detrimental impacts, but the current ways we discuss them are undoubtedly problematic and impede, rather than facilitate, effective communication and serve to harm our relationship with the natural world. We have not provided an exhaustive list of strategies in which we can work to improve the way we study, discuss, and manage introduced species, but rather, we have started an illustrated framework of doing so through the story of introduced earthworms in North America. Earthworms are an exemplary group to have this discussion with as they do provide us with critical agricultural services, yet also significantly impact ecosystems into which they are newly introduced, which leads to complexities in communicating messages to different audiences. The goal of this article is to further an ongoing conversation about the impacts of language within the field of invasion ecology. Although there are many leading efforts for meaningful change to improve our relationships with introduced species and management outcomes, there is still much work to be done. Reframing the discourse will allow for diverse and novel perspectives and approaches that would otherwise not be possible with the current state of discourse.

2.6 Figure



Figure 1. An infographic summary of the comparison between the current state of discourse regarding the field of invasion ecology and recommended reframing of perspectives and discourse through two-eyed seeing. Earthworm introductions in North

America are exemplary for describing the frequent disconnect in communication between differing audiences.

2.7 References

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3 Exploring potential impacts of introduced earthworms (Annelida: Lumbricidae) on sap chemistry in sugar maple (*Acer saccharum*) in the Great Lakes region

The robust maple syrup industry in North America generates significant annual economic revenue thus impacts on maple sap quality could have major implications. The introduction of earthworms has led to significant ecological impacts in northern hardwood forests, especially on sugar maples. Sugar maple health has been correlated with introduced earthworm activity, but there is much we still do not understand about how tree chemistry and physiology are affected. For this study, we used tree sap collected during the spring of 2023, soil sampling, and earthworm population data to explore potential impacts of introduced earthworms on overall sap sugar content using linear regression models. Introduced earthworms were found to create a complex, dynamic chain of interactions that have a bottom-up effect on sugar maple health that impair the capacity of trees to produce quality sap for maple syrup production. Most notably, the presence of anecic earthworms at sites with established earthworm communities is negatively correlated with overall sugar content in sap ($p = 0.038$). This highlights the potentially detrimental implications for production of maple syrup and sugar. It will be beneficial to increase awareness of the impacts of introduced earthworms with producers for consideration in sugarbush management regimes.

3.1 Introduction

The production of maple syrup is a growing lucrative industry in North America, bringing in millions of dollars in revenue annually from both commercial and private producers (Farrell & Chabot, 2012; Ramadan et al., 2021). Based on the most recent United States Department of Agriculture (USDA) data, total maple syrup production in the United States in 2022 was 5.03 million gallons, with 14.3 million reported taps, or the number of tap holes in trees collecting sap (USDA 2022). Overall value of production for the 2022 season totaled \$172 million, with the average price per gallon being \$34.70 (USDA 2023). The 2023 season yielded 4.18 million gallons of syrup from 13.4 million reported taps, despite the season being less favorable for production (USDA 2023). None of these values include the Canadian maple syrup industry, which is on an even larger scale (Ramadan et al., 2021; Wild & Yanai, 2015). Despite the economic importance of this lucrative industry, there are still many unknowns when it comes to potential impacts by earthworm activity to the chemistry (and thus quality) of maple sap. In general, the causes of the wide variability seen between individual trees and entire stands are not well understood (West et al., 2023).

3.1.1 Sugar Maple Sap Chemistry

Sugar maple (*Acer saccharum* Marsh.) sap is chemically complex, containing more than 200 identified compounds that contribute to the quality of syrup produced such as sugars, phenolics, organic acids, minerals, nutrients and more (Ball, 2007; Lagacé et al., 2015; Mohammed et al., 2020; Ramadan et al., 2021). Even compounds that occur in minute quantities in sap can have important implications for the quality and flavor of the syrup

(Mohammed et al., 2022). Sucrose is the dominant compound found in sap, comprising 96-100% of the organic content (Ball, 2007; Lagacé et al., 2015). Sap is also slightly acidic (avg. 5.9) and tends to become more acidic as the season progresses (Ball, 2007; Lagacé et al., 2015). Sap is almost 98% water, which means that much of it must be evaporated away to create maple syrup, or almost all liquid is removed for maple sugar. It takes roughly 40-50 gallons of sap to make one gallon of syrup, which is usually 66-77 % sugar (sucrose) and 33-34% water (Ball, 2007; Lagacé et al., 2015; Ramadan et al., 2021; West et al., 2023). The quality of maple syrup depends on the sugar content and chemical compounds that influence flavor, taste, and odor (Ball, 2007; Lagacé et al., 2015; Ramadan et al., 2021). Though some syrup compounds are formed during the evaporation process (mainly by heating), many are present from the start in sap (Ball, 2007).

There are many factors that can influence the chemical composition of maple sap, including, but not limited to, the individual genetics of a tree, the overall health and vigor of a tree, the time of day or the point within the season, weather conditions, and ambient temperature (Lagacé et al., 2015; van den Berg et al., 2019; West et al., 2023). There are also many possible influences from overall forest health and composition, such as stand density and age, canopy density, and species assemblages (both in the canopy and on the forest floor), but these factors are not well understood (West et al. 2023). The most influential factor on overall tree health and sap chemistry is the chemical composition of the soil, which is where maple trees obtain the required nutrients for physiological functions like sugar production (West et al., 2023; Wild & Yanai, 2015).

3.1.2 The Importance of Soil to Sugar Maple Sap Chemistry

Disturbance and subsequent changes within the soil, such as shifts in the balance and cycling of soil nutrients, has been found to have negative impacts on sugar maple health and vigor, contributing to dieback and decline (West et al., 2023). Microbial communities within sap, which originate from soil microbial communities, have important impacts on the flavor and color of maple syrup, which impacts the overall quality (Filteau et al., 2011). Sugar maples have been found to have symbiotic relationships with different species of arbuscular mycorrhizal fungi (AMF), which allows them to gain essential nutrients even in less than ideal soil conditions (West et al., 2023). The hydrolysis of sucrose into glucose and fructose also occurs due to microbial activity, and changes in the content of these three sugars can be seen throughout the season as microbial communities shift during different flow periods, with glucose and fructose levels increasing as the season progresses and sucrose levels decreasing (Filteau et al., 2011; Garcia et al., 2020; Lagacé et al., 2015).

This is true for other chemical compounds as well (Ramadan et al., 2021). Vanillin, which is an important phenolic compound for the flavor, taste, and odor of syrup due to the characteristic vanilla notes it provides, is believed to possibly be produced through the activity of particular microorganisms in the sap that are introduced through the tapping process (Lagacé et al., 2015; Mohammed et al., 2022). Additionally, syringaldehyde is a compound with antioxidant properties that contributes to the flavor

and taste of sap, and it is produced through the microbial hydrolysis of lignin (Lagacé et al., 2015; Mohammed et al., 2022). The phenolic content of sap decreases over the course of the season, likely due to decreases in the microbes that produce them despite an overall increase in microbe activity (Lagacé et al., 2015). The content of organic acids like lactic and fumaric acid, which are important to the flavor of syrup, increase over the course of the season (Lagacé et al., 2015; Mohammed et al., 2022).

Increased availability of N, P, K, Ca, and Mg in the soil has been shown to lead to a short-term increase in overall sap yield, but this has not yet been reported in regards to sap chemistry (West et al., 2023). N has also been documented to be the limiting nutrient for sap sweetness in sugar maples, which may be because sugar maples grow faster on soils with higher N content (Wild & Yanai, 2015). The mineralization of N in the soil leads to sweeter sap, as it increases the ability of N for uptake by sugar maples (Wild & Yanai, 2015). High foliar N allows for higher rates of photosynthesis and subsequently higher sugar production (Wild & Yanai, 2015). Surprisingly, P is negatively correlated with sap sweetness, despite being associated with higher plant productivity and greater overall sap yield (West et al., 2023; Wild & Yanai, 2015). Changes to soil composition and health, especially with nutrients and moisture, are likely a driving force behind maple dieback and decline, which has significant implications for the yield of maple sap for syrup production, and potentially for the quality of sap, although that is not yet known (West et al., 2023). Introduced earthworms, which have extensive impacts on the soils in northern hardwood forests, may be a contributing factor to undescribed or undetected changes in maple sap chemistry and quality.

3.1.3 Introduced Earthworm Impacts in Northern Hardwood Forest Soils

During the most recent ice age, the Laurentide ice sheet covered most of Canada and the northern parts of the Midwest and New England in the United States. The southward progression of the ice sheet removed any material in its path, leaving behind bare bedrock and depositing sediment as it receded (Tiunov et al., 2006). Through the removal of the soil, all soil-dwelling organisms were removed as well, including any endemic species of earthworms that may have been present at that time (Gates, 1982). Forest succession, then, proceeded in the absence of earthworm activity. Soil-dwelling annelids were not reintroduced to previously glaciated areas, such as the Great Lakes region, until European earthworms were brought over between 100 and 300 years ago by settlers through transportation of horticultural material and ship ballast during the colonization of North America (Gates, 1982; James & Hendrix, 2004). Today, earthworms continue to be spread by human activity, e.g. building of infrastructure and roads, timber harvesting, global trade, and agriculture (Cameron & Bayne 2009). In sugarbushes, which are regularly managed through methods like thinning, earthworms may be introduced by numerous human activity such as on the wheels of vehicles (West et al., 2023).

