

Tests of the Invasional Meltdown Hypothesis in invasive
herbaceous plant species in southern Ontario

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Abstract

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According to the Invasional Meltdown Hypothesis (IMH), invasive species may interact in their introduced range and facilitate future invasions. This study investigated the possibility that *Alliaria petiolata*, an invasive allelopathic herbaceous plant in Ontario, is facilitating invasions by additional alien species. Two allelopathic focal species were chosen for this study: the native *Solidago canadensis* and the invasive *A. petiolata*. Field surveys in southern Ontario that quantified plant biodiversity in plots that included one or both focal species revealed no support for the IMH, although fewer species co-existed with *A. petiolata* than with *S. canadensis*. A year-long recruitment experiment in Peterborough, Ontario, also produced results inconsistent with the IMH, although did provide some evidence that *A. petiolata* limited recruitment of other species. These results collectively show negative impacts on regional biodiversity by *A. petiolata*, even in the absence of an invasional meltdown.

Keywords

Alliaria petiolata, *Solidago canadensis*, invasional meltdown hypothesis, invasive species, allelopathy, non-native species, germination, co-occurrence surveys.

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Glossary

Allelopathy: The suppression of growth of a plant by a chemical released from another plant.

Arbuscular mycorrhiza: A type of mycorrhizal association where there is a symbiotic relationship between a fungus and the roots of a plant.

Invasional Meltdown: Mutualistic interactions between non-native species in which they benefit from each other's presence (Simberloff and Von Holle 1999). The Invasional Meltdown Hypothesis (IMH) predicts that non-native species could interact in their introduced range and aid each other's invasions (Simberloff and Von Holle 1999).

Chapter 1: General Introduction

An invasive species is defined as a non-native species that is harmful to the environment, economy, or human health in its introduced range. A non-native, or introduced, species is a species that has been introduced to a new range but does not necessarily have known negative consequences. Only 10% of the introduced species become established and only 10% of those become invasive (Williamson 1996). Some of those may pose a risk to human health, such as giant hogweed, which has sap that can cause photodermatitis upon contact (Rzymiski et al. 2015). Others mainly pose an environmental threat, by altering the ecosystem into which they are introduced (Tang et al. 2012). Overall, once established, invasive species are very costly to control and even if control is successful, their effects on the ecosystem are often irreversible (Ontario Ministry of Natural Resources 2012).

There are many ideas as to how introduced species become invasive in their new range. Blumenthal (2005) argues that many of the theories regarding the invasiveness of non-native species revolve around the differences between their native range and the new introduced range. For example, the “resource availability theory” suggests that non-native species are able to invade communities that have an excess of resources available and thus are more likely to invade disturbed areas (Davis et al. 2000). The “enemy release hypothesis” is another possible mechanism, which explains that exotic species become invasive because they are not regulated by their natural enemies in their new range (Keane and Crawley 2002). It is also possible that these two theories can

interact by way of the “resource-enemy release hypothesis”, where non-native species compete even better in resource rich communities because they are not controlled by herbivores and can utilize the extra resources without being penalized (Blumenthal 2005). Another mechanism is the “novel weapons hypothesis” which explores the possibility that invasive species possess certain competitive mechanisms that are new to their introduced communities, thus allowing them to successfully invade (Callaway and Aschehoug 2000). Overall, although there are many competing ideas, it may be that each case is different and one theory cannot explain all invasions (Davis et al. 2000; Keane and Crawley 2002). However, one idea that is starting to gain attention is that invasive species can themselves create favourable conditions for future invasions (Simberloff and Von Holle 1999; Braga et al. 2018).

Invasive species can affect the environment in their introduced range in four main ways: through competition, by changing resource availability, by altering nitrogen processes, or by modifying disturbance regimes in an ecosystem (Crooks 2002; Tang et al. 2012). For example, one way to explain the success of *Alliaria petiolata*, an allelopathic invasive plant species present in much of eastern North America, is that it was preceded by the introduction of invasive earthworms that are facilitating the introduction of other non-native species (Flinn 2017). Invasive European and Asian earthworms are able to alter the soil properties to the advantage of certain non-mycorrhizal species, such as *Alliaria petiolata*, by changing the composition of microbial and fungal communities (Frelich et al. 2006). Now *A. petiolata* is one of the most common invaders of forest understories in Ontario. Therefore, it is vital to understand

factors that affect and facilitate establishment of newly introduced species in the province.

The invasional meltdown may be a crucial component in understanding the establishment of invasive species in the future. The invasional meltdown is a process in which invasive species interact in their introduced range and aid each other's invasions (Simberloff and Von Holle 1999). With rates of invasions on the rise, the likelihood of systems with multiple invasive species is increasing; however, currently only about 6% of invasive species research looks at the possibility of these species interacting (Kuebbing, Nunez, and Simberloff 2013). Forest invasion research, even more so than marine and grassland research, is lagging by almost exclusively focusing on single invasion scenarios (Kuebbing, Nunez, and Simberloff 2013). It is time to shift the focus from research on single invasive scenarios, to research that include the possible additive or synergistic interactions of invasive species in their new ranges. My study examines one possible mechanism for the establishment of invasive species: whether invasive species facilitate other invasions through allelopathy.

Biology and Ecology of Focal Species

Alliaria petiolata (Brassicaceae) is an invasive biennial herbaceous plant, which is thought to have been brought to North America from England as an edible herb in the late 1800s, as it has a strong garlic smell. It has since escaped from gardens and has spread aggressively throughout much of southern and eastern Ontario, as well as northeastern and midwestern USA (Van Driesche et al. 2002). In the first growing year A.

petiolata appears as a basal leaf, which then overwinters and develops into a single growing stalk early in the spring (Anderson et al. 1996). White flowers then form in April, and linear fruit appear in early summer, encompassing black cylindrical seeds (Anderson et al. 1996). There are several possible reasons for its successful invasion. The first is its ability to flower and seed while all other species are still dormant (Cavers et al. 1979). The second is its shade tolerance, which allows it to effectively invade forest understories (Van Driesche et al. 2002; Anderson et al. 1996). It can also grow in various conditions, and quickly spreads through seeds transported by both humans and animals (Anderson et al. 1996). Allelopathy has been proposed as an additional mechanism which allows *A. petiolata* to invade so effectively in its introduced range (Stinson et al. 2006).

Past studies have found that *A. petiolata* can inhibit germination of native species. One study looked at the effects of soil contaminated by *A. petiolata*, on two congeneric species: one European (*Geum urbanum*) and one North American (*Geum laciniatum*) (Prati and Bossdorf 2004). They found that germination was inhibited by *A. petiolata* soil, with much stronger effects observed in the North American species, concluding that *A. petiolata* contaminated the substrate through root exudates (Prati and Bossdorf 2004). *Alliaria petiolata* has also been shown to disrupt the mutualistic relationship between native seedlings and arbuscular mycorrhizal fungi (Stinson et al. 2006; Cantor et al. 2011). This may be one reason behind the success of *A. petiolata* in its introduced range, as it can suppress the growth of native plants in this way (Stinson et al. 2006).

Although not all potential allelochemicals released by *A. petiolata* have been identified (Poon and Maherali 2015), it is known that its roots and leachate from its leaf litter contain sinigrin, which breaks down into allyl isothiocyanate (AITC) (Vaughn and Berhow 1991). This chemical has a half life of somewhere between 1-40 days, depending on the substrate (Barto and Cipollini 2009; Tsao et al. 2000). *Alliaria petiolata* was also found to release benzyl isothiocyanate (BzITC), which along with allyl isothiocyanate has been shown to inhibit growth of certain species (Vaughn and Berhow 1991). Benzyl isothiocyanate has a half-life of around 2 days, again depending on substrate (Barto and Cipollini 2009; Gimsing et al. 2007). Allelochemicals seems to provide an effective strategy in gaining a competitive advantage over its neighbours since *A. petiolata* itself does not use mycorrhizae (Stinson et al. 2006), whereas most woodland herbs do (Whigham 2004). It also releases cyanide (Cipollini and Gruner 2007), a cyanoallyl glycoside named alliarinoside (Barto and Cipollini 2009; Haribal and Renwick 2001), and several flavonoid glycosides (Barto and Cipollini 2009; Haribal and Renwick 2001).

Solidago canadensis (Asteraceae) is a perennial herbaceous plant that is native to most of Canada and much of the USA, with several varieties included in the taxon. *Solidago canadensis* can spread through seed, seedlings and rhizomes (Pavek 2011). Flowers typically bloom from July to October with panicles located at the end of each stem (Pavek 2011). As *S. canadensis* grows aggressively through rhizomes, it can colonize new and disturbed areas effectively and is difficult to control (Pavek 2011). This may be one of the reasons that *S. canadensis* is now considered an invasive species in

Europe, Asia, Australia and New Zealand (Abhilasha et al. 2008). In Ontario, *S. canadensis* is found in moist soil habitats ranging from damp meadows to roadside ditches to deciduous and evergreen forests, and can also exist in many soil types, a wide range of moisture regimes, and soil fertility conditions (Pavek 2011). As it can occur in both full sun or partially shaded habitats in southern Ontario, it can sometimes co-occur with *A. petiolata* (personal observations June 6th, 2018).

One possible reason for the successful spread of *S. canadensis* around the globe is allelopathy. One study found that *S. canadensis* releases four main chemical compounds into the soil that could be considered as allelochemicals (Abhilasha et al. 2008) [more information on the chemistry of these four compounds can be found in detail in the original article (Abhilasha et al. 2008)]. Flavones, Phenolics and saponins are the main groups of allelochemicals produced by *S. canadensis* (Zhang et al. 2006 as cited by Zhang et al. 2011), although it is possible that there are other currently unknown allelochemicals in play (Yuan et al. 2013). Like *A. petiolata*, the allelochemicals released by *S. canadensis* affect mutualistic relationships between native plants and mycorrhizal fungi (Zhang et al. 2010).

Previous experiments have shown that *S. canadensis* inhibits germination of other herbaceous plants. For example, one study looked at how *S. canadensis* interacted with co-occurring species in both its native and introduced ranges (Ledger et al. 2015). Ledger et al. (2015) found that native plant diversity in the introduced range was negatively affected by higher densities of *S. canadensis*, while the same effect was not observed in North America (Ledger et al. 2015). Another study also found that both

growth and germination of competing species in Europe were inhibited by root exudates of *S. canadensis* (Abhilasha et al. 2008). The same trend has been observed in China, where Yuan et al. (2013) determined that *S. canadensis* exerted more allelochemicals in its introduced range than in its native one. Another study found that *S. canadensis* extracts inhibited both growth and germination of four target species, including *Morus alba*, *Pharbitis nil*, *Triticum aestivum* and *Brassica campestris* (Sun et al. 2006). They also noted that higher concentrations of aqueous extracts had stronger inhibitory effects on these species (Sun et al. 2006). Overall, although *S. canadensis* is shown to inhibit the germination and growth of herbaceous species in its introduced range, it seems that its allelopathic effects are somewhat diminished in its native range (Abhilasha et al. 2008).

Hesperis matronalis (Brassicaceae) is a herbaceous biennial or perennial plant, and like *A. petiolata*, is also in the mustard family. It has purple, pink, or white flowers, with flowering stems dying back in July, and with the rosettes persisting throughout the growing season (Pavlovic et al. 2009). *Hesperis matronalis* is native to Eurasia and is found in all southern parts of Canada, including Ontario (NatureServe 2016b). More information on the biology of *H. matronalis* can be found in an article by Francis et al. (2009).

Hesperis matronalis has a similar life history to *A. petiolata* (Francis et al. 2009) and has intermediate shade tolerance like that of *A. petiolata* (USDA, NRCS 2018c). In Ontario, *H. matronalis* grows in disturbed habitats ranging from roadsides, old fields, and disturbed riparian woodlots, to disturbed deciduous wooded areas (Francis et al. 2009). Here it has been found in disturbed riparian woodlands for over 40 years and has

been seen growing in areas with *A. petiolata* (Francis et al. 2009). The mechanisms behind *H. matronalis* invasions are not well understood (Leicht-Young et al. 2012) and this plant may contain some allelopathic chemicals (Francis et al. 2009). Although several flavonoid glycosides (Tatsuzawa 2012; Tatsuzawa 2013) and glucosinolates (Larsen et al. 1992) have been identified in the past, exact allelopathic effects are unclear at this point (Francis et al. 2009) and they are likely not as allelopathic as *A. petiolata* (Brouwer et al. 2015). *Hesperis matronalis* was used as a control in this experiment, as it is similar in its life history to *A. petiolata* but is not considered to be as allelopathic as *A. petiolata* (Brouwer et al. 2015).

Objective 1

Firstly, I looked at the co-occurrence of invasive species in southern Ontario, as it is home to the greatest number of invasive species in Canada (Ontario Ministry of Natural Resources 2012) and so the co-occurrence of invasive species should be more likely here and thus should provide sufficient observational data for this study. The first step is understanding the co-occurrence of invasive species through observational studies that could confirm the existence of patterns among invaders (Kuebbing, Nunez, and Simberloff 2013). I tested one prediction of the invasional meltdown hypothesis, which states that invasive species that facilitate other invasions should co-occur with other non-native species. Thus, the first objective of my study was to find if such patterns in co-occurring invasive species exist. **My first hypothesis was that *Alliaria petiolata* would co-occur more often with other non-native species than with native ones, compared to its native counterpart *S. canadensis*.**

To satisfy the first objective, I conducted field surveys in several provincial parks in southern Ontario. The purpose was to determine whether *A. petiolata* tends to co-occur more often with non-native species than with native species. To do so, I compared plots with *A. petiolata* to plots with the native *S. canadensis*. Both plants release allelochemicals and occupy similar habitats in the study area, making this an informative comparison.

In each plot, I recorded all herbaceous plant species that were present, and the percent cover of the non-native and native herbaceous plant species identified. I then analysed the data to determine any correlation between co-occurring non-native species, to ascertain whether there is a positive relationship between *A. petiolata* and other non-native species in Ontario. **I expected to find more non-native species in plots with *Alliaria petiolata* than with the native *Solidago canadensis*.**

Objective 2

My second objective was to investigate one of the mechanisms that could be responsible for the co-occurrence of invasive species in Ontario. I intended to examine the effect *Alliaria petiolata* has on the recruitment of other invasive species. **My second hypothesis was that invasive pioneers create favourable environments for the future establishment of new non-native species into the ecosystem.**

For my second objective, I compared the effects that *A. petiolata* and *S. canadensis* have on the recruitment of other herbaceous plants. To test the effects that allelopathic species have on the recruitment of both native and non-native species, I

conducted an experimental garden study in several different habitats. A quarter of the pots in each habitat were planted with *A. petiolata*, a quarter with *S. canadensis*, a quarter of the pots with a non-allelopathic invasive control (*Hesperis matronalis*), and the rest remained unplanted and contained only soil. These pots were monitored for a full year for recruitment of new species. The proportion of native to non-native species was noted in each of the pots, along with the stem density. **I predicted that pots planted with the invasive *A. petiolata* would recruit more non-native species than pots with native species, non-allelopathic alien species, or control pots.**

To test for allelopathic potential of the two focal species (*A. petiolata* and *S. canadensis*), a germination experiment was also conducted, in which radish seeds were germinated in soil collected from each of the aforementioned pots. Radish seeds are often used in germination experiments testing for allelopathy (Pisula and Meiners 2010). After five days, the germination rates of radish seeds from the various pots were compared to germination rates in uncontaminated standard soil (soil control). **I predicted that germination rates will be higher in soil from control pots, the soil control and *H. matronalis*, than in *A. petiolata* and *S. canadensis* soil.**

Overall this research aims to establish whether there is evidence for facilitative effects of *A. petiolata* on herbaceous non-native species in southern Ontario that are consistent with the predictions of the invasional meltdown hypothesis. Synergistic interactions between invasive species are now more likely and thus studies that look at multi-invasion scenarios on a community level are vital (Braga et al. 2018). It is important to identify invasive species that are possibly facilitating other invasions

because their effects may exceed those that are expected under single species invasion scenarios.