Earthworms can drastically alter the ecosystems into which they are introduced (either areas with no previous earthworms or where only different types of earthworms were previously present), causing significant disturbances to the biogeochemical composition

and processes within soil (Frelich et al., 2006). As a region without endemic earthworms, the northern hardwood forests around the Great Lakes region and eastern North America evolved over the last 11,000-12,000 years since the glaciers receded in the absence of any earthworm activity (James & Hendrix, 2004). As a result, this has led to a slow cycling, nutrient-limited environment that relies on the activity of macroinvertebrates and microbes, like mycorrhizal fungi, in the soil for nutrient cycling (Frelich et al., 2006; Hale & Host, 2005). Earthworms, once introduced, fill an ecological niche that previously stood unfilled, which leads to greater negative impacts than if they were to be entering an existing niche (Keller et al., 2007). This is because the subsequent changes to soil chemistry and composition occur over a few short years, as opposed to the thousands of years it took for the soil to form (Resner et al., 2015).

Earthworms shift these systems through a bioturbation process, in which they take material from different soil horizons and homogenize it, also changing the overall soil composition and physical conditions (Fahey et al., 2013; Ferlian et al., 2020). This leads to an increase in the rate of nutrient cycling and leaching, a decrease in water retention capacity, and an increase in aeration in the soil (Frelich et al., 2006; Hale & Host, 2005; Richardson et al., 2018). This aeration of the soil also contributes to the shift in soil microbial communities, favoring aerobic bacteria that accelerate decomposition of organic matter (De Menezes et al., 2018; Fahey et al., 2013; Ferlian et al., 2020). This can negatively impact soil mycorrhizal communities, such as the AMF sugar maples rely on for nutrient uptake (Ferlian et al., 2020; West et al., 2023).

Different earthworm ecotypes also have differing impacts on the soil, and community assemblages change over time and with differing degrees of human activity at a site (Ferlian et al., 2020). Introductions often occur along road edges and expand outward at a rate of anywhere between 2-10 meters per year on average depending on species (Cameron et al., 2007; Cameron & Bayne, 2009; Hale et al., 2005; Resner et al., 2015). Epigeic species, which reside in the leaf litter and have minimal impacts on the soil, are typically introduced first, followed by epi-endogeic and endogeic species that create horizontal burrows in the upper soil horizons and have slightly greater soil impacts; lastly come the anecic species, which can burrow more than a meter vertically into the deeper soil horizons and have the greatest impacts on soil (Resner et al., 2015). The most complex and diverse species assemblages, as well as the greatest impacts observed, are in areas with the longest time since introduction, which is often near roads and in association with higher levels of human activity (Cameron et al., 2007; Cameron & Bayne, 2009; Resner et al., 2015).

3.1.4 Introduced Earthworm Impacts on Sugar Maples

The passage of soil through the gut of earthworms and subsequent gut microbial activity also alters soil microbe community composition and distribution, as well as availability and cycling of soil nutrients (De Menezes et al., 2018; Ferlian et al., 2020). Earthworms have a negative relationship with C:N ratios (Drouin et al., 2016; Fahey et al., 2013). Earthworm mucus has a higher N content that can increase N abundance in these typically N-limited ecosystems (De Menezes et al. 2018). Sugar maple (*Acer saccharum*)

tree rings show a sudden, but short-lived, increase in growth rate for only a few seasons shortly following introduction of earthworms, as P and N are rapidly released through the mixture of soil horizons (Larson et al. 2010). However, these same tree rings studied by Larson et al. 2010 showed a longer-term decrease in growth rate that reflects the previously documented loss of nutrients (e.g. Ca, Mg, K, & P) from the system over time. Sugar maples are healthier and more vigorous on sites with higher Ca and Mg availability, with low Ca, Mg, K, and P being associated with maple dieback and decline (Frelich et al., 2006; Resner et al., 2015; West et al., 2023).

One of the most notable impacts of earthworms in northern hardwood forests is the removal of the duff layer by consuming or burying it (Frelich et al., 2006; Hale & Host, 2005). Sugar maples, which have shallow root systems within the top 60 cm of soil, depend on this duff layer for protection and for obtaining nutrients (West et al., 2023). Without it, roots are exposed to extreme temperatures, seeds are exposed to desiccation and consumption, and many species are outcompeted by species that are more adapted to take advantage of the increased nutrient availability (Dobson et al., 2017; Frelich et al., 2006). Sugar maples also face detrimental impacts and have been shown to experience decreases in seedling regeneration and increasingly stressed crown conditions, as the mineralization of soil nutrients and loss of rooting substrate and root protection lead to stressful conditions (Dobson et al., 2017). This stress also impedes the ability of sugar maples to uptake the nutrients they need to survive (Richardson et al., 2018).

There is much we do not know yet about the impacts of introduced earthworms, as the degree to which they are causing damage has only relatively recently come to the attention of the scientific community. It was not understood that earthworms were indeed introduced, at least in the northern regions of North America, until the 1980s (Gates, 1982). As recently as the 1990s, scientific literature still held the perspective that earthworms are positive indicators of biodiversity and good health in forests where they were previously not present (e.g. Coderre et al., 1995). There is a notable gap in the literature regarding the impacts of introduced earthworms on tree physiology, especially regarding economically important species like sugar maple (*Acer saccharum*). Although studies exist showing that sugar maple decline and dieback are correlated with introduced earthworm presence (e.g. Bal et al., 2017), there are currently no studies exploring the potential implications for changes to sap chemistry along gradients of introduced earthworms.

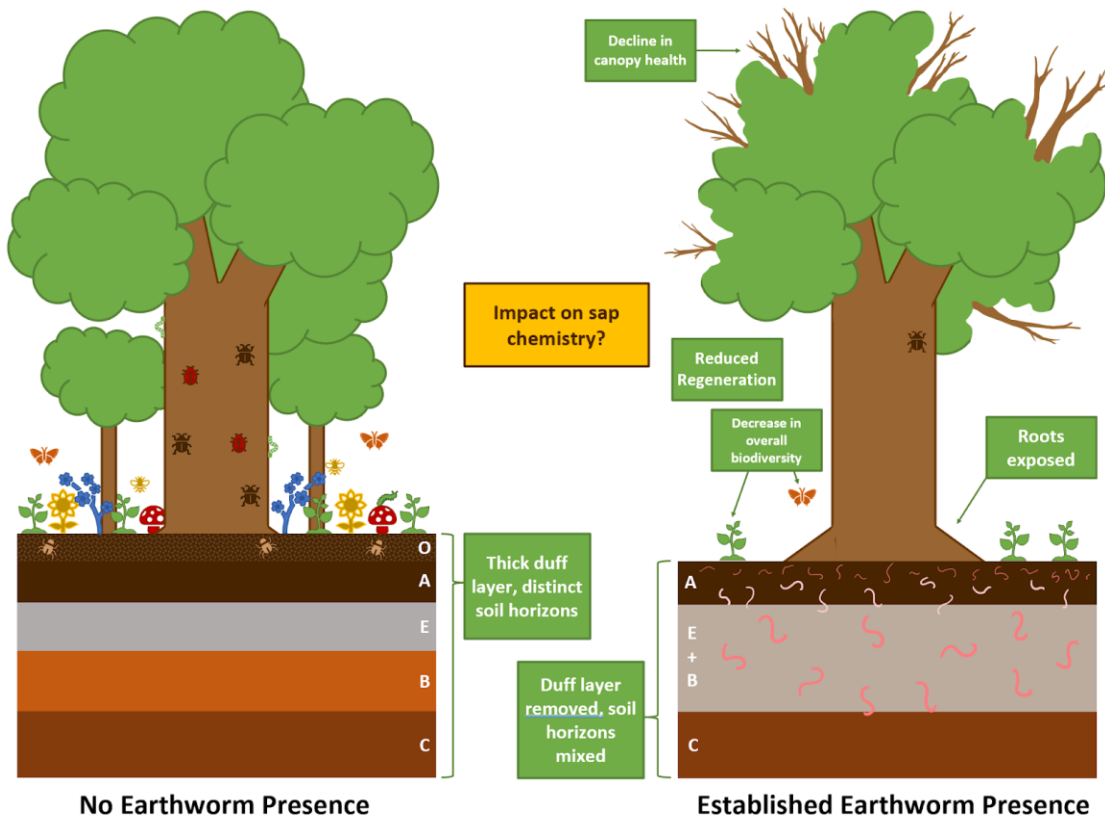


Figure 2. Illustrated summary of the ecological changes associated with introduction of earthworms into previously earthworm-free northern hardwood forests in North America.

3.1.5 Objective Statements

Changes in the quantities of chemical compounds in soil, especially nutrients like Ca, Mg, K, & P, and shifts in soil microbial communities due to introduced earthworm activity may have the potential to change the overall quality of maple syrup. The goal of this study is to evaluate gradients of introduced earthworm activity, species assemblages, and forest floor impacts to sugar maple sap chemistry collected in spring, as it would be for maple syrup production. We anticipate seeing an associated decline in sugar content and changes in other compounds within the sap, due to the reduced capacity of the trees to produce these compounds in the presence of increasing earthworm activity.