Chapter 2: Tests of the Invasional Meltdown Hypothesis using Co-occurrence Surveys and a Seedling Recruitment Experiment

Introduction

With urbanization and the ever-increasing movement of people and goods around the world, the spread of invasive species significantly increased in the past 200 years (Seebens et al. 2017). Invasive species are one of the biggest threats to biodiversity worldwide, and depending on the declining taxa, are sometimes considered the most important driver of population decline (McGeoch et al. 2010). Invasive species pose a significant risk to Ontario's biodiversity, affecting about 20% of species identified as 'at risk' under the Endangered Species Act of Ontario (Ontario Ministry of Natural Resources 2012). Ontario is considered a hub for invasive species due to its population and geographic location, which makes it a centre for trade and transportation. In fact, it has 441 introduced naturalized plant species, the most recorded out of all Canadian provinces (Ontario Ministry of Natural Resources 2012). With rates of invasions still increasing globally (Seebens et al. 2017), there has been an exponential increase in worldwide efforts to combat future invasions (McGeoch et al. 2010). Similarly, invasive species are the focal point of many conservation initiatives in the province (Ontario Ministry of Natural Resources 2012).

With the number of invasions on the rise, it is now more likely than ever that invasive species will co-occur (Rauschert and Shea 2012; Kuebbing, Nunez and Simberloff 2013). Even so, most invasion research has been focused on single species

invasions (Kuebbing, Nunez, and Simberloff 2013). The concept of interactions between invasive species is not new; however, in comparison with single-species studies little research has been conducted on this subject (Rauschert and Shea 2012; Kuebbing, Nunez and Simberloff 2013). Nevertheless, studies are now focusing more on co-occurrence of non-native species in terrestrial ecosystems (Barga et al. 2018; Kuebbing and Nunez 2015). Although multi-species invasion studies looking at community or ecosystem levels are still lacking (Braga et al. 2018). With invasive plant species having such a devastating effect on terrestrial ecosystems, and the chances of co-occurrence on the rise, a shift in research from single species invasions, to research of pairs or clusters of invasive species is now in demand (Kuebbing, Nunez and Simberloff 2013). However, first there is a need to identify the types of interactions that occur between invasive species and the mechanisms underlying those interactions.

Three types of interactions between species are possible: facilitative, neutral, or competitive (Rauschert and Shea 2012; Kuebbing, Nunez and Simberloff 2013). Based on the concept of 'biotic resistance' of an ecosystem, competitive interactions would prevent new species from becoming established and thriving (Simberloff and Von Holle 1999). Conversely, neutral, or simply additive interactions, include no additional impacts on the introduced range, other than the combined individual impacts of the invasive species (Kuebbing, Nunez and Simberloff 2013). For example, Griffen and Byers (2009) examined how predation rates on native prey communities differed once an invasive crab, *Hemigrapsus sanguineus*, was introduced to a region in the coast of New England that another species of crab, *Carcinus maenas*, had already invaded. They found that the

two invasive crab species had additive consequences on the invaded marine ecosystem because their interactions made their effects on the community redundant (Griffen and Byers 2009). If effects of invasive species are redundant based on the species already present, then impacts from those invasions may be diminished (Griffen and Bayers 2009; Crooks 2002). Overall, it stands to reason that, unlike competitive interactions, neutral interactions could lead to co-occurrence of invasive species.

Of most concern for the management of native ecosystems are facilitative, or superadditive, interactions, as these could lead to the process of an 'invasional meltdown' (Simberloff and Von Holle 1999). An invasion meltdown is defined by mutualistic interactions between invasive species in which they benefit from each other's presence (Simberloff and Von Holle 1999). In this scenario invasive species facilitate each other's invasion by increasing the likelihood of survival, amplifying the ecological impact and accelerating the accumulation of invasive species (Simberloff and Von Holle 1999). Facilitative interactions can have devastating consequences on native ecosystems, bigger even than the combined individual impacts of all the persisting invasive species (Simberloff and Von Holle 1999). For instance, Kuebbing, Souza and Sanders (2014) investigated how the number of invasive species affected both native and non-native species composition in 1-m² plots in an old field habitat and found that synergistic interactions between invasive species were responsible for altering successional dynamics in an old field setting. They noted that composition changed to random assemblages of species once the number of invasive species exceeded 3, and not only did increased rates of invasion alter interactions between native species, but it

also changed the ratio of herbaceous to woody foliar cover in the observed plots (Kuebbing, Souza and Sanders 2014). Studies like this suggest that when there are several invasive species established in the same ecosystem there is a chance for them to coexist with one another, and even have magnified consequences on their new range.

Past studies have shown instances where invasional meltdowns are occurring, but according to one meta analysis of studies looking at the Invasional Meltdown Hypothesis (IMH) there is a lot of bias and research gaps that need to be addressed (Braga et al. 2018). Although mechanisms involved in invasional meltdowns have not yet been clearly identified, especially on a community scale (Braga et al. 2018), one proposed factor that could contribute to invasional meltdowns is the presence of habitat modifying abilities in invasive species (Simberloff and Von Holle 1999). Plants that are able to alter the environment in their introduced ranges may be able to facilitate the further invasion of that environment (Simberloff and Von Holle 1999). In eastern North America, *Alliaria petiolata*, a biennial plant originating from Eurasia, may be responsible for facilitating further establishment of invasive species (Callaway et al. 2008). This plant is one of the most damaging invasive species in the region as it releases allelochemicals that inhibit the growth of mycorrhizal fungal mutualists (Stinson et al. 2006). Of the known allelochemicals, *A. petiolata* predominately produces 2-Propenylglucosinolate (sinigrin) which is part of the glucosinolates family (Vaughn and Berhow 1991), and alliarinoside, an allelochemical in the hydroxynitrile glycosides class (Barto and Cipollini 2009; Haribal and Renwick 2001). These characteristics are likely one of the reasons behind this species' successful invasion (Olsen et al. 2014) and given the

prevalence of *A. petiolata* in Ontario and elsewhere, it is important to investigate whether *Alliaria petiolata* might facilitate a future invasional meltdown.

My study had two objectives that address the invasional meltdown hypothesis and the mechanisms underlying invasions by terrestrial plants. My first objective was to determine whether there are non-random patterns of co-occurrence between *A. petiolata* and other invasive species. This was done through an observational study consisting of field surveys of four provincial parks and an urban park in southern Ontario. My second objective was to identify mechanisms that could help explain patterns of co-occurrence between *A. petiolata* and other non-native species. Because of the potential role for allelochemicals to alter patterns of species co-occurrence with *A. petiolata*, this study investigated whether allelopathy affects patterns of native versus non-native seed recruitment. I used replicated experimental gardens to ascertain whether pots with *A. petiolata* recruited a higher proportion of non-native to native herbaceous species, compared to pots with *S. canadensis*. The rationale for both objectives is based on the novel weapons hypothesis which proposes that non-native species become aggressive invaders because they possess novel traits (e.g. allelopathic chemicals) that suppress the growth of other (native) species in their new range (Callaway and Ridenour 2004). This study thus assumes that *A. petiolata* co-occurs with other non-native species from its native range, which is probable considering its vast range in Eurasia (NatureServe 2016a). This research aims to establish whether there is evidence for non-additive effects of *A. petiolata* on native plants in southern Ontario that are consistent with the predictions of the invasional meltdown hypothesis.

Methods

Biology and Ecology of Focal Species

Alliaria petiolata (Brassicaceae) is a biennial herbaceous plant and a prominent invader of forests in North America. In the first growing year, *A. petiolata* consists of sharply toothed basal leaves, and in the second year the plant typically produces a single growing/flowering stalk (Anderson et al. 1996). Flowers are produced as early as April, although more can grow in mid-summer, and black, cylindrical seeds can be found in linear fruits in early summer (Van Driesche et al. 2002; Anderson et al. 1996). First-year plants that successfully overwinter can quickly grow in early spring while other plants are still dormant, giving *A. petiolata* a competitive advantage over other herbaceous plants (Cavers et al. 1979). It is also shade tolerant and thus can invade forest understories in its introduced range (Van Driesche et al. 2002; Anderson et al. 1996). *Alliaria petiolata* has been shown to outcompete native plant species, including native seedlings (Stinson et al. 2006; Cantor et al. 2011), and reduce seed germination of some native herbaceous species (Prati and Bossdorf 2004; Cipollini and Greenawalt Bohrer 2016). It appears that native species vary in their response to *A. petiolata* invasions, but overall it has been observed that native species diversity is lower when *A. petiolata* density is higher, and with its removal, species diversity increases (Stinson et al. 2006).

Another possible reason for the invasiveness of this species is its allelopathic properties: it releases allelochemicals that allow *A. petiolata* to displace native plants that rely on mycorrhizal fungi (Poon and Maherali 2015; Roberts and Anderson 2001; Stinson et al. 2006). Root exudates and leaf litter leachate include 2-

propenylglucosinolate (sinigrin) which is part of the glucosinolate family (Vaughn and Berhow 1991). Sinigrin suppresses the growth of mycorrhizal fungi that plants typically use to receive nutrients (Poon and Maherali 2015; Stinson 2006); however, *A. petiolata* itself does not appear to have mycorrhizal associations and thus has a potential advantage over plants that do (Stinson et al. 2006). This 'biochemical weapon' that is novel in its introduced range, combined with its potential to uptake nutrients more effectively than other competitors, may partly explain the invasiveness of this species (Poon and Maherali 2015).

Solidago canadensis (Asteraceae) is a herbaceous plant that is native to most of Canada and much of the USA, and invasive in Europe, Asia, Australia and New Zealand (Abhilasha et al. 2008). It is a perennial plant, with flowers blooming from July to October (Pavek 2011). *Solidago canadensis* can grow in a variety of conditions, soil types, light conditions, and habitats, and in Ontario its habitats range from damp meadows to roadside ditches to deciduous and evergreen forests (Pavek 2011). For these reasons, *A. petiolata* and *S. canadensis* can co-occur in southern Ontario, often in partly shaded habitats (personal observation June 6th, 2018).

Solidago canadensis is also considered allelopathic, which may be one reason for its success in its introduced range. At least four chemicals that *S. canadensis* releases have been identified and are considered allelochemicals (Abhilasha et al. 2008). Zhang et al. (2010) found that *S. canadensis* alters the soil arbuscular mycorrhizal fungal communities in its favour and reduces associations with native species in China, similarly to *A. petiolata*. Past studies have shown that *S. canadensis* can inhibit germination of

native herbaceous plants in its introduced range (Abhilasha et al. 2008; Zhang et al. 2010), and negatively affects densities of native species (Leger et al. 2015). Based on previous studies it does seem that its allelopathic affects are more pronounced in its introduced range (Abhilasha et al. 2008; Leger et al. 2015).



Figure 1. Photograph of *A. petiolata* and *S. canadensis* growing together in Trent's Nature Areas in Peterborough, Ontario.

Hesperis matronalis (Brassicaceae) is a herbaceous biennial or perennial plant. *Hesperis matronalis* is native to Eurasia and is found in all southern provinces of Canada, including Ontario (NatureServe 2016b). Like *A. petiolata*, *H. matronalis* is also in the mustard family, and overall the two species have a similar life history (Francis et al. 2009). In Ontario it prefers open, disturbed habitats such as roadsides, old fields, disturbed riparian woodlots, and disturbed deciduous wooded areas (Francis et al. 2009). Like *A. petiolata*, it has intermediate shade tolerance (USDA, NRCS 2018c) and so can be seen growing in areas with *A. petiolata* in Ontario (Francis et al. 2009). Some chemicals that could be allelopathic have been identified in the past (Tatsuzawa 2012,

2013; Larsen et al. 1992), but currently, it is unclear whether this species is allelopathic, and is unlikely to be as allelopathic as *A. petiolata* (Francis et al. 2009; Brouwer et al. 2015). *Hesperis matronalis* was used as a control plant in the *Recruitment Experiment* in this study; similarly, it has been used as a control in previous experiments which examined the allelopathic effects of *A. petiolata* (Brouwer et al. 2015; Hale et al. 2015).

Co-occurrence Surveys

For these surveys only *A. petiolata* and *S. canadensis* were considered as focal species. The distribution of *A. petiolata* in Ontario was reviewed on the EDDMaps Ontario website (EDDMapS Ontario 2018a) prior to choosing parks for the co-occurrence surveys. Provincial parks in southern Ontario within 150 KM of Trent University with *A. petiolata* reports were then visited for an initial survey. The park had to contain at least 10 *A. petiolata* or *S. canadensis* plots to be chosen for these surveys. In this case, plots are defined as 1m² quadrats that contain any amount of either, or both, of the focal species. Based on these criteria, Sibbald Point, Balsam Lake, and Sandbanks Provincial Parks were chosen for *A. petiolata* surveys. Indian Point and Sandbanks Provincial Parks were chosen for *S. canadensis* surveys. In addition, surveys in Peterborough Ontario were conducted for both focal species. See Figure 2 for the surveyed locations.



Figure 2. A map displaying the locations of the parks used for the co-occurrence surveys. Sibbald Point, Balsam Lake, and Sandbanks Provincial Parks were chosen for *A. petiolata* surveys. Indian Point and Sandbanks Provincial Parks were chosen for *S. canadensis* surveys. An urban park in Peterborough was surveyed for both focal species. Map made in BaseCamp (Garmin, Olathe, Kansas, USA).

At each location, up to 50 1m² plots which contained the focal species were chosen based on visual surveys, with each plot being at least 15m away from any other plot. Plots were given a unique code and their locations were recorded in a GPS device (GPSMap 64s, Garmin, Olathe, Kansas, USA). Then a maximum of 20 of the 1m² plots were randomly chosen to be surveyed. Balsam Lake Provincial Park contained only 13 plots with *A. petiolata* and therefore all sites were surveyed. All herbaceous species

were recorded, as well as percent cover estimates for each. Percent cover of bare ground and litter were estimated and recorded, except for *A. petiolata* sites in Balsam Lake Provincial Park (N=13) and *Solidago canadensis* sites in Indian Point Provincial Park (N=20) due to logistical reasons. Also recorded was whether *A. petiolata* were first year plants, second year plants, or whether there was a mix of both in the plot. In cases where species could not be identified on the spot, a photo was taken for later identification and the genus name was recorded where possible. Once the surveys were completed, it was determined whether each species listed was native or non-native. If a species was not identified, it was recorded as NA instead of native or non-native (5 out of 134 plots each contained a species recorded as NA). Categorising was based on best available information from plant identification guides, the USDA website (USDA, NRCS 2018a), EDDMaps Ontario (EDDMaps Ontario 2018b), or the NatureServe website (NatureServe 2018). Data were imported from the GPS device into BaseCamp (Version 4.6.2; Garmin Ltd., 1996-2018). In total, 73 *A. petiolata* sites were surveyed in 4 locations, and 61 *S. canadensis* sites were surveyed in 3 locations.

Data were analyzed using R (Version 3.5.0; R Core Team, 2017). All diversity data were first square root transformed to meet model assumptions, while cover data remained the same. A mixed-effects model was used for each of the response variables, with focal species and focal percent cover acting as the fixed effects, and sampling location as a random effect. The function `lmer()` from the 'lme4' package (Version 1.1.15; Bates et al. 2015), was used for these response variables: percent cover (native, ground) and diversity (total, native, non-native). The total diversity, or species richness,

included the total number of herbaceous species, other than the focal species, found in each plot. The proportion of native:non-native diversity was analysed using the `glmer()` function from the same package. Non-native percent cover data were zero inflated and thus the `glmmTMB()` zero inflation function was used from the `glmmTMB` package (Version 2.1.0; Brooks et al. 2017). An analysis of variance (ANOVA) was then used to determine the significance of the focal species on the response variables. For a more detailed description of data analysis see the R-markdown report attached (Appendix 1).