3.2 Materials & Methods

3.2.1 Site Descriptions

This study has a total of five sites: one in Marquette County, Michigan at Huron Mountain Club property (Big Bay, MI) and four sites (one in Houghton County, MI, two in Ontonagon County, MI, and one in Baraga County, MI) in the Ottawa National Forest, MI (Table 1). None of the sites established are managed as a sugarbush or have been used for modern maple sap or syrup production as they are generally managed for timber

production. The Marquette County site experiences mean annual temperature variations of -7.8°C to 18.9°C (average max. 33.9°C, average min. -22.8°C), average rainfall of 93.8 cm annually, and average snowfall is 363.5 cm annually (NOAA records from 2000-2023). The sites in Ottawa National Forest sites generally experience mean annual temperature variations of -10°C to 17.8°C (average max. 31.7°C, average min. -27.2°C), average rainfall of 81.3 cm annually, and average snowfall is 309.1 cm annually (NOAA records from 2000-2023). The forests at all five sites had similar forest composition, with some variation due to soil class (Table 1); all were representative of northern hardwood forest communities. Most stands had a high percentage of sugar maple (Table 1), but other common trees were white ash (*Fraxinus americana*), hop-hornbeam (*Ostrya virginiana*), yellow birch (*Betula alleghaniensis*), eastern hemlock (*Tsuga canadensis*), basswood (*Tilia americana*), red maple (*Acer rubrum*), and quaking aspen (*Populus tremuloides*), as well as some less common inclusions of white spruce (*Picea glauca*), balsam fir (*Abies balsamea*), and black ash (*Fraxinus nigra*).

Table 1. Descriptive site characteristics for soil, earthworm, and maple sap chemistry collections in northern hardwood forests in Upper Michigan, 2023

Site	County/ Ownership	Soil Class	Average DBH (in)	Sugar Maple Basal Area (ft ²)	% Sugar Maple Basal Area	Basal Area (ft ²)	Trees /acre	Average % Crown Density	Average % Dieback	Average % Canopy Density
1	Marquette/ Huron Mountain Club	Buckroe very channery loamy sand, stony	12.22	50.81	89.23	56.94	740	65.33	3.47	90.22
2	Ontonagon/ Ottawa National Forest	Negwegon silty clay; Allendale loamy fine sand	10.49	19.47	25.01	77.85	1170	54.50	10.70	81.78
3	Houghton/ Ottawa National Forest	Graveraet fine sandy loam, stony; Lupton and Cathro soils	10.12	16.39	26.00	63.03	960	64.50	3.67	57.89
4	Ontonagon/ Ottawa National Forest	Zandi-Karlin complex	11.27	34.15	59.67	57.23	730	62.17	4.70	90.56
5	Baraga/ Ottawa National Forest	Nunica silt loam	9.10	26.78	40.49	66.13	1350	54.67	9.20	80.89

Each site overall represents an earthworm introduction gradient, with three circular fixed plots (11.3 m radius plot size) established along a transect that follows the gradient (Figure 3). At each plot, ten sugar maples of at least 20 cm diameter at breast height (DBH) were selected for sap sampling and stem mapped relative to plot center (note: some tapped trees exceed the 11.3 m distance from center, which was done to ensure 10 trees of appropriate size were available; trees more than 11.3 m from center were not included in calculations for plot basal area). All 30 trees per site were then assessed for overall health, which included overall crown, canopy, and bole condition. Percentage of

crown density, which estimates the average density of the foliage, was assessed, as well as percentage of crown dieback, which considers how much of the canopy has recently died, especially fine twigs near the top of the canopy (Bal et al., 2017; Talient-Halsell, 1994). Canopy density was assessed using CanopyApp, which quantifies the amount of canopy cover. This was done at each of the plots within three subplots at five paces N, SE, and SW of plot center. Forest productivity metrics for each site are included in Table 1. Sites were established during the summers of 2021 and 2022, in order to map out earthworm gradients, and measure tree canopy variables.

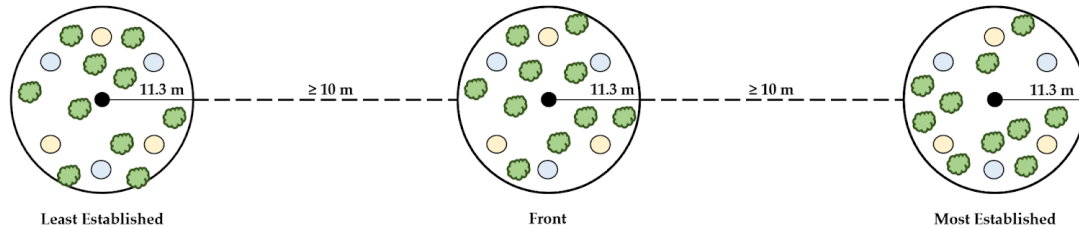


Figure 3. A replication of the method with which transects are established. Each of the three plots along the transect is 1/10th acre. Distance between plots varied based on the introduction gradient at each site but were a minimum of 10 m apart.

3.2.2 Sap Collection and Processing

Sap was sampled from all 10 trees per plot, per site (n=147, as three trees did not produce sap) within a one-week period in spring 2023 (April 7-14). Average daily temperatures that week ranged from -2.2°C to 14.4°C, with a minimum temperature of -15.6°C and a maximum temperature of 27.8°C, reflecting a wide swing in spring weather patterns; but all sap values reported here were collected from within a 6-day period. A total of 100 mL sap per tree was collected using a 5/16” drill bit and 5/16” OD clear vinyl tubing. Sap was then stored in plastic 50 mL vials, kept cool at 4°C for less than one week and then filtered using 3 mL plastic luer-lock syringes (Agilent) and 25 mm 0.2 µm polyethersulfone (PES) luer-lock syringe filters (Agilent) to prepare samples for analysis by high performance liquid chromatography (HPLC). A pocket pH meter (Apera Instruments SX-620 pH tester) was used to measure the pH of the sap and a digital refractometer (Misco Palm Abbe digital refractometer) was used to measure the overall sugar content (% Brix) of the sap in the field.

Standard solutions of sugars (glucose, fructose, & sucrose), organic acids (fumaric acid & lactic acid), and phenolics (vanillin & syringaldehyde) were prepared in ultra-pure water at a concentration of 2 g/L. Sugars and organic acids were analyzed using the refractive index detector (RID) while phenolics were analyzed using the diode-array detector (DAD) at 300 nm due to their optimum linear standard curves observed at these wavelengths. The stock solutions were further diluted with ultra-pure water and filtered through a 0.2 µm polyethersulfone filter to give three different concentrations for calibration curves. The calibration curves were obtained by plotting the peak height of each standard to their known concentrations.

The analysis was performed with an Agilent 1200 liquid chromatography system equipped with a G1311A quaternary pump, G1322A degasser, G1329A autosampler, G1315B DAD detector, G1362A RID detector, and G1316A temperature column controller. The separations using the RID were carried out using an Aminex HPX-87H column (300 mm × 7.8 mm) with a column temperature of 50°C, and a mobile phase of 5 mM H₂SO₄ at a flow rate of 0.6 mL/min and an injection volume of 10 µL for 20 min. The DAD separations were carried out using a Waters µBondapak C18 column (3.9 mm × 300 mm, 10 µm) with a column temperature of 45°C. The mobile phase consisted of 0.2% formic acid-water solution (A) and 0.1% formic acid-acetonitrile solution (B) with a flow rate of 0.6 mL/min and an injection volume of 10 µL for 20 min.

Samples were also analyzed for nutrient/mineral content using inductively coupled plasma analysis (P, Cu, Ca, K, Mg, Mn, Zn, Fe) using a Perkin Elmer Optima 7000 DV ICP-OES, which did not require prior filtering.

3.2.3 Soil Collection and Processing

Soil samples were collected from three subplots within each plot at five paces N, SE, and SW to prevent conflict with collecting earthworm specimens. Soil horizons were also measured using cores from a 2.5 cm soil corer. Soil samples were sifted using a 1.00 mm sieve and then digested in a CEM Mars 6 microwave digester in Express Plus vessels using the EPA 3051 method (9 mL nitric acid and 1 mL hydrochloric acid) before being analyzed using inductively coupled plasma (Perkin Elmer Optima 7000 DV ICP-OES) to quantify P, Cu, Ca, K, Mg, Mn, Zn, and Fe content, as well as by elemental combustion analysis using a Costech 4010 Elemental Analyzer (calibrated with atropine with the left furnace at 1000 C, the right furnace at 650 C, and the column at 75 C) to quantify C and N content. Soil pH was also determined using a pocket pH meter (Apera Instruments SX-620 pH tester).

3.2.4 Earthworm Collection, Identification, & Measurement

Introduction gradients were confirmed using a visual soil impact rating scale (Appendix Table A.1) as well as using a standard mustard pour procedure (4 L of mustard solution, made by mixing 40 g of mustard powder into 4 L of water) to perform population counts and to collect specimens for further identification using a dissecting scope to determine ecotype and ecological impact (Hale et al., 2005; Hale et al., 2008). Earthworms were sampled from three 0.11 m² subplots at five paces S, NW, and NE to give a representation of each plot as a whole. Earthworms collected in situ were euthanized using ethyl alcohol (200 proof) and taken to the lab for identification (using Hale, 2013) and to quantify wet biomass and ash-free dry mass (Hale et al., 2004). Earthworms that were determined to be juveniles of *Lumbricus spp.* were put into a separate class due to the inability to distinguish between juveniles of the species *L. terrestris* and *L. rubellus*, which are classified as two different ecotypes (anecic and epi-endogeic, respectively). It should be noted that *L. terrestris* is typically only found at long-established sites with complex bioassemblages, so many of the juveniles found at least established and front plots are likely *L. rubellus*, but juveniles were kept them separate for comparisons (Resner et al., 2015).

3.2.5 Statistical Analysis

Statistical analysis for this project was completed using R (version 4.2.2) and analyses used core R functions unless stated otherwise. Exploratory analysis was performed in two ways: principal component analysis (PCA) and sparse partial least squares analysis (sPLS). It should be noted that sap glucose, lactic acid, and fumaric acid content were excluded from analysis due to a lack of output from HPLC analysis (values too low in some samples). For PCA, which was completed using mixOmics (version 6.22.0), the data set was broken into four dataframes (Forest Composition, Earthworms, Soil, and Sap) and each dataframe was analyzed to determine correlation between the variables. PCA plots were created using factoextra (version 1.0.7) and correlation plots were created using ggplot2 (version 3.4.2). For sPLS analysis, which was also completed using mixOmics (version 6.22.0), separate dataframes were also used. A total of 6 sPLS analyses (Forest Composition-Earthworms, Sap-Earthworms, Soil-Earthworms, Forest Composition-Soil, Forest Composition-Sap, and Soil-Sap) were performed to determine the magnitude of relatedness between variables, which then allowed for more targeted selection of which associations to analyze further using linear regression. Clustered image maps (CIMs) were created using mixOmics (version 6.22.0). Finally, linear regressions were performed on all highly associated variables (both positive and negative) to determine the significance of the association. For variables that were 0 inflated (Anecic earthworm counts), generalized linear models with a Poisson distribution were performed instead. Multiple linear models were attempted but did not produce significant results. Linear models were checked for validity using performance (version 0.10.4).

3.3 Results & Discussion

A total of 10 species of earthworms were observed out of the 16 species that have been introduced in the Great Lakes region (Hale, 2013), and these are listed with their associated ecotypes in Table 2. Assessments of earthworm biomass, forest floor impact, and bioassemblages all confirmed the validity of the introduction gradients at each site (Table 3). Established plots had the greatest sum of total earthworm presence across all 5 established plots (290 earthworms) and the lowest average impact rating (1.07/5, with a rating of 1 being the most impacted forest floor), as well as more complex bioassemblages with more endogeic and anecic species, which is typical of longer-standing earthworm establishments. Least established plots had the lowest sum of total earthworm presence across all 5 least established plots (115 earthworms) and the highest average impact rating (4.53/5, with a 5/5 being an intact forest floor), as well as less complex bioassemblages with predominately epigeic species, which is typical of more recent earthworm introductions. Values from plots on the introduction front fell in between, which was expected. Due to how widespread earthworms have become in the Great Lakes region due to human activity (Holdsworth et al., 2007), it is becoming rarer to find areas that have zero earthworm presence, so none of the least established plots were completely absent of earthworms.

When comparing individual sites, site 3, which is located in the Ottawa National Forest, MI, had the highest average overall sugar content (2.62 %Brix) and also the highest average earthworm count (57.67 earthworms) between the three plots along the introduction gradient. Site 3 also had the highest overall sugar content from a single tree (6.7 %Brix). Site 1, which is located in the Huron Mountains, MI, had the lowest overall sugar content (1.87 %Brix) and also the lowest average earthworm count (10.33 earthworms) between the three plots along the introduction gradient.

There is an abundance of literature that explores the chemical composition of maple syrup, but a notable scarcity of literature that does so for raw sugar maple sap, so it should be noted that there were a select few papers to which we could compare our results, and the methods used varied greatly between them. When comparing average sap values from each plot class (Table 3) to average chemistry values found in published literature (Lagacé et al., 2015, van den Berg et al. 2019, Yuan et al. 2013; Table 5), there are some differences, but most values were within ranges of those reported previously (which were not associated with earthworm presence in any way). Average values by plot class for sap pH (5.95-6.03), overall sugar content (2.16-2.25 % Brix), and sucrose content (20.02-21.15 g/L) all fell within the average range reported within previous literature (Table 3 & 5). Our average fructose values from each plot class (0.4441-0.4981 g/L) seem relatively high, which may be due to interference due to the presence of malic acid in our samples, as the peaks produced for malic acid and fructose using the HPLC methods utilized can be too close together for the software to distinguish. This may have led to an overinflated report on the content of fructose. Additionally, the average values from each plot class for vanillin (0.0008-0.0009 g/L) and syringaldehyde (0.0001 g/L) were much higher than those reported in the literature. This may be due to major differences between the methods used in this study, which used fresh, raw sap samples for HPLC analysis, and those used by Lagacé et al., 2015, which used dehydrated sap samples that were mixed with methanol before analysis by HPLC.

When comparing average sap nutrient content from each plot class, Ca (36.87-37.65 mg/L), Fe (0.021-0.048 mg/L), and Zn (0.12-0.17 mg/L) were lower than average, but still within the typical range reported in literature. Average sap Cu (0.015-0.023 mg/L) was lower than average, being at the low end or below the reported range. Average sap P (1.48-1.82 mg/L) and Mn (2.25-4.03 mg/L) were higher than average but still within the reported range. Average sap K (87.68-96.45 mg/L) was much higher than average, being above the high end of the reported range. Average sap Mg (4.88-5.14 mg/L) was very close to the reported average.

Table 2. Summary of all earthworm species observed and their associated ecotypes.

Ecotype	Species
Epigeic	<i>Dendrobaena octaedra</i>
	<i>Dendrodrilus rubidus</i>
	<i>Eiseniella tetraedra</i>
	<i>Eisenia fetida</i>
Epi-Endogeic	<i>Lumbricus rubellus</i>
Endogeic	<i>Aporrectodea caliginosa</i>
	<i>Aporrectodea longa</i>
	<i>Aporrectodea rosea</i>
	<i>Aporrectodea trapezoides</i>
Anecic	<i>Lumbricus terrestris</i>
Epi-Endogeic/Anecic	Juvenile <i>Lumbricus spp.</i>

Table 3. Summary of results for soil and earthworm collection at each of the three plot classes. Included are average impact ratings, total sums of earthworm counts and biomass measurements for each site class, and average soil chemistry values for each site class.

	Established	Front	Least Established
Earthworm Values (totals unless noted as average)			
Average Impact Rating (1-5)	1.07	2.73	4.53
Earthworm Count	290	191	115
Ash-Free Dry Mass (g)	8.91	5.74	3.95
Wet Mass (g)	55.41	35.35	23.19
Epigeic Count	51	71	62
Endogeic Count	77	31	10
Epi-Endogeic Count	10	16	11
Anecic Count	3	3	1
Juvenile <i>Lumbricus spp</i> Count	149	70	31
Average Soil Chemistry Values			
pH	5.03	4.7	4.43
N (%)	0.26	0.22	0.24
C (%)	3.83	3.29	4.55

Soil P (mg/kg)	273.51	261.59	189.19
Soil Cu (mg/kg)	8.07	8.63	6.99
Soil Ca (mg/kg)	1851.28	1076.77	1005.52
Soil K (mg/kg)	560.56	501.06	370.52
Soil Zn (mg/kg)	30.69	23.33	20.13
Soil Mg (mg/kg)	1136.93	1106.88	567.89
Soil Mn (mg/kg)	449.2	329.41	193.57
Soil Fe (mg/kg)	6130.13	6408.93	3503.93

Table 4. Average total sugar content (% Brix), average soil impact rating (on a 1-5 scale, with 1 being highest impact and 5 being lowest impact), and average earthworm comparison between each of the five sites. Averages are based on three plots per site.

Site	Sugar Content (% Brix) (min-max)	Soil Impact Rating (1-5 scale)	Earthworm Count (min-max)
1	1.87 (1.2-2.8)	2.67	10.33 (6-15)
2	2.41 (1.5-3.5)	2.78	36.67 (5-57)
3	2.62 (1.4-6.7)	2.78	57.67 (43-76)
4	1.97 (1.2-3.4)	3.11	43.67 (31-59)
5	2.12 (1.3-3.5)	2.56	50.33 (30-83)

Table 5. Average chemistry values from fresh liquid sugar maple sap reported in literature compared with average sap chemistry values for each site class (minimum to maximum range reported in parentheses).