Recruitment Experiment

Site choice and description

The experiment was conducted in the Trent Nature Areas, which are natural areas belonging to Trent University in Peterborough, Ontario. Trent Nature Areas include 566 hectares of land on the outskirts of Peterborough, Ontario. They contain several ecosystems types including forest, woodland, plantation, swamp, marsh, agricultural and old field habitats (Jones, Fox, & Marsh 2002). The areas were scouted in early spring of 2017 for five suitable locations for this experiment. The sites had to meet several criteria. First, they had to be able to sustain both focal species. As discussed in the *Ecology of Focal Species* section, *Alliaria petiolata* prefers shaded conditions and *Solidago canadensis* prefers open fields and fully sunny conditions. However, both focal species can survive in habitats that are semi-shaded and often co-occur in semi-shaded habitats in the study region, including the Trent Nature Areas. Thus, the chosen locations had to be semi-shaded, and were often placed on a boundary between a field and a hedgerow. Additionally, the locations had to be remote enough to minimize

disturbance from people. For logistical reasons they also had to be located near an area that is accessible by vehicle. Using these criteria 5 sites were chosen and can be seen in the map provided (Figure 3; Table 1).



Figure 3. Map displaying all five sites chosen for the recruitment experiment. Sites are found within the Trent Nature Areas in Peterborough, Ontario. Map made in ArcMap with data provided by Trent University.

Table 1. Description of the locations of the 5 sites chosen for the recruitment experiment. All five sites are found in the Trent Nature Areas in Peterborough ON.

Site #	Site Name	Site Location (latitude/longitude)	Site Description
Site 1	DNA Building	44.359594, -78.283157	Open field, semi shaded from hedgerow, near a university building and surrounded by farmland
Site 2	Trailer	44.359133, -78.283564	Open field, semi shaded from hedgerow, near a university building and surrounded by farmland
Site 3	Hydro lines	44.360292, -78.295034	Hydro lines corridor, drumlin, semi shaded from small patch of mixed deciduous woodland
Site 4	Wetland	44.364111, -78.292696	Open field, wetland, semi shaded from hedgerow, near Otonabee river
Site 5	Forensic House	44.367177, -78.282786	Farmland, semi shaded from hedgerow, near a residential house

Experimental Design

Plants were collected on May 1st and 2nd and potted within 24 hours of collection. Enough plants were collected for 15 pots of each of the two focal species: *Solidago canadensis* and *Alliaria petiolata*, as well as *Hesperis matronalis*. *Solidago canadensis* was collected from a field on Trent University property in Peterborough,

Ontario; *A. petiolata* was collected near a trail on Trent University property; *H. matronalis* was collected from a backyard in Peterborough, Ontario. All were dug out, with emphasis on preserving as much of the root as possible at the beginning of the growing season, when plants were young. Each plant was potted into a separate 11.75"x10.2" pot and kept in the greenhouse. In total there were 15 pots per focal species, and one plant per pot. The pots containing *A. petiolata*, each contained a second-year plant. In addition, 100 smaller pots were each potted with a single first-year *A. petiolata* plant and kept in a secure outdoor location. It was expected that second-year plants in the pots would die off throughout the summer, thus the first-year *A. petiolata* plants would be needed to replace the second-year plants halfway through the summer. Finally, 15 pots were designated as control pots and were not planted with anything, but rather filled solely with soil. Sunshine's Universal soil mix was used for potting in all cases. In the greenhouse plants were monitored and watered daily. The greenhouse conditions were set to 12 hours sun/day.

On May 18th all plants other than the first-year *A. petiolata*, were moved into a secure outdoor area near Sites 1 and 2 in order to transition from the greenhouse conditions, at which point 33g of Osmocote's 5-6 month nursery mix fertilizer was added on top of the soil. On May 23rd and 24th, the pots were moved to the five sites. At each site 3 pots containing *A. petiolata*, 3 pots containing *H. matronalis*, 3 pots containing *S. canadensis*, and 3 control pots were randomly placed in the manner displayed in Figure 4. The pots were placed 0.5 meters apart from one another and were dug into the ground with approximately 5cm of the pot above ground so that the soil

level in the pot matched the soil level of the ground. The area was marked off with flagging tape. Throughout the summer, pots were monitored twice weekly and watered as needed. Flowers were removed from all 3 species within a week of appearing, to prevent seeds from being released. Four first year *A. petiolata* plants were added to each pot mid-August to replace the withered second year plants in order to achieve biomass and ground cover similar to that of the second-year plants. It was also expected that not all four first-year plants would survive the winter and at least 2 plants were needed to approximately match *S. canadensis* biomass and percent cover the following spring.

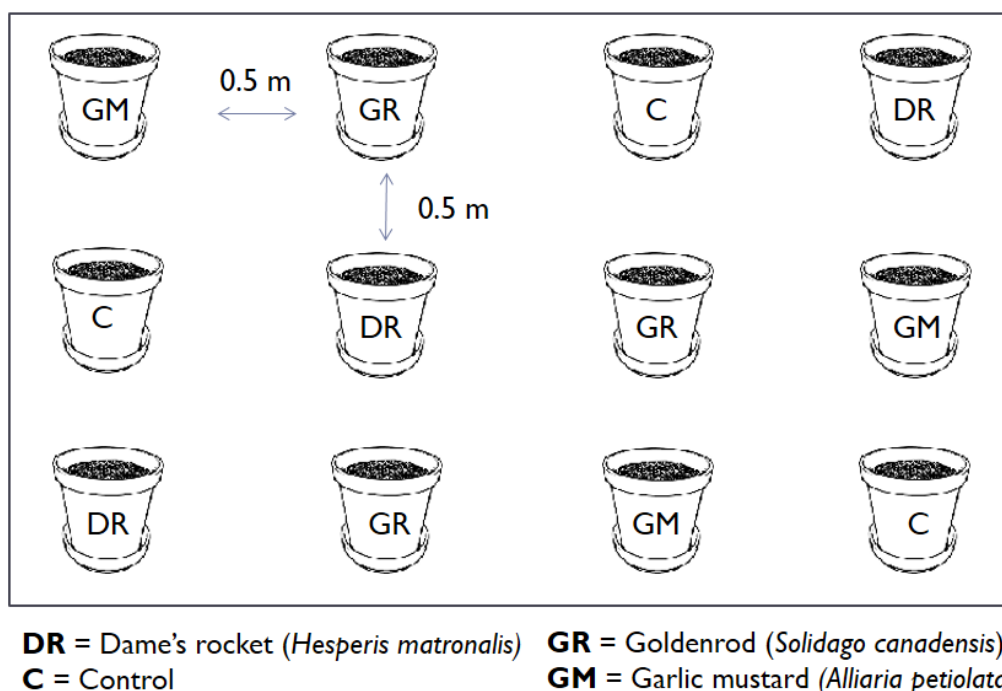


Figure 4. A representation of what each site looks like, noting however, that the placement of pots was randomized at each of the five locations.



Figure 5. A photograph of Site 5 (Forensic House Site).

The following spring, for every pot, the species identities, the numbers of emerged stems, and the percent cover of non-focal species were recorded on June 6th, 2018. Non-focal species, and percent cover of each were recorded for each pot. Photographs were taken when species identification was not possible, and genus names were recorded when known. If identified, species were then categorized as “native” or “non-native”, or as “NA” when identification was not possible. Categorising was based on best available information from plant identification guides, the USDA website (USDA, NRCS 2018a), EDDMaps Ontario (EDDMaps Ontario 2018b), or the NatureServe website (NatureServe 2018).

Data were analyzed in R (Version 3.5.0; R Core Team, 2017). The ‘lme4’ package (Version 1.1.15; Bates et al. 2015) was used for the mixed-effects models, with focal species as the fixed effect, and the site as the random effect. A model was created using

the `glmer()` function for each of the response variables: non-focal diversity (total, native, non-native) and non-focal total stem density. The total diversity represented the total number of herbaceous species, other than the focal species, found in each pot. A type II analysis of variance (ANOVA) from the “car” package (Version 3.0.0; Fox & Weisberg 2011) was then used to determine whether the focal species had a significant effect on the response variables. Finally, a Tukey HSD test was used as a post hoc analysis, using the ‘multcomp’ package (Version 1.4.8; Hothorn et al. 2008). For more details regarding this data analysis see the appended R-Markdown report (Appendix 2).

Diversity Surveys of the Trent Nature Areas

The area surrounding each pot was surveyed to evaluate the species richness and abundance of the plants that because of their proximity should be most likely to disperse seeds into the pots in the experiment. This was done using the Modified Whittaker Plot Design, as described by Stohlgren and Falkner (1995). The plot was always set in the same position at each site (Figure 6), so that the plot would start on a gradient between two habitats. All sites bordered a hedgerow or woodland edge that provided shade to each experimental site. Accordingly, each Whittaker Plot was set up in parallel to the hedgerow or woodland edge, as described by Stohlgren and Falkner (1995). Surveys were conducted at the beginning (June 7th-27th, 2017) and at the end of summer (August 21st-25th), to enable identification of all species growing at the sites.

The protocol was followed as described by Stohlgren and Falkner (1995), other than the recording of the data. In the A plots all species present were recorded, as well as an estimate of their percent cover. However, in plots B, C, and D only the species

present were recorded. The proportion of native:non-native species at each site was also calculated, using the same protocol as described in the *Experimental Design* section.

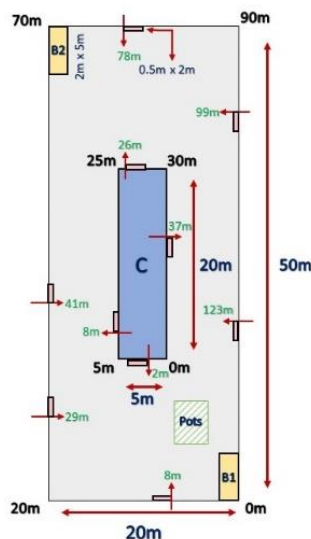


Figure 6. A modified Whittaker plot design, showing the location of the pots at each site.

Evaluation of Allelopathic Potential

For the germination experiment, I set out to measure the allelopathic potential of the plants in each of the pots from the *Recruitment Experiment*. I followed the methods set by Przepiorkowski and Gorski (1994) with a few minor changes. On July 10th, 2018 I collected approximately 5g of soil from each of the 12 pots at all five locations, using a small gardening spade, and placed the soil into labeled zip lock bags. This time of year was chosen for the collection of soil as *A. petiolata* plants were beginning to senesce, and were roughly the same size as the *S. canadensis* plants. In between each collection, the spade was wiped with diluted alcohol to prevent allelochemicals from transferring between samples. Soil was taken from near the root of the biggest focal plant in each pot, dug from approximately 5cm from the surface level

of the pot. In control pots, soil was collected from the middle of the pot. One sample was collected from each pot, for a total of 12 samples per site, or 15 samples per focal species.

Five grams of soil were weighed from each sample and placed it at the bottom of a separate 60mm x 15mm petri dish. All petri dishes were previously labeled with a code that corresponded to the pot number. Five grams of dampened Sunshine's Universal soil mix were placed in 15 separate petri dishes to act as a soil control. Water was added to the control soil until it appeared to be approximately as moist as the field samples. The soil in each petri dish was then covered with a 55mm filter paper that would absorb the allelochemicals in the soil and provide a base on which to place the seeds.

For this germination experiment, Early Scarlet Globe radish (*Raphanus sativus*) seeds were used, purchased from Ontario Seeds Co. Limited (Kitchener, ON, CA). Radish seeds are quick to germinate, are sensitive to allelopathic inhibition, and have been used in many allelopathy experiments in the past (Pisula and Meiners 2010). One hundred seeds were placed on top of the filter paper in each petri dish, 1mL of tap water was added to each petri dish, lids were placed on the petri dishes and sealed with parafilm tape, and the dishes were placed in germination trays in an environmental chamber at Trent University (25C, 50% humidity, 12h light/dark cycle). After 5 days, germination rates were calculated for each petri dish, with seeds counted as germinated if the radicle was visible.

Germination rates were analyzed in R (Version 3.5.0; R Core Team, 2017).

Germination rates were compared for pots with *S. canadensis*, *A. petiolata*, *H. matronalis*, control pots, and the soil control. A mixed effects model was created using the `glmer()` function from the 'lme4' package (Version 1.1.15; Bates et al. 2015). The number of germinated seeds served as the response variable, the focal species as a main effect and the sites as a fixed effect. Data appeared to meet model assumptions and so an analysis of variance (ANOVA) was used to determine whether the plant treatment had a significant effect on germination rates (Appendix 3).

Results

Co-occurrence Surveys

Total herbaceous species diversity within plots was affected by the focal species (Figure 7). Plots with *Solidago canadensis* as the focal species had significantly higher total diversity than *Alliaria petiolata* plots (Estimate: 0.789742, Standard Error: 0.139167, $t = 5.675$, $P = 8.89e-08$). Higher total diversity in *Solidago canadensis* plots was driven by both significantly higher native (Estimate: 0.3496, Standard Error = 0.1399, $t = 2.499$, $P = 0.0138$; Figure 8) and non-native diversity (Estimate: 0.665537, Standard Error: 0.143778, $t = 4.629$, $P = 9.07e-06$; Figure 9). However, the proportion of native:non-native herbaceous plant species was significantly higher in *A. petiolata* plots (Estimate: -0.8972, Standard Error: 0.2441, $z = -3.676$, $P = 0.000237$; Figure 10).

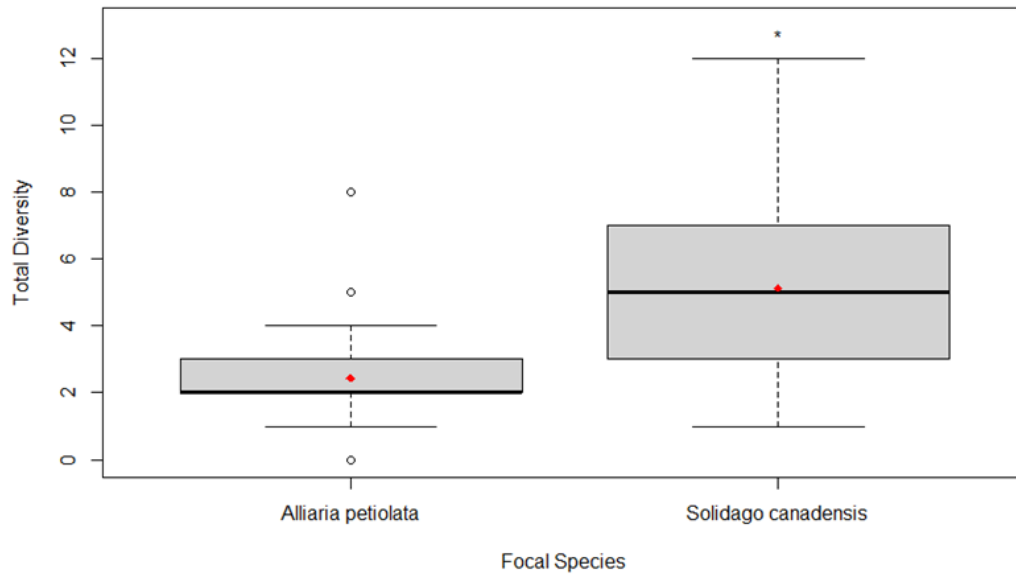


Figure 7. The total herbaceous species diversity in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. Plots with *Solidago canadensis* had higher total diversity than plots with *Alliaria petiolata*. Black lines represent the median and the red dots represent the means.