	Values From Literature *	Established	Front	Least Established
pH	7.13 (5.18-8.11) ^A	6.03 (5.78-6.29)	6.00 (5.79-6.34)	5.95 (5.63-6.14)
Sugar Content (% Brix)	2.03 (1.10-3.10) ^A	2.16 (1.75-2.36)	2.25 (1.79-2.64)	2.18 (1.74-2.9)
Sucrose (g/L)	22.6094 ± 3.8887 ^C	20.53 (17.12-22.66)	21.15 (15.86-25.01)	20.02 (16.18-26.26)
Fructose (g/L)	0.1267 ± 0.1621 ^C	0.4441 (0.3511-0.4981)	0.4835 (0.4240-0.5369)	0.4981 (0.3927-0.7249)
Syringaldehyde (g/L)	0.00003 (<0.00003-0.00007) ^A	0.001 (0.00086-0.00112)	0.001 (0.00086-0.00110)	0.001 (0.00086-0.00106)

Vanillin (g/L)	0.00003 (<0.00001-0.00007) ^A	0.0008 (0.00032-0.00113)	0.0009 (0.00031-0.00115)	0.0009 (0.00032-0.00114)
Ca (mg/L)	49.10 (24.4-67.6) ^B	37.65 (29.38-47.54)	42.47 (28.60-53.66)	36.87 (33.33-38.43)
P (mg/L)	1.20 (0.45-2.20) ^B	1.61 (1.03-2.21)	1.48 (0.89-2.08)	1.82 (1.05-2.30)
K (mg/L)	64.6 (54.0-80.2) ^B	91.34 (80.50-100.16)	96.45 (90.76-101.80)	87.68 (62.24-104.94)
Mg (mg/L)	5.17 (3.12-7.25) ^B	4.88 (4.15-5.81)	5.09 (3.86-6.31)	5.14 (4.37-5.85)
Fe (mg/L)	0.19 (0.01-2.17) ^B	0.021 (0.004-0.042)	0.048 (0.005-0.099)	0.042 (0.003-0.138)
Mn (mg/L)	3.84 (0.78-7.92) ^B	2.25 (0.87-3.44)	3.84 (1.24-6.45)	4.03 (2.47-5.66)
Cu (mg/L)	0.16 (0.02-1.03) ^B	0.016 (0.012-0.021)	0.023 (0.013-0.035)	0.015 (0.012-0.020)
Zn (mg/L)	0.26 (0.15-0.63) ^B	0.12 (0.09-0.17)	0.16 (0.11-0.18)	0.17 (0.12-0.22)

^ALagacé et al., 2015; ^Bvan den Berg et al. 2019; ^CYuan et al. 2013

Of all the clustered image maps (CIMs), the ones showing earthworm-sap (Figure 4), soil-sap (Figure 5), and forest composition-sap (Figure 6) interactions produced the most notable results, with numerous strong positive and negative associations being shown. However, there were significant correlations found in all 6 CIMs. Using these maps, we selected the most noteworthy pairings and ran linear regressions, the results of which can be found in Tables 6 and 7.

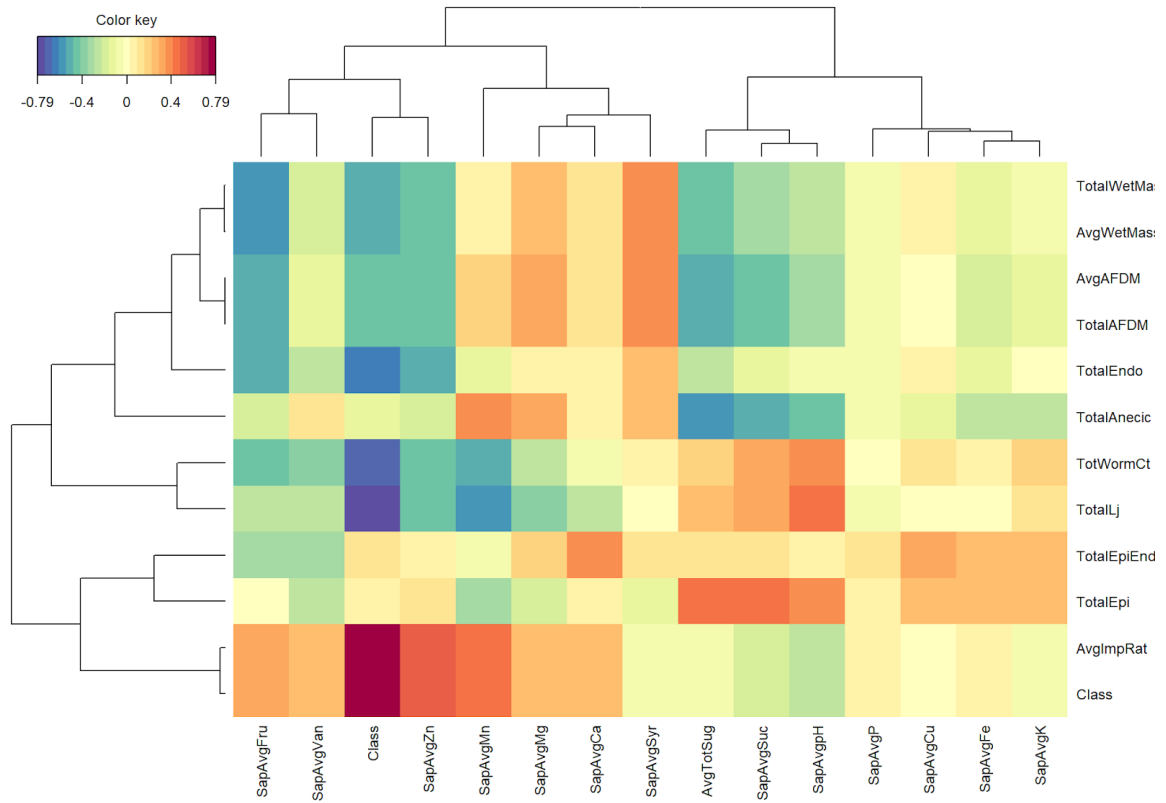


Figure 4. sPLS clustered image map of the association between variables in the Sap dataframe and those in the Earthworm dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using mixOmics (version 6.22.0).

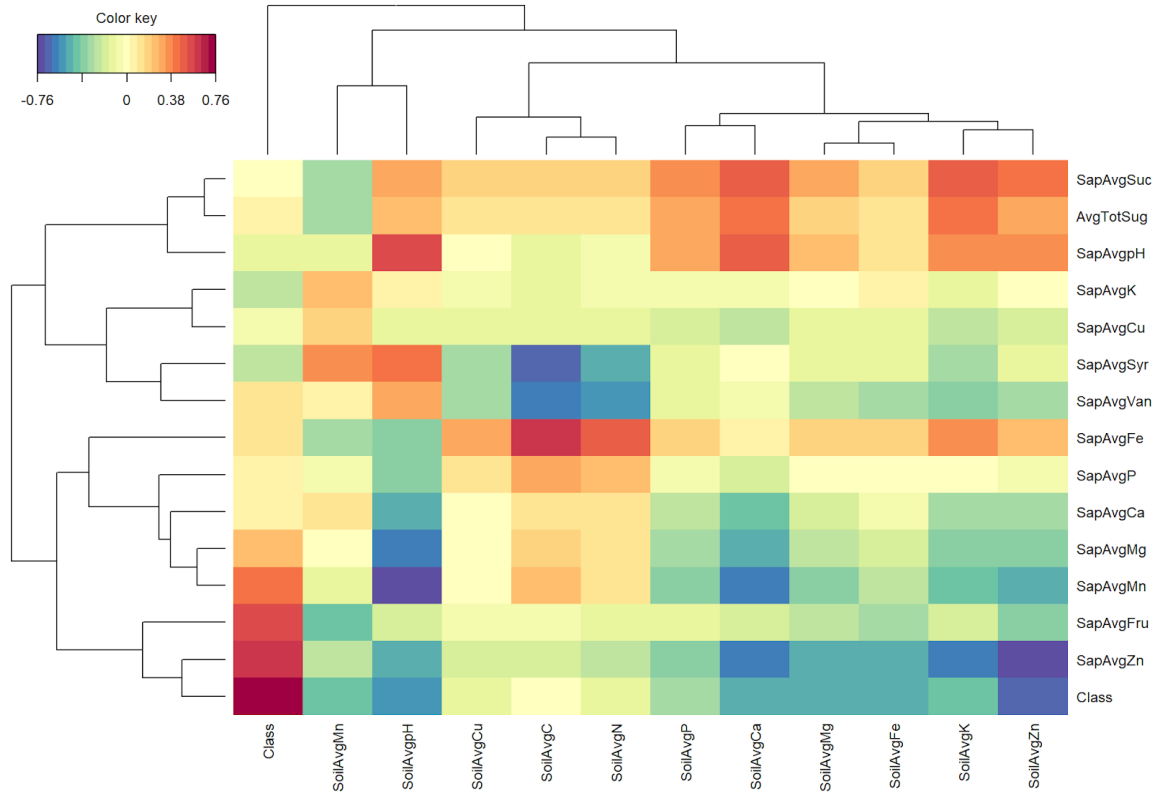


Figure 5. sPLS clustered image map of the association between variables in the Soil dataframe and those in the Sap dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using mixOmics (version 6.22.0).

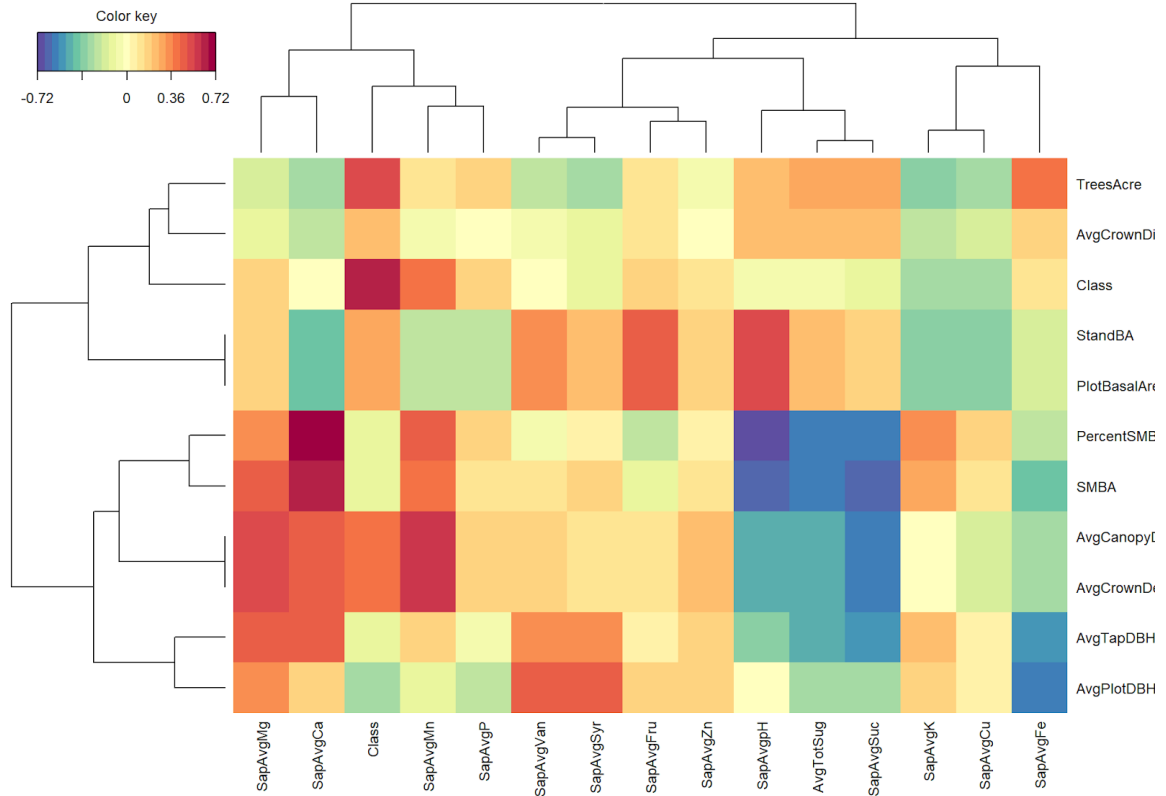


Figure 6. *sPLS clustered image map of the association between variables in the Forest Composition dataframe and those in the Sap dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using mixOmics (version 6.22.0).*

There was a significant positive correlation between average soil pH and average sap pH ($R^2 = 0.33$, $p = 0.014$; Figure 5, Table 6). There was also a significant positive correlation between the presence of epigeic earthworms and the average pH of sap ($R^2 = 0.22$, $p = 0.045$; Figure 4, Table 6), as well as a significant positive correlation between total earthworm counts and average soil pH ($R^2 = 0.35$, $p = 0.011$; Appendix Figure 8, Table 6). The influence of soil pH on earthworms, and vice versa, is well-documented, as less acidic soils are more favorable for earthworm habitation and earthworms also amend the soil over time and increase the pH, which better suits them (Ferlian et al., 2020; Frelich et al., 2006). These associations point to the possibility that earthworm activity in the soil, especially that of epigeic earthworms, may be having an impact on the pH of the sap.

There is a similar dynamic that can be seen with average sap Ca, which is negatively correlated with average soil pH ($R^2 = 0.31$, $p = 0.018$; Figure 5, Table 6), but positively correlated with the presence of epi-endogeic earthworms ($R^2 = 0.21$, $p = 0.048$; Figure 4, Table 6), which perform similar functions within the soil as epigeic earthworms. Ca ions are rapidly leached from soil that is more acidic (West et al., 2023), but short-term increases in Ca availability are associated with earthworm activity (Resner et al., 2015). Additionally, sugar maple basal area is also positively correlated with sap Ca content (R^2

= 0.21, $p = 0.049$; Figure 6, Table 6), which could also be due to the short-term increase in Ca availability, as sugar maples tend to be healthier and more vigorous at sites with higher Ca availability (West et al., 2023).

Average soil Zn was highly correlated with total earthworm counts ($R^2 = 0.67$, $p = 0.001$; Appendix Figure 8, Table 6), which has previously been documented in literature, as earthworms immobilize Zn in their castings, which artificially inflates Zn presence in soil samples by bringing Zn from other soil horizons to the surface (Lévêque et al., 2015; Richardson et al., 2018). This is also shown in that average impact rating is negatively correlated with average soil Zn ($R^2 = 0.48$, $p = 0.002$; Appendix Figure 8, Table 6), which means that as impact ratings get lower (signifying greater earthworm impact on the forest floor), soil Zn content increases. It appears that endogeic earthworms are the most associated with increase in soil Zn ($R^2 = 0.42$, $p = 0.005$; Appendix Figure 8, Table 6), which is likely due to their activity being in the A horizon, which causes greater movement of nutrients and minerals (Resner et al., 2015). Interestingly, average soil Zn is negatively correlated with average sap Zn ($R^2 = 0.32$, $p = 0.016$; Figure 5, Table 6) as well as average crown density ($R^2 = 0.25$, $p = 0.033$; Appendix Figure 9, Table 6), which likely shows that the activity of earthworms is decreasing the bioavailability of Zn for uptake by the sugar maples, either through immobilizing it or through increased root stress, which has previously been documented in literature (Richardson et al., 2018).

Higher average soil Mn content was associated with higher total ash-free dry mass biomass values ($R^2 = 0.27$, $p = 0.028$; Appendix Figure 8, Table 6). High levels of Mn can be problematic for sugar maples, as it can impair photosynthetic capabilities as well as hinder the uptake of other important ions (St. Clair et al., 2008). Higher earthworm biomass quantifications were negatively associated with average sap fructose content, both for total ash-free dry mass ($R^2 = 0.24$, $p = 0.038$; Figure 4, Table 6) and total wet mass ($R^2 = 0.27$, $p = 0.027$; Figure 4, Table 6). Although there were probable issues with our methods regarding fructose measurements, it is worth noting that there may be some associated impacts between earthworm presence and sap fructose content.

Average soil K was positively correlated with total earthworm counts ($R^2 = 0.27$, $p = 0.028$; Appendix Figure 8, Table 6), which has also been previously documented in literature as a short-term increase associated with earthworm activity, although this increase in soil availability is not consistently associated with uptake, despite higher K availability being associated with sugar maple growth and canopy health (Dobson et al., 2017; Resner et al., 2015; St. Clair et al., 2008). Although average sap Zn is negatively correlated with average soil K ($R^2 = 0.34$, $p = 0.013$; Figure 5, Table 6), this is likely a superficial association due to the increase in soil Zn and soil K both being associated with total earthworm counts and then average sap Zn being negatively correlated with average soil Zn, as discussed earlier. Most notably, average sap sucrose content is positively correlated with average soil K ($R^2 = 0.21$, $p = 0.047$; Figure 5, Table 6), which could signify that the increase in soil K due to earthworm activity could be having positive implications for the ability of the trees to produce more sugars. Although average overall sugar content was not significantly correlated with average soil K, it is important to note that sucrose makes up 96-100% of the sugar content of sap, which can be seen in the

close association between average overall sugar content and average sucrose content in Figures 4 and 5 (Ball, 2007; Lagacé et al., 2015).

Average sap vanillin is positively correlated with average plot DBH ($R^2 = 0.22$, $p = 0.046$; Figure 6, Table 6), and average sap syringaldehyde was negatively correlated with average soil C ($R^2 = 0.29$, $p = 0.023$; Figure 5, Table 6). There is no clear explanation for why these associations may have been significant, especially due to possible issues in the methods used to detect these compounds, but because the production of vanillin and syringaldehyde in the sap is likely due to microbial activity, it is likely that there are some soil-related factors at play that contribute to the presence of the responsible microbes (Lagacé et al., 2015; Mohammed et al., 2022).

Table 6. Results of linear regression models for all statistically significant correlations. Included are y-variables, x-variables, R^2 values, p-values, and whether the correlation is positive or negative.

y-variable	x-variable	R^2	p-value	+/-
Epigeic Earthworms	Average Sap pH	0.22	0.045	+
Average Soil Mn	Total Earthworm Ash-Free Dry Mass	0.27	0.028	+
Average Soil Zn	Total Earthworms	0.67	0.001	+
Average Soil pH	Total Earthworms	0.35	0.011	+
Average Soil K	Total Earthworms	0.27	0.028	+
Average Sap Vanillin	Average Plot DBH	0.22	0.046	+
Average Sap pH	Average Soil pH	0.33	0.014	+
Average Sap Sucrose	Average Soil K	0.21	0.047	+
Average Sap Ca	Epi-Endogeic Earthworms	0.21	0.048	+
Average Sap Fructose	Total Ash-Free Dry Mass	0.24	0.038	-
Average Sap Fructose	Total Wet Mass	0.27	0.027	-
Average Soil Zn	Endogeic Earthworms	0.42	0.005	+
Average Soil Zn	Average Impact Rating	0.48	0.002	-
Average Sap Zn	Average Soil Zn	0.32	0.016	-
Average Crown Density	Average Soil Zn	0.25	0.033	-
Average Sap Ca	Sugar Maple Basal Area	0.21	0.049	+
Average Sap Zn	Average Soil K	0.34	0.013	-
Average Sap Ca	Average Soil pH	0.31	0.018	-
Average Sap Syringaldehyde	Average Soil C	0.29	0.023	-

Anecic earthworms, which were heavily zero inflated, required analysis using generalized linear models with a Poisson distribution. This is likely due to the fact that anecic earthworms, such as *L. terrestris*, are typically only present at long-established sites, but it is important to note that the mustard extraction method used is the most effective method for sampling anecic earthworm populations (Cameron et al., 2007; Cameron & Bayne, 2009; Resner et al., 2015). Anecic earthworms were negatively associated with average sap pH ($R^2 = 0.28$, $p = 0.019$; Figure 4, Table 7). Most notably, anecic earthworms were negatively correlated with both average overall sugar content ($R^2 = 0.52$, $p = 0.038$; Figure 4, Table 7) and average sap sucrose content ($R^2 = 0.41$, $p = 0.029$; Figure 4, Table 7). This could be an indicator that the presence of anecic earthworms marks a point at which sugar maples begin to have a decreased capacity to produce sugars, although there is not a clear direct explanation as to specific mechanisms why in regards to anecic earthworms, other they are typically present in more established introduction zones.