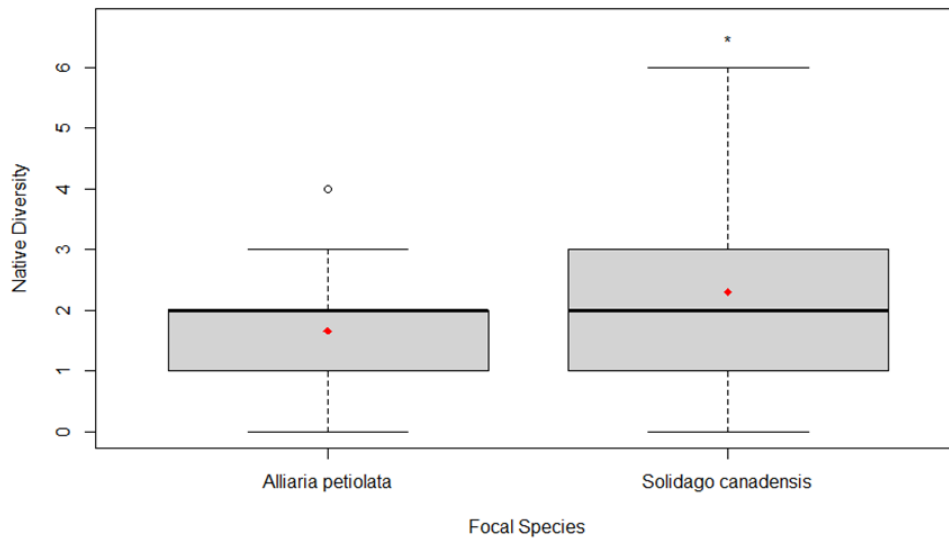


Figure 8. Native herbaceous species diversity in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. Native diversity was significantly higher in plots with *Solidago canadensis*, than those with *Alliaria petiolata*. Black lines represent the median and the red dots represent the means.

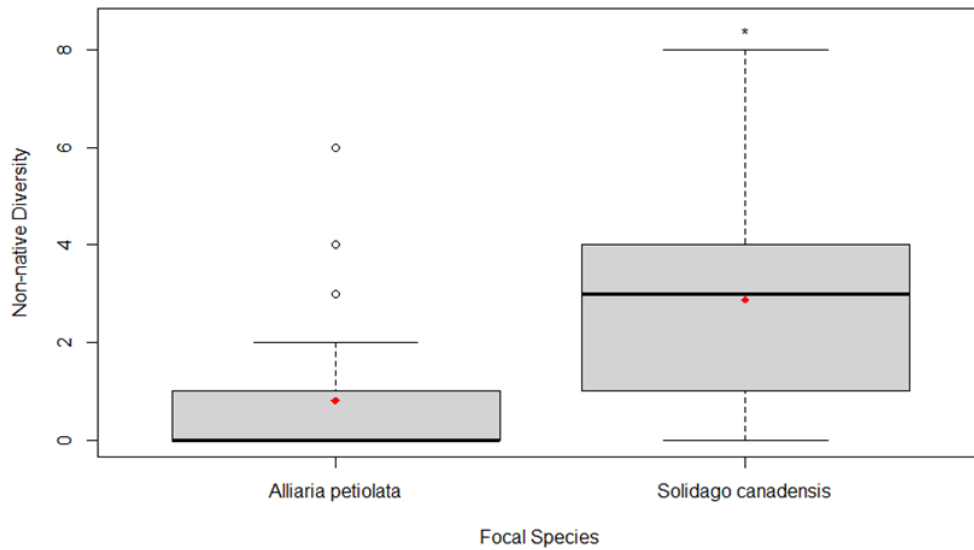


Figure 9. Non-native herbaceous species diversity in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. Plots with *Solidago canadensis* showed higher non-native diversity than plots with *Alliaria petiolata*. Black lines represent the median and the red dots represent the means.

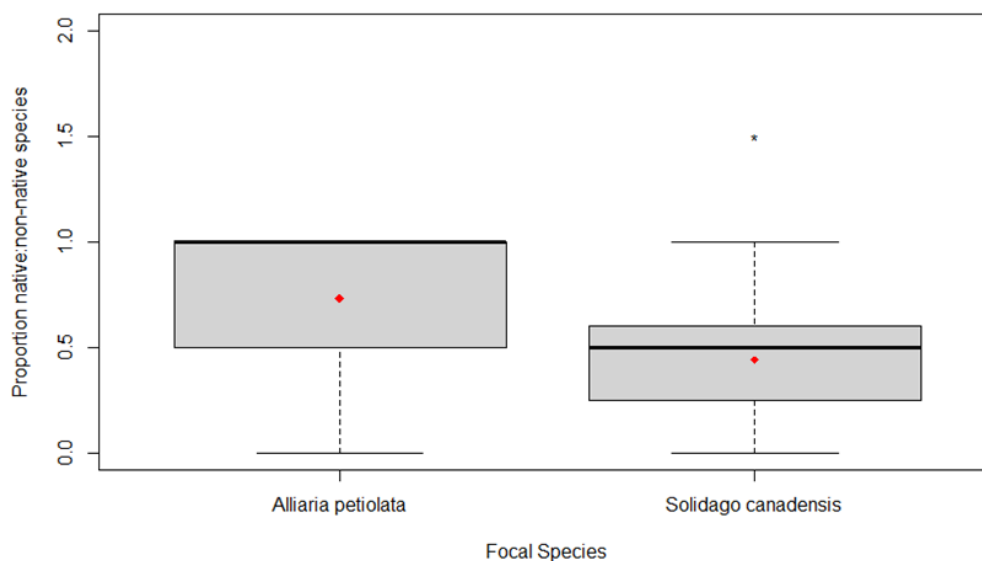


Figure 10. The proportion of native:non-native herbaceous species in plots with *Alliaria petiolata* and *Solidago canadensis*. The proportion of native:non-native herbaceous species was significantly higher in plots with *Alliaria petiolata* than those with *Solidago canadensis*. Black lines represent the median and the red dots represent the means.

In terms of percent cover of native herbaceous species, an analysis of variance (ANOVA) showed that there was no significant effect of focal species ($X^2(1, N=129) = 0.3690, P = 0.5435$; Figure 11). There was a significant focal species effect on the percent cover of non-native herbaceous species, with *Solidago canadensis* plots having a higher percent cover of non-native species than plots with *Alliaria petiolata* (Estimate: 0.824675, Standard Error: 0.331578, $z = 2.487, P = 0.0129$; Figure 12). *Alliaria petiolata* plots had a significantly higher percent bare ground cover than plots with *Solidago canadensis* (Estimate: -33.4985, Standard Error: 6.3861, $t = -5.246, P = 1.25e-06$; Figure 13).

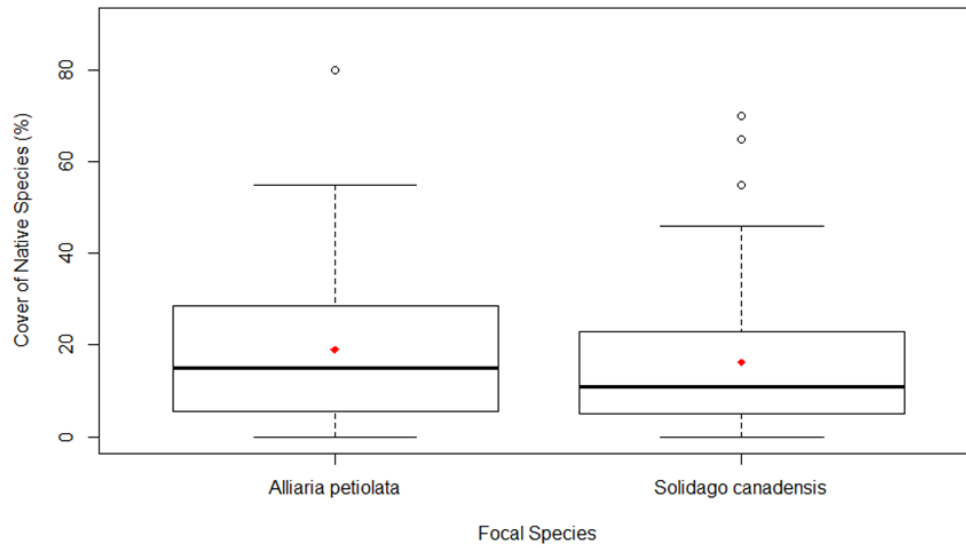


Figure 11. Percent cover of native herbaceous species in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. *Solidago canadensis* and *Alliaria petiolata* plots did not vary significantly in the percent cover of native herbaceous species. Black lines represent the median and the red dots represent the means.

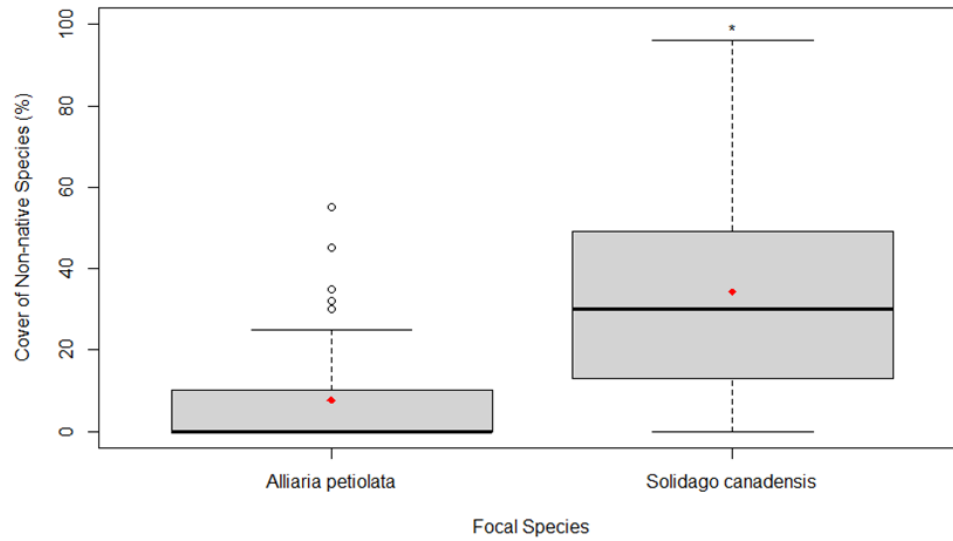


Figure 12. Percent cover of non-native herbaceous species in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. Plots with *Solidago canadensis* had a significantly higher percent cover of non-native species than plots with *Alliaria petiolata*. Black lines represent the median and the red dots represent the means.

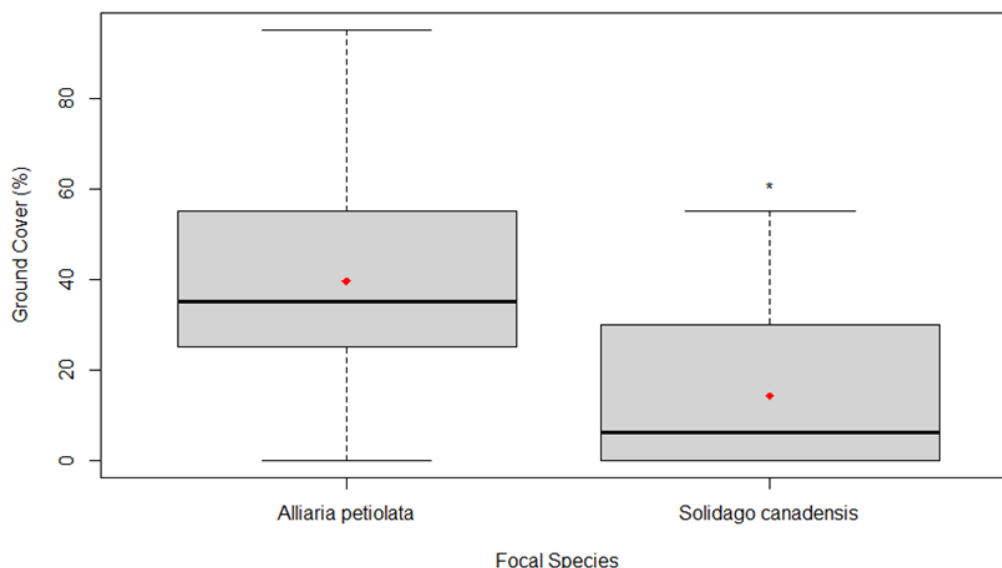


Figure 13. Percent cover of bare ground in plots with *Alliaria petiolata* and *Solidago canadensis* as the focal species. Bare ground cover was significantly lower in plots with *Solidago canadensis* than in plots with *Alliaria petiolata*. Black lines represent the median and the red dots represent the means.

Circaea canadensis (Enchanter's nightshade) was the most common native species in *A. petiolata* plots, found in 30 out of 73 plots (41%). *Geranium robertianum* (herb-robert) was found in 30% of the plots, *Parthenocissus inserta* (Virginia creeper) in 29% of the plots, and *Geum aleppicum* (yellow avens) in 20%. *Rhamnus cathartica* (common buckthorn; 23%), and *Taraxacum officinale* (dandelion; 15%) were the most common non-native species in *A. petiolata* plots. In *S. canadensis* plots, the most common native species was *Toxicodendron radicans* (poison ivy), which appeared in 44% of the 61 surveyed plots, followed by *Fragaria vesca* (woodland strawberry; 25%) and *Symphotrichum lanceolatum* (lance-leaf aster; 21%). As for non-native species, *Poa*

pratensis (Kentucky bluegrass; 48%), *Vicia cracca* (cow vetch; 38%), and *Phleum pratense* (Timothy; 38%) were most common. Although *Phleum pratense*, *Vicia cracca*, *Symphotrichum lanceolatum* and *Toxicodendron radicans* were very common in *S. canadensis* plots, none of these species appeared in any of the *A. petiolata* plots. *Rhamnus cathartica*, *Parthenocissus inserta* and *Toxicodendron radicans* were not included in the analysis though since they are not herbaceous plants. Furthermore, it is important to note that *Solidago canadensis* appeared in 3 out of the 73 *A. petiolata* plots, as did *Hesperis matronalis*.

Germination Experiment

An analysis of variance (ANOVA) showed that the focal species did not have a significant effect on germination rates ($F(4,67) = 1.381$; Figure 14). The germination rates were consistent between treatments.

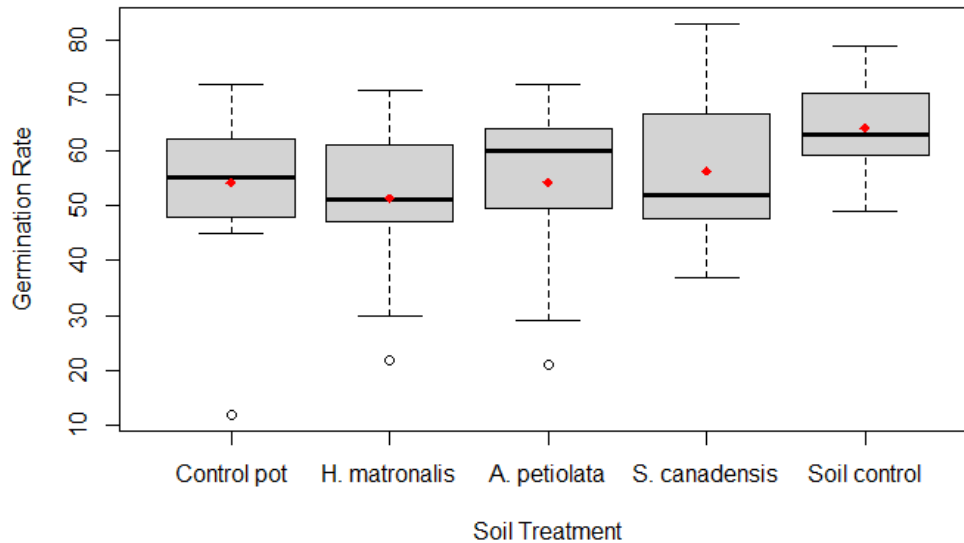


Figure 14. The germination rates on day five for radish seeds grown in soil from pots of the three focal species, the control pots, and the soil control. Germination rates did not significantly differ between treatments. Black lines represent the median and the red dots represent the means.