Table 7. Results of generalized linear regression models for relevant statistically significant correlations. Included are y-variables, x-variables, R² values, p-values, and standard errors.

y-variable	x-variable	R²	p-value	Est. Slope and Std. Error
Anecic Earthworms	Average Sap pH	0.28	0.019	-5.35 ±2.29
Anecic Earthworms	Average Total Sugar	0.52	0.038	-5.40 ±2.60
Anecic Earthworms	Average Sap Sucrose	0.41	0.029	-0.42 ±0.19

3.4 Conclusion

Introduced earthworms appear to be correlated with significant changes in the chemistry of sugar maple sap due to complex, dynamic interactions they have with the soil. This dynamic is bottom-up in nature, where the earthworms change the chemistry of the soil by altering things like soil texture, pH, and availability of soil nutrients, which leads to impacts on the overall health of the trees, possibly through increased rooting stress and decreased bioavailability of nutrients like Zn and K that are essential for tree health and vigor. These impacts on tree health and vigor ultimately lead to a long-term decline in the capacity of impacted trees to produce typical quantities of important compounds found within maple sap that are important for maple syrup quality, such as vanillin, syringaldehyde, and sugars like sucrose. The presence of anecic earthworms, which are typically only present at sites with longer-standing earthworm communities, are associated with significant decreases in sap sugar content which may be an indicator that late-stage earthworm colonizations mark a point where sugar maples begin to experience a quantifiable decline in ability to produce quality sap. This is possibly due to this being a point at which essential nutrients, such as Ca, P, Mg, and K, are leached out of the system at a rate they cannot be effectively replenished, creating deficiencies.

This study, as a pilot exploration, provides an important jumping off point for a previously unstudied subject and lays the groundwork for future research. Ultimately, this study provides a snapshot in time for each of these individual sites. Further exploration is needed of the impacts that introduced earthworms are having on sugar maple health. There were limitations using the HPLC methods for sap analysis that could be improved, thus more thorough chemical analysis of sap along these gradients may provide additional insight into changes in phenolic and organic acid content. Conducting long-term studies on these five sites will also provide more clarity by allowing analysis of trends while accounting for the natural yearly variation in sap chemistry. Additionally, it may prove beneficial to assess changes in soil microbial communities along earthworm introduction gradients with specific focus on soil microbes that are important to the chemical processes that take place within sugar maple sap. There is also much that is still unknown about the physiological changes that may occur in sugar maples along these gradients, so it could be of interest to study potential changes in foliar concentrations of nutrients as well. Additionally, it would be a beneficial next step to assess the impacts of differing bioassemblages of earthworm ecotypes on sap chemistry, e.g. combining endogeic and anecic earthworm counts and assessing associated changes (Hale & Host, 2005).

This study highlights the potentially detrimental implications for the production of maple syrup and sugar, and it will be beneficial to increase awareness of the impacts of introduced earthworms with producers for consideration in sugarbush management regimes. Although management for introduced earthworms could look like increased cost and labor for producers, for example by checking that tires of equipment are cleaned regularly or monitoring throughout their sugarbush for earthworm presence, these actions will act as extra tools that producers can use to monitor the health of their trees and ensuring more efficient production. Additionally, it would be beneficial for future studies to conduct a cost-benefit analysis of how extra cost of management or mitigation practices regarding introduced earthworms would compare to the impacts of earthworm activity on production costs and potentially reduced sugars in trees over time. This would provide an important tool for outreach and education for producers that will allow them to make informed decisions on what are best practices for their sugarbush.

3.5 References

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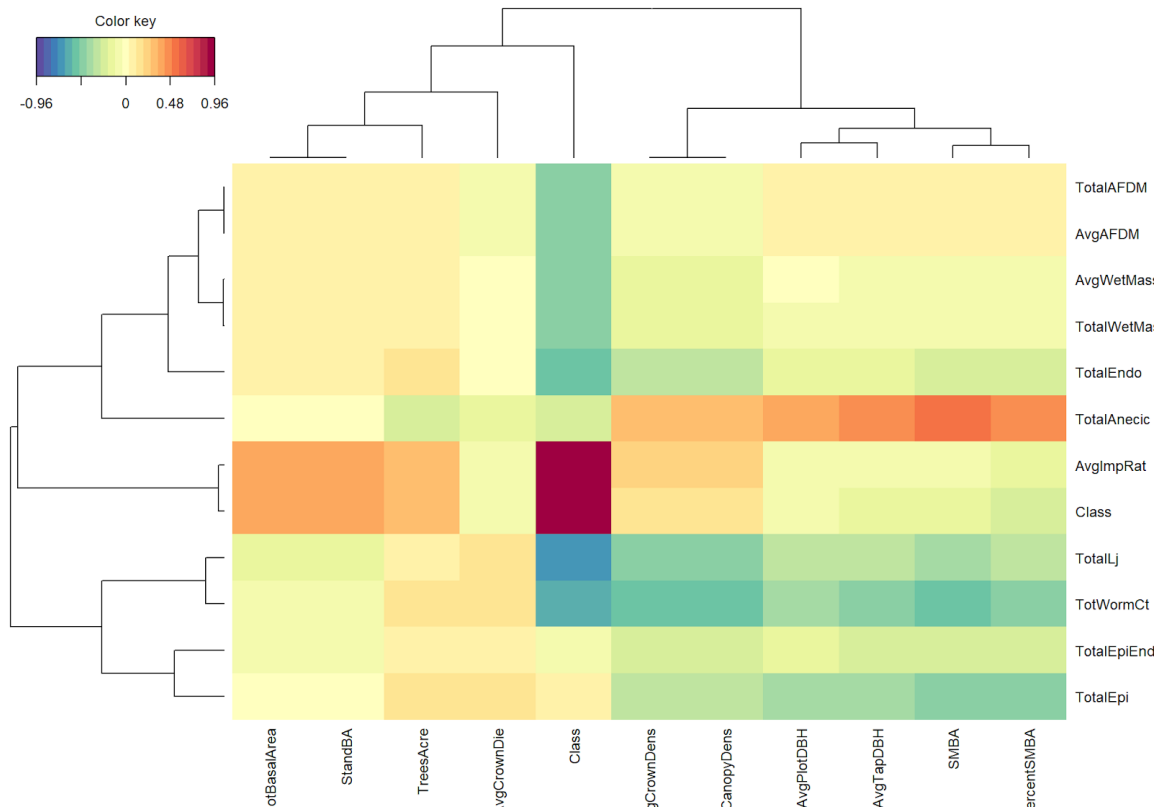
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3.6 Appendix

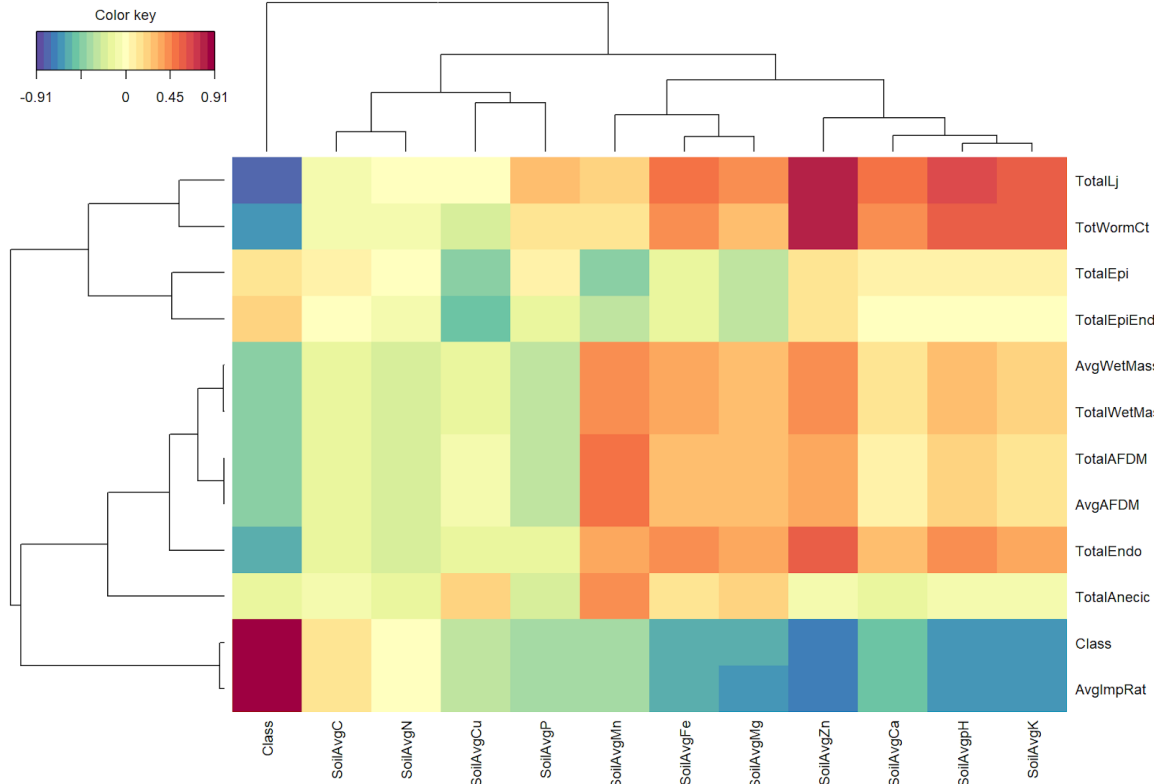
Appendix Table 1. Rating scale used to do visual assessments of earthworm impacts on the forest floor.