Recruitment Experiment

The focal species had a significant effect on the total diversity of germinating plants within the pots ($\chi^2(3, N = 51) = 10.156, P = 0.01728$; Figure 15). *Alliaria petiolata* pots had significantly lower total diversity than pots with *Hesperis matronalis* (Estimate: -1.06784, Standard Error: 0.34874, $z = -3.062, P = 0.0116$) and control pots (Estimate: -1.00331, Standard Error: 0.35171, $z = -2.853, P = 0.0218$). None of the other comparisons were significant.

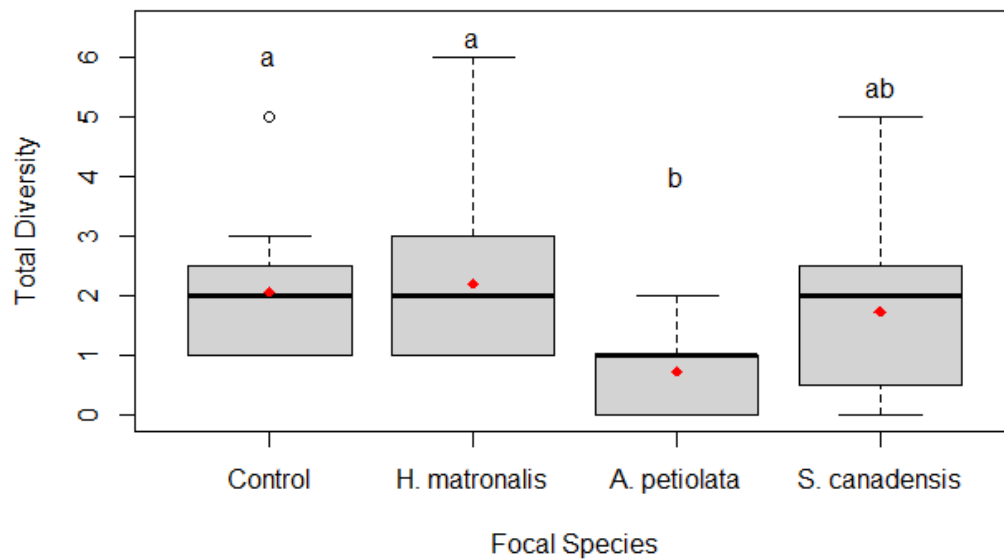


Figure 15. The total diversity of newly established herbaceous plant species for each of the three focal species and the control pots. *Alliaria petiolata* pots had significantly lower total diversity than pots with *Hesperis matronalis* and control pots but did not significantly vary from *Solidago canadensis* pots. Black lines represent the median and the red dots represent the means.

The Wald Chi-square test revealed that there was not a strong relationship between the focal species and the native diversity in the pots ($\chi^2(3, N = 51) = 5.2268, P = 0.1559$; Figure 16). The same test showed that focal species did appear to affect the non-native diversity within pots ($\chi^2(3, N = 51) = 8.1008, P = 0.04397$); however, the post-hoc analysis did not indicate which levels of the species treatment were driving this response (Figure 17). Specifically, although *A. petiolata* pots had slightly lower non-native diversity than the control pots, this difference was not significant according to the Tukey HSD test (Estimate: -2.5638, Standard Deviation: 1.0328, $z = -2.482, P = 0.0561$).

Similarly, the proportion of native:non-native species was also not significantly affected by the focal species ($X^2 = (3, N=51) = 6.048, P = 0.1093$; Figure 18).

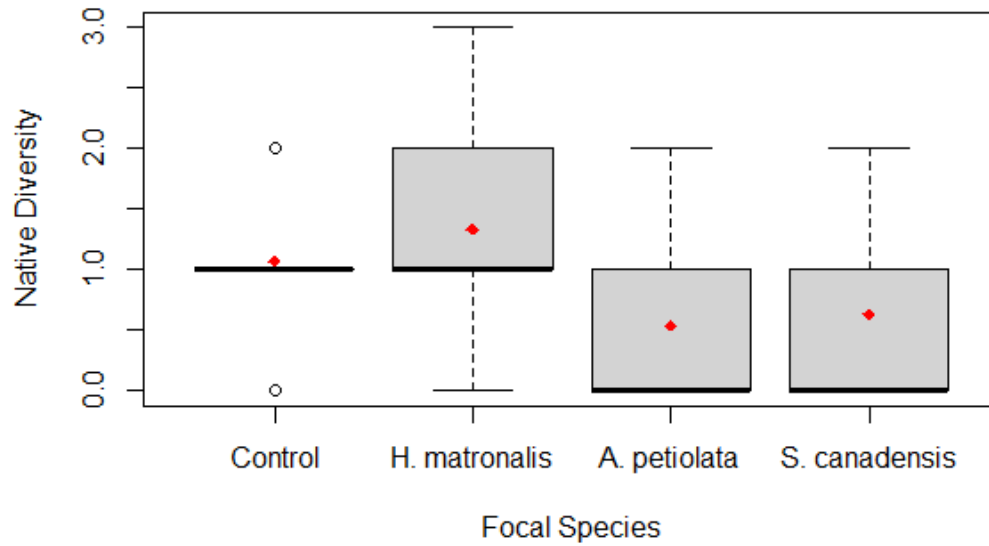


Figure 16. Native diversity of newly established herbaceous plant species for each of the three focal species and the control pots. Differences between treatments were not significant. Black lines represent the median and the red dots represent the means.

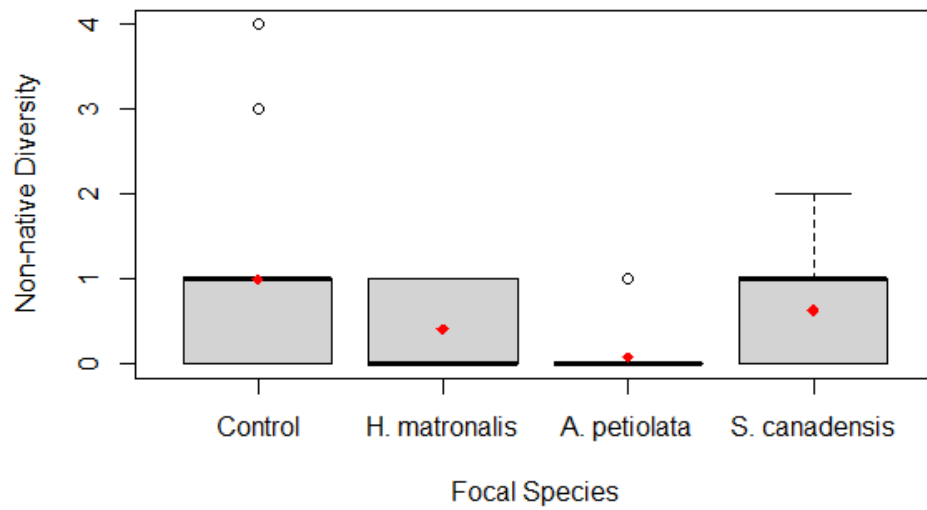


Figure 17. Non-native diversity of newly established herbaceous plant species for each of the three focal species and the control pots. Non-native diversity was consistent when comparing the treatments. Black lines represent the median and the red dots represent the means.

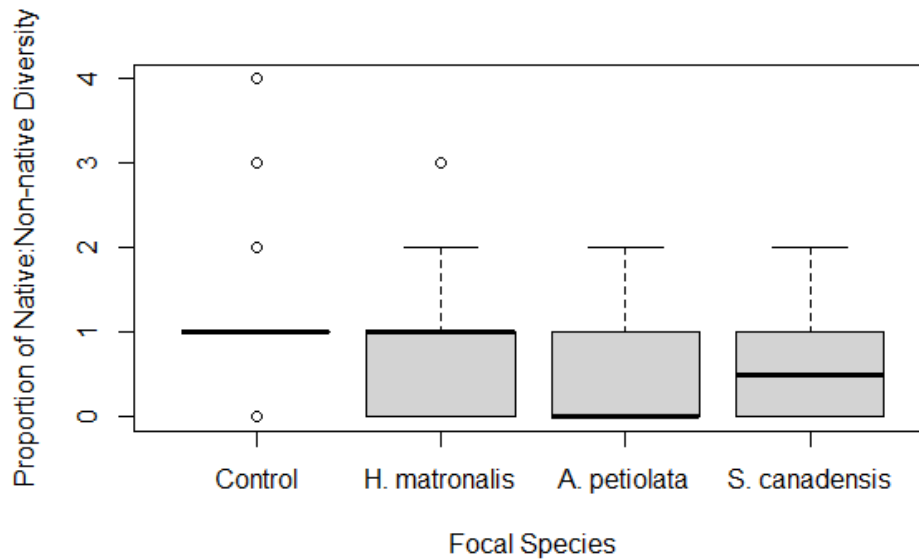


Figure 18. The proportion of native:non-native diversity of newly established herbaceous plant species for each of the three focal species and the control pots. The means did not vary significantly. Black lines represent the medians.

Finally, the focal species affected the total stem density of new herbaceous plant species in the pots ($\chi^2(3, N=51) = 225.23$, $P < 2.2e-16$; Table 2; Figure 19). A post hoc analysis revealed that the *H. matronalis* pots had a significantly higher stem density than pots with any other focal species, whereas *S. canadensis* had a lower stem density than all other treatments (Table 2). *A. petiolata* had a lower stem density than *H. matronalis* and control pots.

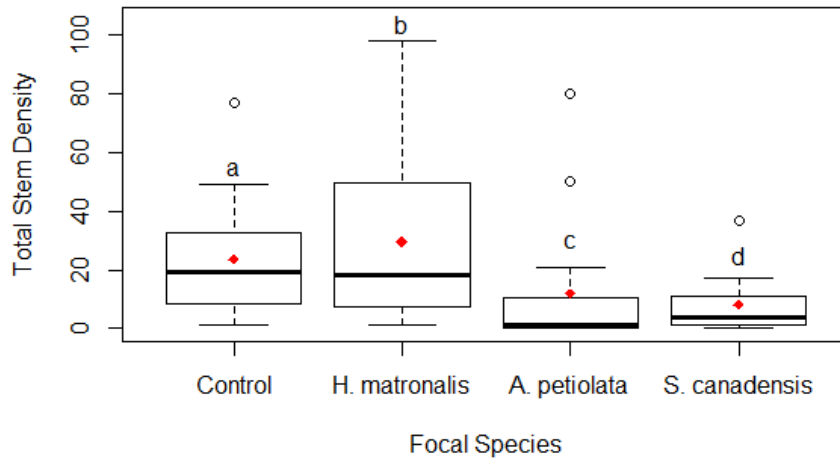


Figure 19. The total stem density of newly established herbaceous plant species for each of the three focal species and the control pots. *Solidago canadensis* pots had significantly lower total stem density than all other pots, whereas control pots had significantly higher stem density than all other pots. *Alliaria petiolata* pots had a lower total stem density than pots with *Hesperis matronalis*. Black lines represent the median and the red dots represent the means.

Table 2. The results of a post hoc analysis (Tukey HSD) comparing total stem density between treatment pairs. Both *A. petiolata* and *S. canadensis* had a lower stem density than *H. matronalis* and the control pots, with *A. petiolata* pots having a higher total stem density than ones with *S. canadensis*. *H. matronalis* pots were determined to have a higher stem density than all other treatments.

Species	Estimate	Standard Error	z value	Pr(> z)
<i>H. matronalis</i> - control	0.22427	0.07121	3.149	0.00911
<i>A. petiolata</i> - control	-0.68191	0.09161	-7.443	< 0.001
<i>S. canadensis</i> - control	-1.08180	0.10552	-10.253	< 0.001
<i>A. petiolata</i> - <i>H. matronalis</i>	-0.90618	0.08847	-10.243	< 0.001
<i>S. canadensis</i> - <i>H. matronalis</i>	-1.30608	0.10280	-12.705	< 0.001
<i>S. canadensis</i> - <i>A. petiolata</i>	-0.39990	0.11785	-3.393	0.00374

Results of the surveys of the Trent Nature Areas surrounding the pots are shown in Table 3. Regardless of seasons, there was a higher proportion of non-native species found in the Trent Nature Areas.

Table 3. Average proportion of native, non-native and unidentified species found in the Trent Nature Areas in surveys completed in the summer and fall seasons of 2017. The proportion of non-native species was typically higher on average, in both the summer and fall seasons.

Season	Average Proportion of Native Species	Average Non-native Species	Average of Unidentified Species
Summer	0.28835035	0.657688689	0.053960961
Fall	0.312005013	0.66273183	0.025263158

Discussion

The invasional meltdown hypothesis (IMH) was first proposed in 1999 (Simberloff and Von Holle 1999). Since then there have been numerous studies on the subject (Barga et al. 2018), although there still remains a need for research to shift from single-species scenarios to multi-species scenarios (Kuebbing, Nunez and Simberloff 2013). Many previous studies support the IMH while looking at a range of taxa, although some of the results have been mixed (Barga et al. 2018). Furthermore, much of the IMH research to date has been directed towards individual populations, whereas more research is needed on community and ecosystem levels (Barga et al. 2018). This point was addressed in this study, which looked at community level responses to effects of *Alliaria petiolata* invasions and allelopathy on community composition.

This study explored whether *A. petiolata* co-occurred more often with native or non-native species in its introduced range in southern Ontario. I found no support for my hypothesis that *Alliaria petiolata* is facilitating further invasions through allelopathy. Using a focal species approach to control for bias in associations between *A. petiolata* and native plant densities, I found that the proportion of native:non-native species was higher in plots with *A. petiolata* than the native *S. canadensis*. The recruitment experiment, which investigated whether *A. petiolata* created a favourable environment for the establishment of non-native species showed that the proportion of native:non-native species did not vary between focal species. Because of the species surrounding the pots were non-native, according to the surveys of the Trent Nature Areas, the proportion of non-native species within the pots was not limited by seed availability. Thus, the earlier predictions under the IMH, that the proportion of non-native species should be higher in pots or plots with *A. petiolata*, were not supported.

In contrast to my results, Barga et al. (2018) cited numerous studies that support the IMH in different ecosystems, across a range of taxa, and explored various mechanisms that allowed for facilitative interactions between invasive species. One notable study looked at *Lonicera maackii* (amur honeysuckle), an invasive allelopathic shrub from Asia, which has been shown to potentially facilitate invasions in several past studies (McNeish & McEwan 2016). Cipollini et al. (2008) have shown that *L. maackii* releases several chemical compounds that have allelopathic potential and affect the growth of certain target species. Culley et al. (2016) found that *L. maackii* was associated with a higher diversity of invasive species and a higher proportion of non-

native:native species in surveyed sites in Ohio. They noted that this may be connected to its abilities to alter the soil chemistry and light availability in its invaded range, both of which are qualities that *A. petiolata* also possess (Culley et al. 2016).

Although the data do not support the IMH, it does appear that *A. petiolata* has ‘additive’ effects in areas where it is introduced, as previous literature has shown in the past (Stinson et al. 2007; Van Riper et al. 2010). Data from the co-occurrence surveys showed that *A. petiolata* was associated with significantly lower total diversity and significantly higher percent cover of bare ground, whereas *S. canadensis* plots were associated with higher total diversity, driven by both higher native and non-native diversity. Similarly, in the recruitment experiment, pots with *A. petiolata* had lower total diversity and stem density than pots with *H. matronalis* or control pots, although they had comparable total diversity and stem density to *S. canadensis* pots. Thus, the results from both the surveys and the recruitment experiment are consistent with previous literature showing that overall, *A. petiolata* negatively affects plant communities by lowering total diversity. For example, Stinson et al. (2007) found that forested areas in Massachusetts with *A. petiolata* had significantly lower native species diversity, and that *A. petiolata* removal increased diversity drastically after a year. Van Riper et al. (2010) also found a similar pattern during a long term observational study of hardwood forests in Minnesota. Compared to studies where only additive effects were explored, this study also examined the effects *A. petiolata* has on native versus non-native species. Overall, the fact that the results from both the field surveys and the recruitment

experiment largely agree could signify that the recruitment experiment was realistic in terms of its implications for natural processes.

Germination Experiment

Data from the germination experiment show that there was no difference in germination rates between petri dishes with soil samples from *H. matronalis*, *S. canadensis*, *A. petiolata*, and control pots. Previous studies found that allelochemicals from *A. petiolata* (Pisula & Meiners 2010) and *S. canadensis* (Butcko & Jensen 2002) plants suppress radish seed germination. Consequently, I expected soil from *A. petiolata* and *S. canadensis* to suppress radish seed germination and result in lower germination rates compared to seeds growing in soil from *H. matronalis* or control pots. Our results suggest that at the time of sampling, none of the pots contained enough allelochemicals to affect the germination rates. However, coupled with the results from the field surveys and the recruitment experiment, which suggest that *A. petiolata* is inhibiting growth and germination of other species compared to *S. canadensis* and *H. matronalis*, it may be that the methods used here were not appropriate to quantify the allelopathic potential of these species in this way. Allelochemicals in soil have a very short half-life, as low as one day, which also heavily depends on variables that were unaccounted for here, such as levels of insect herbivory and microbial degradation (Barto and Cipollini 2009; Tsao et al. 2000). With no plant material in the petri dishes, the amount of soil may not have been large enough to suppress seed germination. Przepiorkowski and Gorski (1994) used more soil, along with litter mixed into it, and found that after three days, the soil taken from areas grown with an allelopathic rye species suppressed germination of seeds

from several target species, such as horseweed and willowherb. Therefore, given the results of the recruitment experiment, it may mean that the methodology used here was simply not sufficient to capture the allelopathic properties of the focal species.

Conclusions:

Through the co-occurrence surveys we found a higher total diversity of herbaceous plant species at *S. canadensis* plots, along with higher percent cover of non-native species. *A. petiolata* plots were distinguished by a lower total diversity and a higher percent cover of bare ground. Unlike our predictions, the proportion of native:non-native species was higher in *A. petiolata* plots than *S. canadensis* plots. The recruitment experiment had similar results with lower total diversity and stem density in *A. petiolata* pots compared to *H. matronalis* and control pots, although not compared to *S. canadensis* ones. The proportion of native:non-native species did not differ between pots. Finally, the germination experiment resulted in consistent germination rates among focal species treatments. Altogether these results do not support the invasional meltdown hypothesis, they do provide additional support for the additive effects of *A. petiolata* on its invaded communities studied here.

It is important to note that this study only focused on herbaceous invasive species. *Rhamnus cathartica* (common buckthorn) was the most common non-native species in the *A. petiolata* sites, although it is non-herbaceous. It is possible that if non-herbaceous species were included in the analysis, the results would be different. In fact, one study found that *A. petiolata* had the most drastic effects on tree seedlings, out of

all functional groups studied (Stinson et al. 2007). Another study found that as *A. petiolata* cover decreased over time, woody species cover increased, whereas the same pattern was not observed for herbaceous species (Lankau et al. 2009). This may indicate that allelochemicals released by *A. petiolata* affect woody species more than herbaceous species (Lankau et al. 2009). Thus, it is possible that *A. petiolata* is facilitating further invasions, but primarily those of woody species.

Another limitation of the study is the lack of knowledge of the below and above ground biomass in the pots. Since density of *A. petiolata* is known to affect the amount of allelochemicals in the soil (Barto and Cipollini 2009; Lankau 2012), it is reasonable to assume that the above and below ground biomass of the allelopathic species are good predictors of the amount of allelochemicals released by it. However, for this study, these were not measured because of the nature of the experiment and the need to keep the focal species planted in the pots throughout the entirety of the experiment. Although it appeared that the above ground biomass was similar between all three focal species, due to their varying growth patterns, it was not possible to definitively verify that they were always consistent throughout all pots. This could have affected the amount of allelochemicals released into the soil, and therefore the results of both the germination experiment and recruitment study. However, a general pattern can be observed here, and since the results largely match those from the field surveys, it can be concluded that they are representative of natural processes.

Overall the results from the co-occurrence surveys and the recruitment experiment are not consistent with those expected under the invasional meltdown

hypothesis, as sites and pots with *A. petiolata* were not found to have a higher proportion of non-native:native species. However, the data agree with previous studies that found lower diversity associated with *A. petiolata* (Van Riper et al. 2010; Stinson et al. 2007). As found in other studies, *A. petiolata* has additive effects in its introduced range where diversity and percent cover of other species is lower in its presence (Van Riper et al. 2010; Stinson et al. 2007). In the future, factors such as the invasion history, and the identity and biology of co-occurring species, should be considered as well. Furthermore, a similar study focusing on woody species in southern Ontario would also be of importance, as results may differ from herbaceous species co-occurrence.

Chapter 3: General Discussion

The goal of my research was to identify one mechanism that could possibly lead to an invasional meltdown. I investigated whether *Alliaria petiolata*, possibly by way of allelochemicals, paves the way for the establishment of more non-native species, whilst inhibiting the growth of native species. Results from an observation study, looking at species co-occurring with *A. petiolata*, and a recruitment experiment, looking at the establishment of new species at sites with *A. petiolata*, were largely consistent with one another. My findings overall indicate that *A. petiolata* is associated with lower total diversity, total stem density and percent cover of co-occurring herbaceous species. However, there is no evidence for an invasional meltdown, as the proportion of native:non-native species did not significantly differ between sites with *A. petiolata* and the native *Solidago canadensis*. Although, it is possible that invasional meltdowns may lead to lags in impacts, and it may not be immediately obvious that invasive species are facilitating future invasions (Havel et al. 2015). Also, one of the assumptions of the study was that non-native species found in this range were also sympatric with *A. petiolata* in its native range. This may not necessarily be the case, and thus these non-natives would not be more resistant to its allelochemical effects.

In the future it would be valuable to explore whether the native species that most commonly co-occur with *A. petiolata* use mycorrhizal fungi. Since past studies show that *A. petiolata* disrupts mutualistic relationships between species and their mycorrhizal fungi (Poon and Maherali 2015), species that are able to coexist with *A. petiolata* may not depend on mycorrhizae. There already has been one study that

showed that *A. petiolata* affected the native *Geum laciniatum* but not the non-native *Geum urbanum* (Prati and Bossdorf 2004). Such comparisons for the rest of the commonly co-occurring species could also be informative. This would indicate whether there is a reason why *A. petiolata* co-occurs with the species identified through the co-occurrence surveys, or whether patterns of co-occurrence are coincidental. *Geum aleppicum* was the fourth most common native species in *A. petiolata* plots. One possibility is that it was able to withstand the original invasion and continue to co-exist with *A. petiolata* because *A. petiolata* produced fewer allelochemicals over time (Lankau et al. 2009). It is also unknown why *A. petiolata* affects some species more than others (Rodgers et al. 2008), so comparing fitness of species with or without mycorrhizae could lead to novel findings.

It would also be useful to investigate the invasion history of the different communities surveyed for this study and determine whether the invasions are new or old. Some studies have shown that over time effects of *A. petiolata* on native species diminish because of lower concentrations of allelochemicals in older *A. petiolata* populations (Lankau et al. 2009, Lankau 2012). Since the length of invasion is not obvious at the surveyed sites, it is not clear whether these effects are lasting. Past studies showed that eventually *A. petiolata* directs its resources away from allelochemical production and into intraspecific competition (Lankau et al. 2009). Thus, higher allelochemical potential is probable in newly invaded areas, where interspecific competition is high, and lower in areas where the species is established, where intraspecific competition is high (Lankau et al. 2009; Lankau 2012). For example, one

study planted red oak seedlings (*Quercus rubra*) in plots with *A. petiolata* that differed based on the length of their invasion history, from 20 to 50 years (Lankau 2012). They found that sites with a longer invasion history resulted in larger *Quercus rubra* seedlings compared to sites where invasions were more recent (Lankau 2012). Thus, it would be of importance to estimate invasion times for the surveyed sites, to determine whether the allelopathic potential of *A. petiolata* is an important factor to consider in the co-occurrence of herbaceous species. It is possible that *A. petiolata* may affect the proportion of native:non-native species but only in newly invaded sites.

Studies looking at the co-occurrence of invasive species in their new range could help develop scientifically informed strategies for invasive species control within the province. One of the main objectives of the Invasive Species Strategic Plan (2012) is to fill in a knowledge gap on the subject of invasive species in order to improve conservation efforts in the province (Ontario Ministry of Natural Resources 2012). This includes more research on the impacts of invasive species and better strategies for risk assessment. Most invasive species management strategies and policies rely on precise impact and risk assessment analysis. Current risk assessment analyses primarily focus on single species scenarios, as there has not been enough research done to assess the synergistic impacts of multiple invasive species on an ecosystem or community level (Barga et al. 2018; Kuebbing, Nunez, and Simberloff 2013). Overall, it is important to identify patterns of co-occurring invasive species to identify possible mechanisms that lead to their coexistence, which will be beneficial in leading future conservation efforts,

assisting with risk assessment analysis for invasive species, and designing effective management practices within the province.

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Field Surveys to Test the Invasional Meltdown Hypothesis

Maria O'Sullivan

July 23, 2018

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Packages used

- R Version 3.5.0
 - RStudio Version 1.1.423
 - lme4 package #Version 1.1.15
 - Car package #Version 3.0.0
 - lmerTest package #3.0.1
 - glmmTMB package #Version 0.2.1.0
- ```
Loading required package: Matrix
Loading required package: carData
##
Attaching package: 'lmerTest'
```

```
The following object is masked from 'package:lme4':
##
lmer

The following object is masked from 'package:stats':
##
step
```

## Cover Analyses

### Native Percent Cover Mixed Effects Model

```
mm_nat <- lmer(native ~ focalspecies * focal + (1|location), data=data)
Anova(mm_nat, type="III")
```

```
Analysis of Deviance Table (Type III Wald chisquare tests)
##
Response: native
Chisq Df Pr(>Chisq)
(Intercept) 22.2596 1 2.382e-06 ***
focalspecies 0.3690 1 0.5435
focal 0.0792 1 0.7784
focalspecies:focal 0.9736 1 0.3238

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

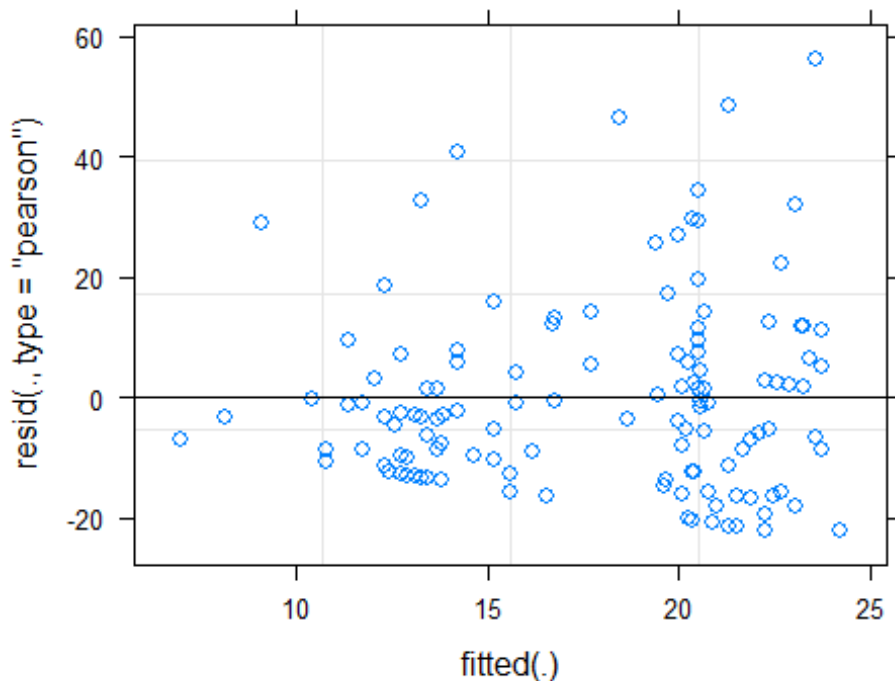
```
summary(mm_nat)
```

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: native ~ focalspecies * focal + (1 | location)
Data: data
##
REML criterion at convergence: 1078.8
##
Scaled residuals:
Min 1Q Median 3Q Max
-1.4013 -0.7121 -0.1806 0.4141 3.5483
##
Random effects:
Groups Name Variance Std.Dev.
location (Intercept) 22.75 4.769
Residual 252.73 15.898
Number of obs: 129, groups: location, 5
##
Fixed effects:
Estimate Std. Error df t value
(Intercept) 20.09940 4.26014 23.77689 4.718
focalspeciesgoldenrod 3.14953 5.18475 121.57932 0.607
focal -0.03456 0.12280 122.86811 -0.281
focalspeciesgoldenrod:focal -0.15654 0.15865 123.23251 -0.987
```

```
Pr(>|t|)
(Intercept) 8.69e-05 ***
focalspeciesgoldenrod 0.545
focal 0.779
focalspeciesgoldenrod:focal 0.326

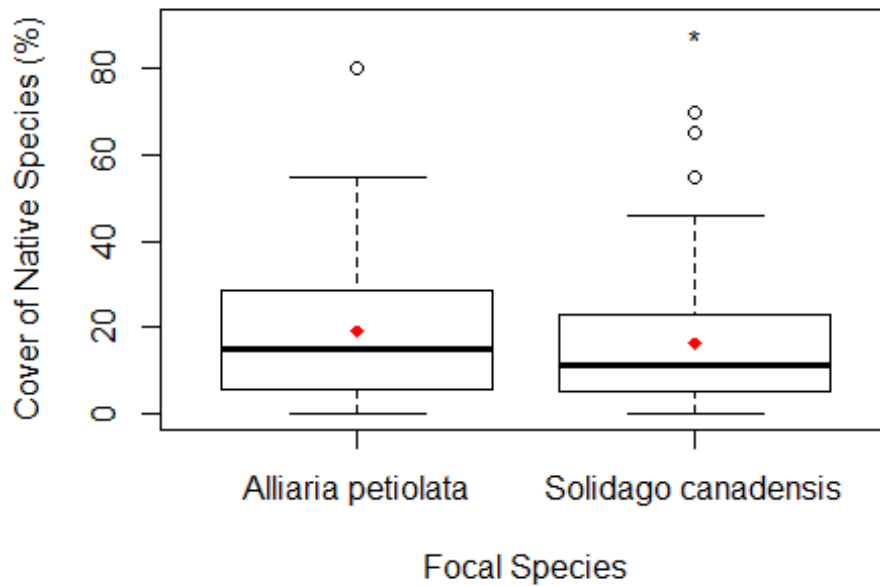
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspc focal
fclspcsgldn -0.612
focal -0.726 0.579
fclspcsgld: 0.569 -0.779 -0.790

#Checking assumptions
plot(mm_nat)
```



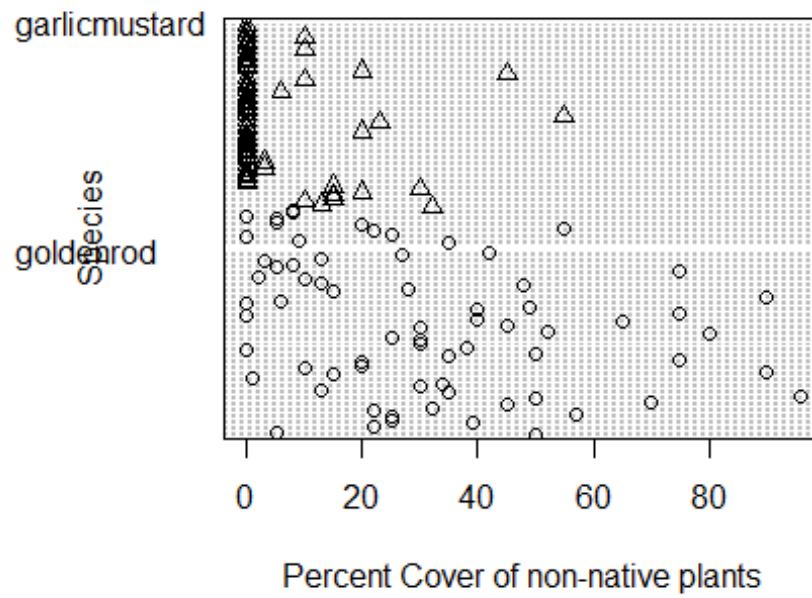
```
#Acceptable as far as overdispersion
```

```
Boxplot of Percent Native Cover by Focal Species
boxplot(native~as.factor(focalspecies), data=data, ylim = c(0,90), xlab
="Focal Species", ylab = "Cover of Native Species (%)", names=c("Alliar
ia petiolata", "Solidago canadensis"))
text(2, 88, "**")
nat_means = tapply(data$native, data$focalspecies, mean, na.rm=TRUE)
points(nat_means, col="red", pch=18)
```