Earthworm Midden Density Scale

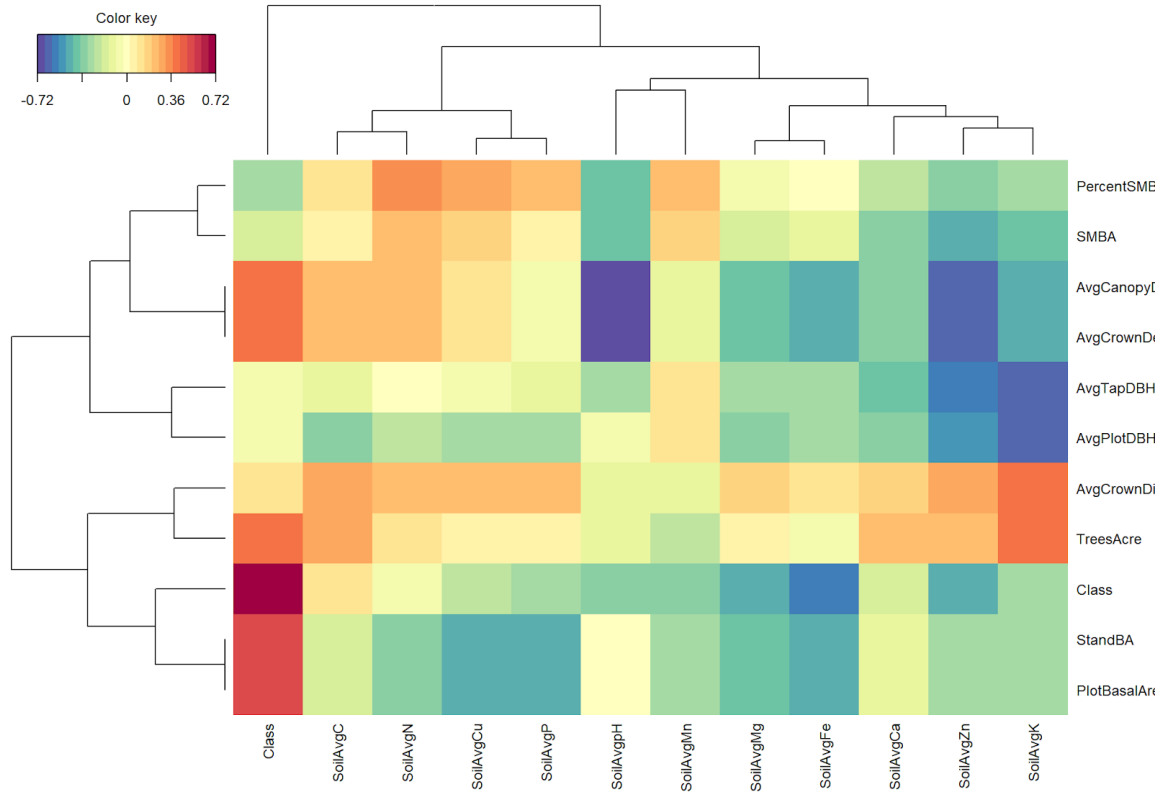
1	No forest floor. Previous year's litter over mineral soil. castings and/or <i>L. terrestris</i> middens abundant.
2	No humus, or small leaf fragments present; larger old leaves may be present under litter. Worm castings present; <i>L. terrestris</i> middens present or absent. Roots absent from forest floor.
3	No humus. Small leaf fragments and larger old leaves present. Sparse to no roots in the forest floor. Some worm castings may be present. <i>L. terrestris</i> likely to be absent or very sparse
4	Humus present in patches, may be slightly mixed with mineral soil, rest of the forest floor is intact. Some roots in forest floor, but not thick. Small worms in forest floor, but no large castings or middens
5	Humus fully intact. Roots present in humus and leaf fragments. Forest floor coherent when picked up with intact recognizable layers. No worms or worm sign present.



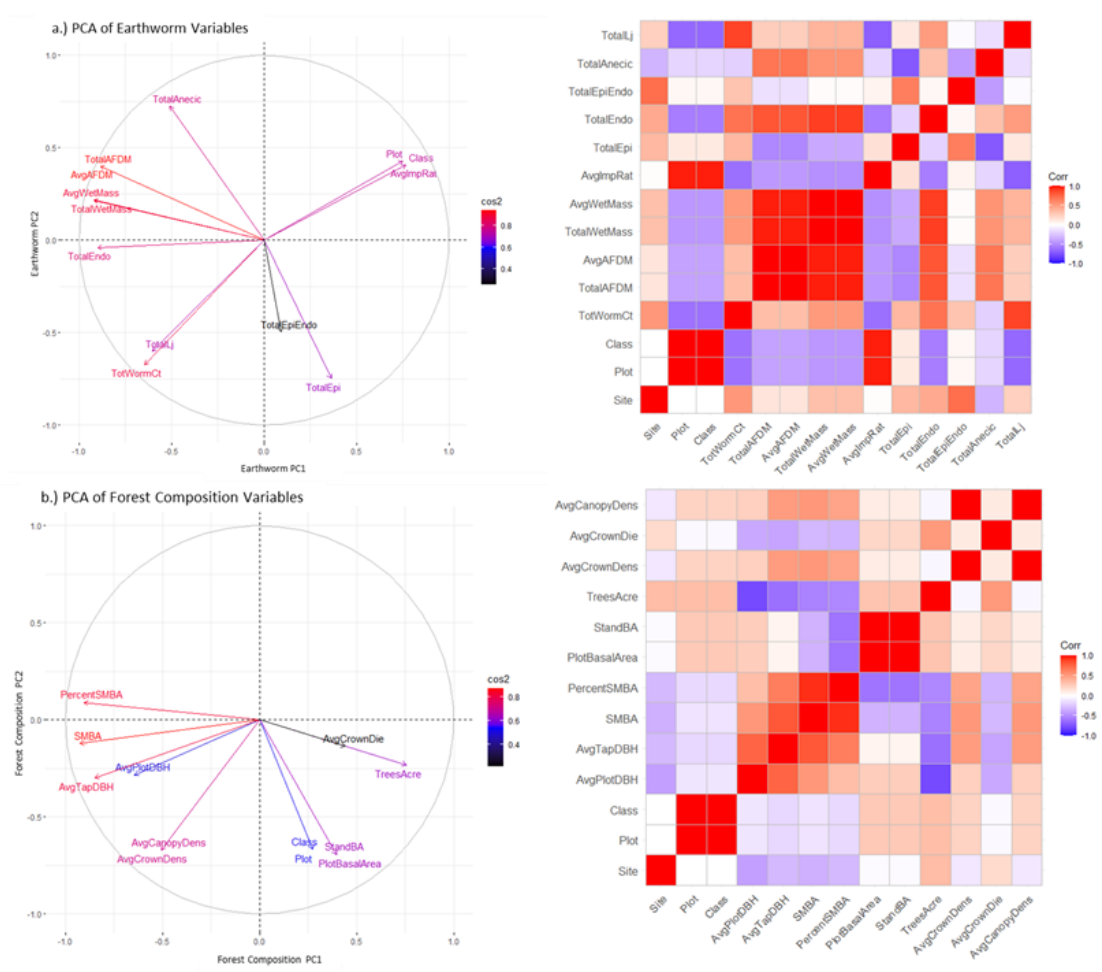
Appendix Figure 1. sPLS clustered image map of the association between variables in the Forest Composition dataframe and those in the Earthworm dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using mixOmics (version 6.22.0).



Appendix Figure 2. sPLS clustered image map of the association between variables in the Soil dataframe and those in the Earthworm dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using mixOmics (version 6.22.0).



Appendix Figure 3. *sPLS* clustered image map of the association between variables in the Forest Composition dataframe and those in the Soil dataframe. Strong positive correlation is represented in deep red and strong negative correlation is represented in deep blue. Created using *mixOmics* (version 6.22.0).



Appendix Figure 4. Results of the PCAs for each of the earthworm and forest composition dataframes. The left figure in each pair, created using factoextra (version 1.0.7), shows the eigenvectors for each variable, where arrows going in similar directions at similar angles are more closely correlated. The cos2 rating for each variable describes the contribution of each variable, i.e. how well it is represented by the principal component. The right figure in each pair, created using ggplot2 (version 3.4.2), is a correlation plot showing how related each variable is to the others in the dataframe.