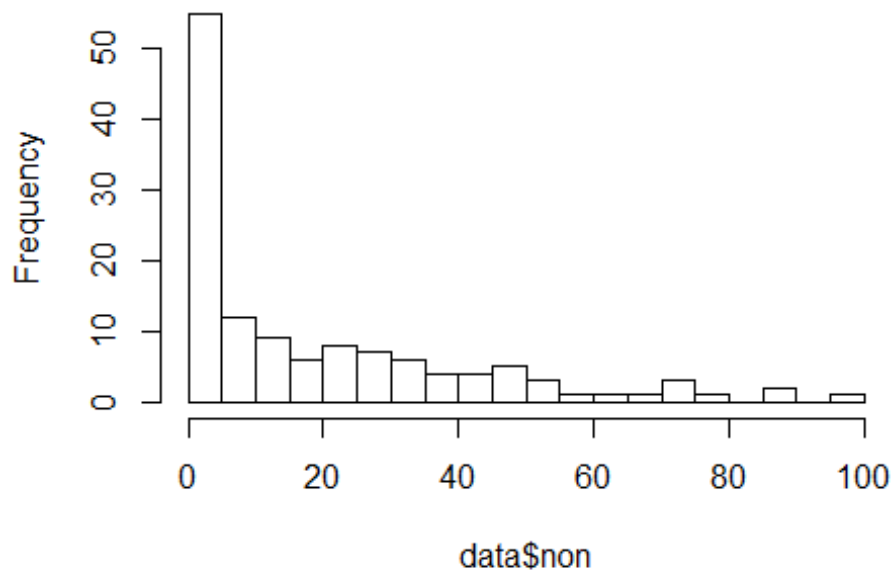
### Non-native Percent Cover Analysis

```
dotchart(data$non, #cover of non-native plants
 groups = factor(data$focalspecies), #groups the dots according
 to species
 xlab="Percent Cover of non-native plants",
 ylab="Species",
 main="",
 pch=as.numeric(data$focalspecies))
```



```
Non-native Percent Cover Mixed Effects Model
hist(data$non, breaks = 20)
```

**Histogram of data\$non**



```
#zero inflated
```

```
non.ZIP1 = glmmTMB(non ~ focalspecies * focal + (1|location), family =
"nbinom2", data = data, na.action=na.omit, ziformula=~1)
summary(non.ZIP1)
```

```
Family: nbinom2 (log)
Formula: non ~ focalspecies * focal + (1 | location)
Zero inflation: ~1
Data: data
##
AIC BIC logLik deviance df.resid
883.6 903.6 -434.8 869.6 122
##
Random effects:
##
Conditional model:
Groups Name Variance Std.Dev.
location (Intercept) 1.211e-09 3.48e-05
Number of obs: 129, groups: location, 5
##
Overdispersion parameter for nbinom2 family (): 2
##
Conditional model:
Estimate Std. Error z value Pr(>|z|)
(Intercept) 3.093443 0.290195 10.660 <2e-16 **
*
focalspeciesgoldenrod 0.824675 0.331578 2.487 0.0129 *
focal -0.008760 0.012063 -0.726 0.4678
focalspeciesgoldenrod:focal -0.004411 0.012894 -0.342 0.7323

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Zero-inflation model:
Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.6208 0.1885 -3.293 0.000993 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

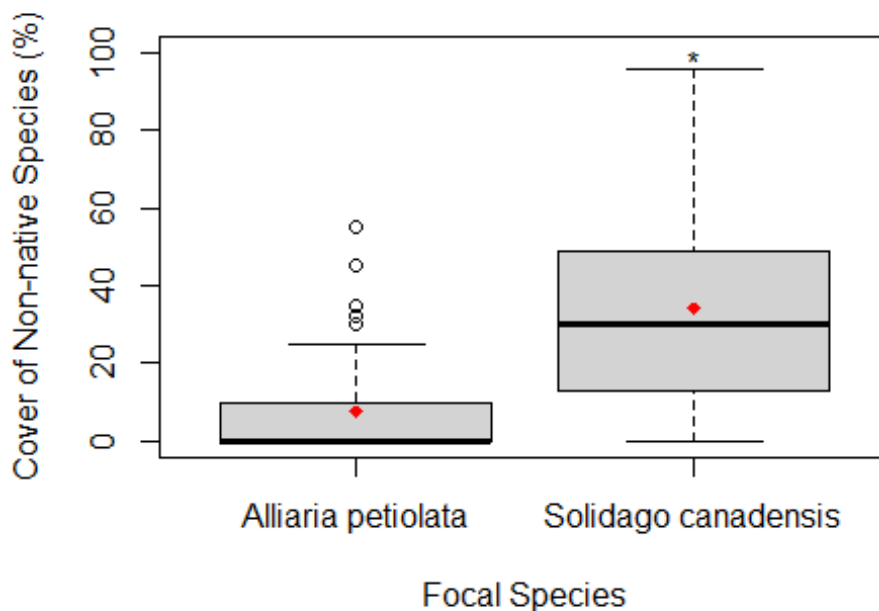
```
non.ZIP2 = glmmTMB(non ~ (1|site), family = "nbinom2", data = data, na.
action=na.omit, ziformula=~1)
anova(non.ZIP1, non.ZIP2)
```

```
Data: data
Models:
non.ZIP2: non ~ (1 | site), zi=~1, disp=~1
non.ZIP1: non ~ focalspecies * focal + (1 | location), zi=~1, disp=~
1
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
non.ZIP2 4 898.42 909.86 -445.21 890.42
```

```
non.ZIP1 7 883.59 903.61 -434.79 869.59 20.832 3 0.0001141

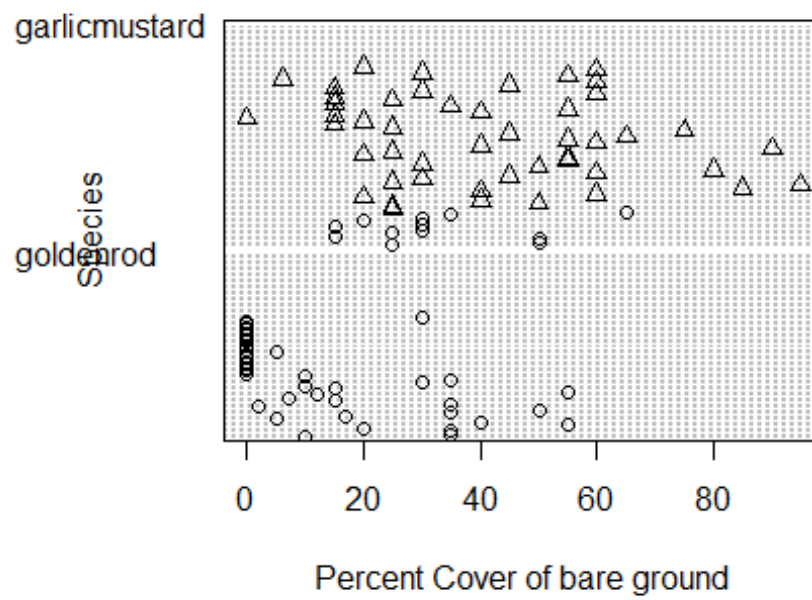
Signif. codes: 0 '****' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Boxplot of Percent Non-native Cover by Focal Species
boxplot(non~as.factor(focalspecies), data=data, col="lightgray", xlab="Focal Species", ylab = "Cover of Non-native Species (%)", names=c("Alliaria petiolata", "Solidago canadensis"), ylim=c(0,100))
text(2,99.5,"*")
non_means = tapply(data$non, data$focalspecies, mean, na.rm=TRUE)
points(non_means, col="red", pch=18)
```



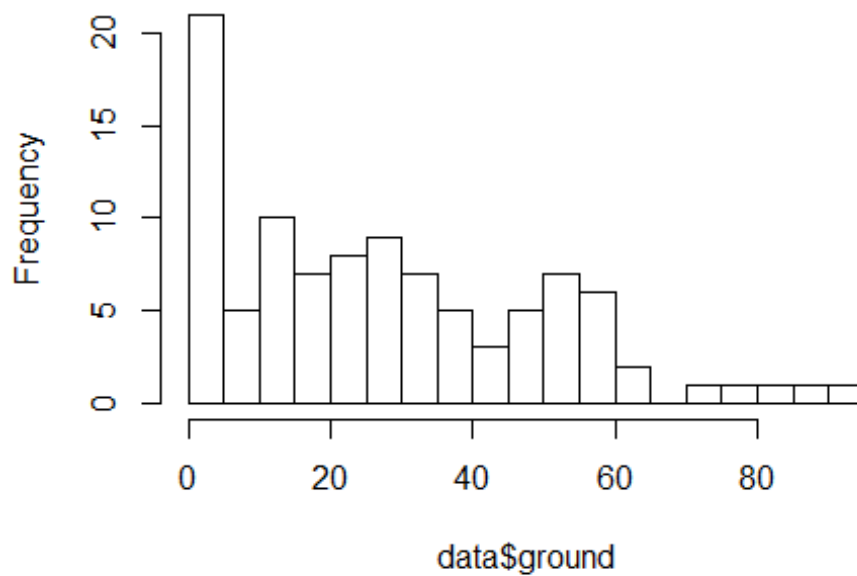
#### Analysis of Bare Ground Cover

```
dotchart(data$ground, #cover of non-native plants
 groups = factor(data$focalspecies), #groups the dots according
 to species
 xlab="Percent Cover of bare ground",
 ylab="Species",
 main="",
 pch=as.numeric(data$focalspecies))
```



```
hist(data$ground, breaks= 20)
```

**Histogram of data\$ground**



```

#not zero inflated

Ground Cover Mixed Effects Model
mm_gr <- lmer(ground ~ focalspecies * focal + (1|location), data=data)
Anova(mm_gr, type="III")

Analysis of Deviance Table (Type III Wald chisquare tests)
##
Response: ground
Chisq Df Pr(>Chisq)
(Intercept) 142.7910 1 < 2.2e-16 ***
focalspecies 27.5159 1 1.558e-07 ***
focal 15.5520 1 8.027e-05 ***
focalspecies:focal 2.2447 1 0.1341

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(mm_gr)

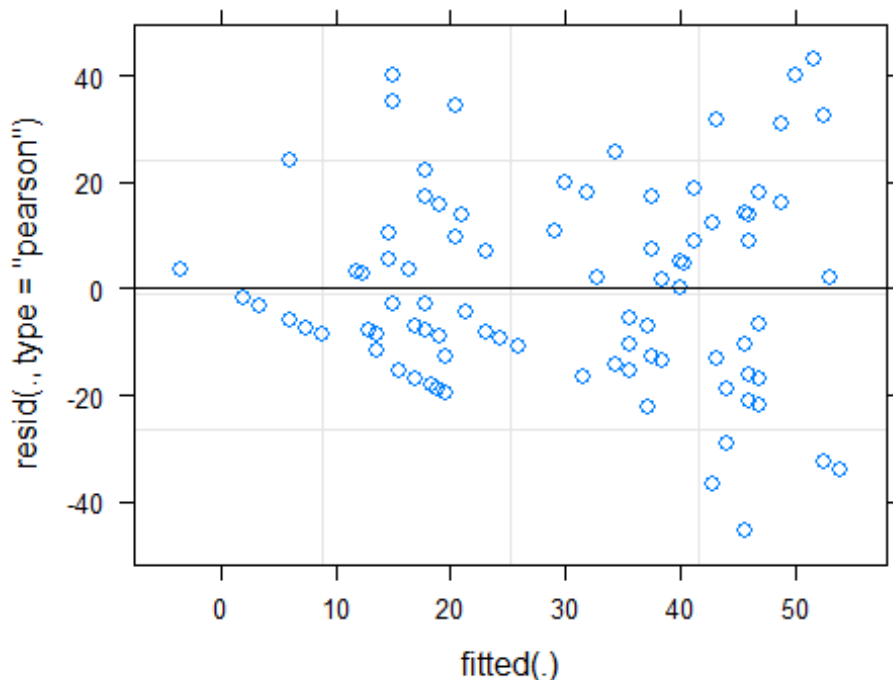
Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: ground ~ focalspecies * focal + (1 | location)
Data: data
##
REML criterion at convergence: 858
##
Scaled residuals:
Min 1Q Median 3Q Max
-2.4992 -0.6996 -0.1566 0.7640 2.3713
##
Random effects:
Groups Name Variance Std.Dev.
location (Intercept) 2.401 1.55
Residual 333.746 18.27
Number of obs: 100, groups: location, 3
##
Fixed effects:
Estimate Std. Error df t value Pr(
>|t|)
(Intercept) 54.6406 4.5726 16.9199 11.950 1.1
4e-09
focalspeciesgoldenrod -33.4985 6.3861 79.7015 -5.246 1.2
5e-06
focal -0.5637 0.1429 69.6864 -3.944 0.0
00189
focalspeciesgoldenrod:focal 0.2911 0.1943 44.0541 1.498 0.1
41209
##
(Intercept) ***
focalspeciesgoldenrod ***

```

```
focal ***
focalspeciesgoldenrod:focal

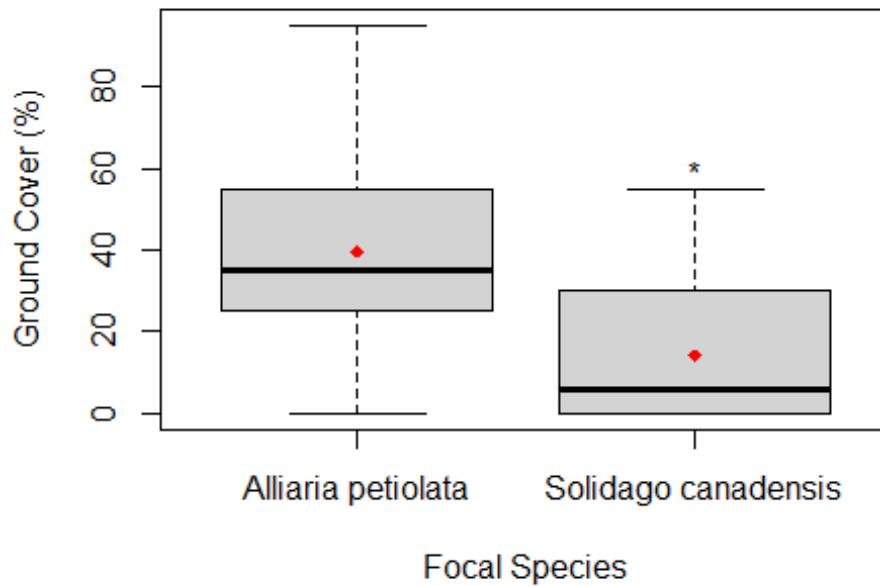
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspc focal
fclspcsgldn -0.684
focal -0.834 0.591
fclspcsgld: 0.619 -0.806 -0.742

#Test Assumptions
plot(mm_gr)
```



```
#Acceptable as far as overdispersion
```

```
Boxplot of Percent Ground Cover by Focal Species
boxplot(ground~as.factor(focalspecies), data=data, col="lightgray", xlab="Focal Species", ylab = "Ground Cover (%)", names=c("Alliaria petiolata", "Solidago canadensis"))
text(2, 61, "**")
gr_means = tapply(data$ground, data$focalspecies, mean, na.rm=TRUE)
points(gr_means, col="red", pch=18)
```

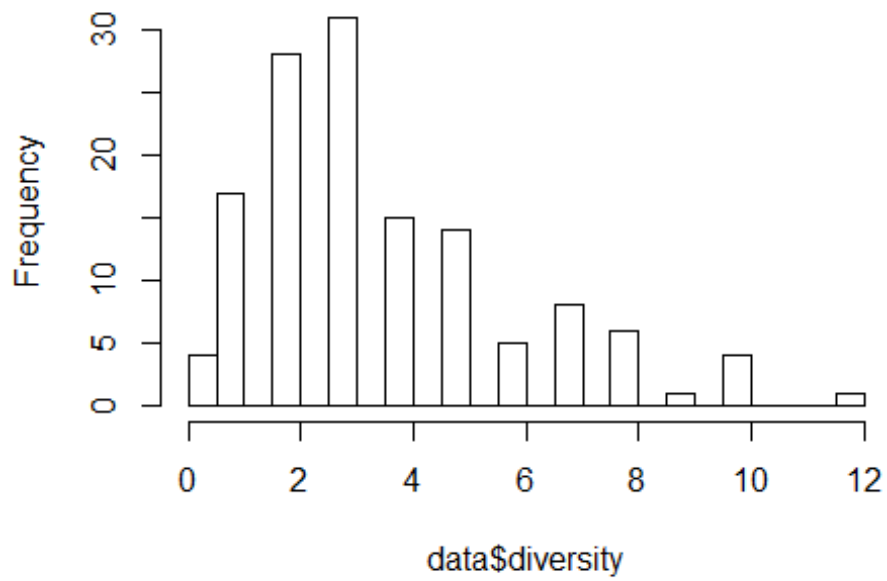


### Conclusions from the cover analyses

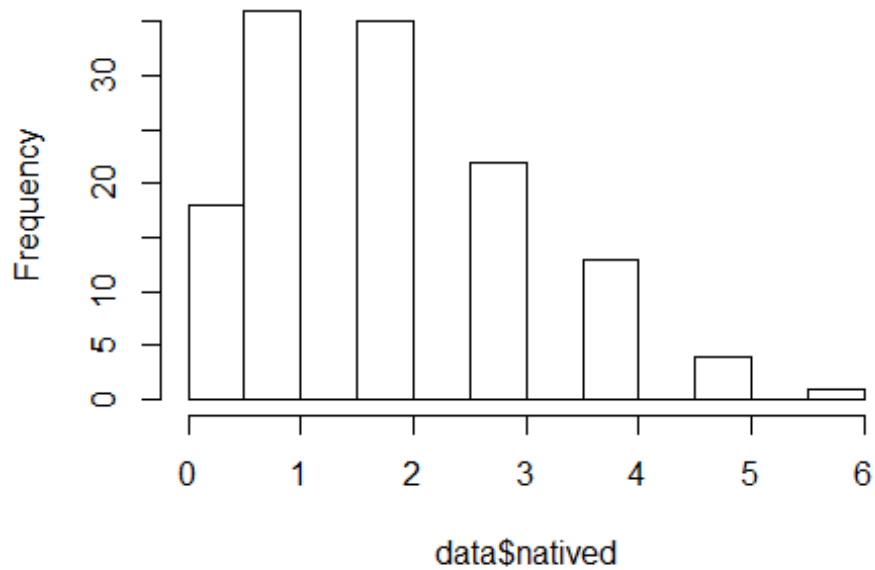
- Goldenrod plots were associated with significantly greater cover of non-native and native plant diversity
- GM is associated with sig greater ground cover
- Ground cover is influenced by the percent cover of the focal species in the plot

### Diversity Analyses

```
Testing assumptions
hist(data$diversity, breaks = 20)
```

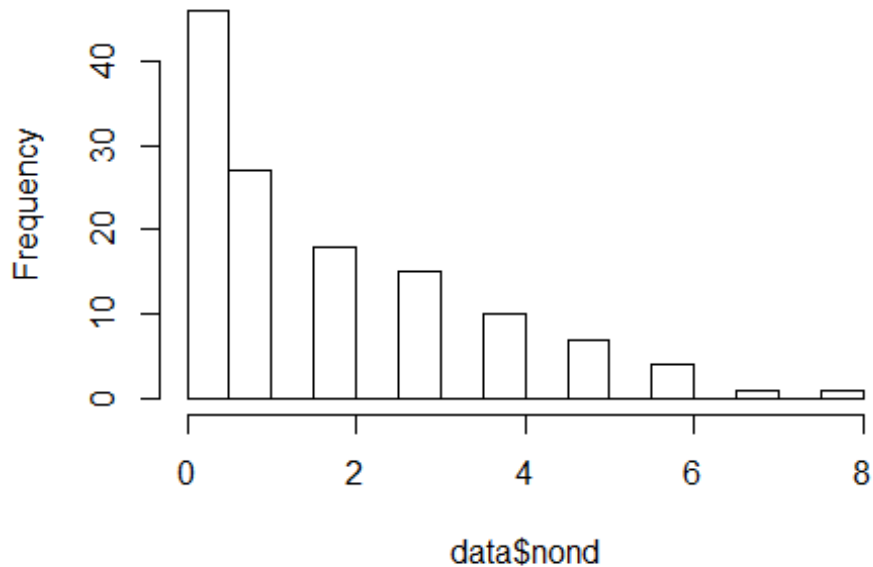
**Histogram of data\$diversity**

```
hist(data$native, breaks=20)
```

**Histogram of data\$native**

```
hist(data$nonD, breaks = 20)
```

## Histogram of data\$nond



```
#Data is not zero inflated
cor(data$natived, data$nond)
```

```
[1] NA
```

```
#correlation coefficient; the two variables are uncorrelated
```

```
#Transform data - not converging without transformations
```

```
sqrt.div = sqrt(data$diversity + 0.5)
sqrt.natived = sqrt(data$natived + 0.5)
sqrt.nond = sqrt(data$nond + 0.5)
```

### Total Diversity Analysis

```
lm.div <- lmer(sqrt.div ~ focalspecies * focal + (1|location), data=dat
a)
```

```
summary(lm.div); anova(lm.div)
```

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
```

```
Formula: sqrt.div ~ focalspecies * focal + (1 | location)
```

```
Data: data
```

```
##
```

```
REML criterion at convergence: 190
```

```
##
```

```
Scaled residuals:
```

```
Min 1Q Median 3Q Max
```

```
-2.8649 -0.6249 0.1262 0.6391 2.6934
```

```

##
Random effects:
Groups Name Variance Std.Dev.
location (Intercept) 0.01435 0.1198
Residual 0.19669 0.4435
Number of obs: 134, groups: location, 5
##
Fixed effects:
Estimate Std. Error df t value
(Intercept) 1.785539 0.115449 28.230340 15.466
focalspeciesgoldenrod 0.789742 0.139167 127.439880 5.675
focal -0.004875 0.003367 127.679608 -1.448
focalspeciesgoldenrod:focal -0.003403 0.004185 126.743304 -0.813
Pr(>|t|)
(Intercept) 2.58e-15 ***
focalspeciesgoldenrod 8.89e-08 ***
focal 0.150
focalspeciesgoldenrod:focal 0.418

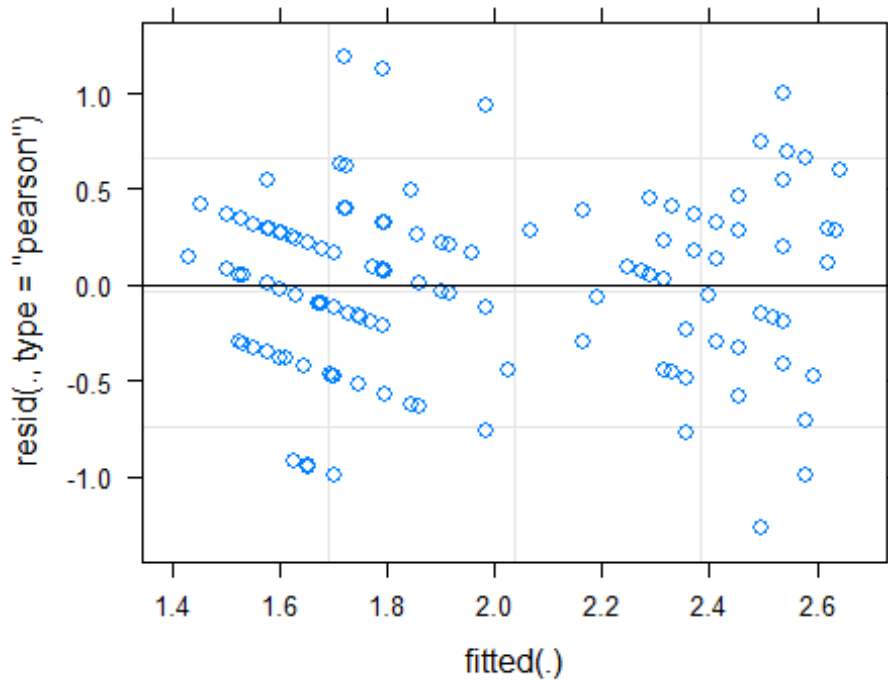
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspc focal
fclspcsgldn -0.639
focal -0.746 0.597
fclspcsgld: 0.597 -0.774 -0.816

Type III Analysis of Variance Table with Satterthwaite's method
Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
focalspecies 6.3340 6.3340 1 127.44 32.203 8.892e-08 ***
focal 2.0202 2.0202 1 129.84 10.271 0.001701 **
focalspecies:focal 0.1300 0.1300 1 126.74 0.661 0.417716

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Testing assumptions
plot(lm.div)

```



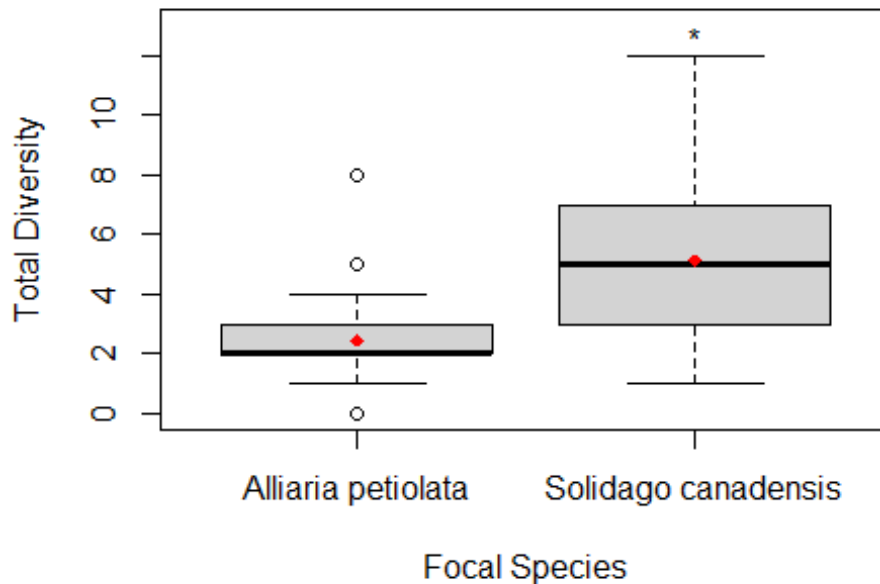
*#Does not appear overdispersed*

*#Total Species Diversity Plot#*

```

boxplot(diversity~as.factor(focalspecies), data=data, col = "lightgray"
, xlab="Focal Species", ylab = "Total Diversity", ylim=c(0,13), names=c
("Alliaria petiolata", "Solidago canadensis"))
div_means = tapply(data$diversity, data$focalspecies, mean)
points(div_means, col="red", pch=18)
text(2,12.8, "*")

```



### Native Diversity Analysis

```
lm.natived <- lmer(sqrt.natived ~ focalspecies * focal + (1|location),
data=data)
```

```
summary(lm.natived); anova(lm.natived)
```

```
Linear mixed model fit by REML. t-tests use Satterthwaite's method [
```

```
lmerModLmerTest]
```

```
Formula: sqrt.natived ~ focalspecies * focal + (1 | location)
```

```
Data: data
```

```
##
```

```
REML criterion at convergence: 176.3
```

```
##
```

```
Scaled residuals:
```

```
Min 1Q Median 3Q Max
```

```
-2.57518 -0.59085 -0.01984 0.65204 2.17428
```

```
##
```

```
Random effects:
```

```
Groups Name Variance Std.Dev.
```

```
location (Intercept) 0.01297 0.1139
```

```
Residual 0.18590 0.4312
```

```
Number of obs: 129, groups: location, 5
```

```
##
```

```
Fixed effects:
```

```
Estimate Std. Error df t value
```

```
(Intercept) 1.442e+00 1.119e-01 2.621e+01 12.886
```

```
focalspeciesgoldenrod 3.496e-01 1.399e-01 1.197e+02 2.499
```

```

focal 1.159e-04 3.315e-03 1.212e+02 0.035
focalspeciesgoldenrod:focal -5.809e-03 4.283e-03 1.214e+02 -1.356
Pr(>|t|)
(Intercept) 7.51e-13 ***
focalspeciesgoldenrod 0.0138 *
focal 0.9722
focalspeciesgoldenrod:focal 0.1776

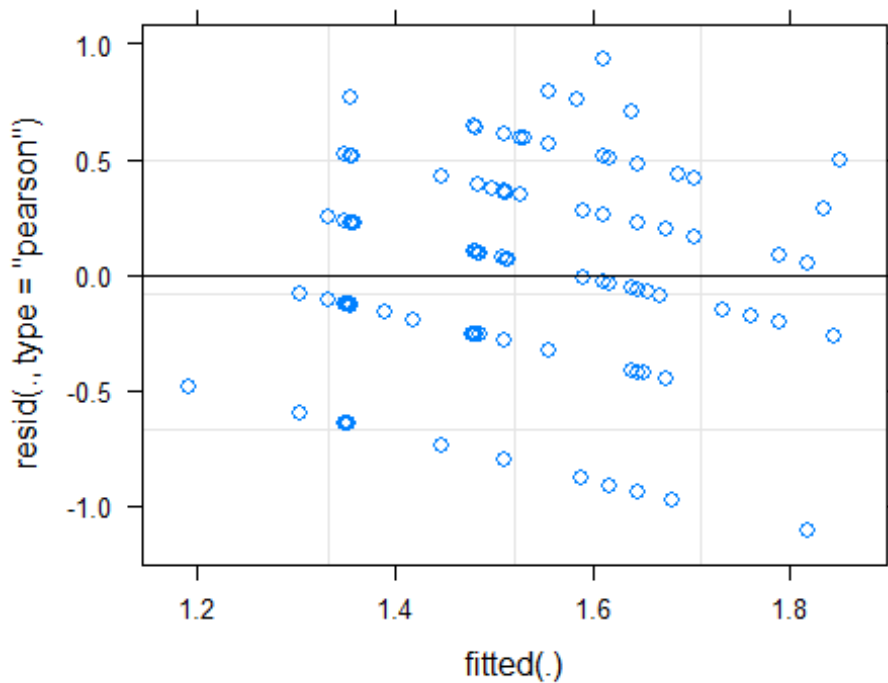
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspc focal
fclspcsgldn -0.631
focal -0.748 0.583
fclspcsgld: 0.586 -0.785 -0.789

Type III Analysis of Variance Table with Satterthwaite's method
Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
focalspecies 1.16074 1.16074 1 119.66 6.2439 0.01382 *
focal 0.33072 0.33072 1 124.87 1.7790 0.18470
focalspecies:focal 0.34192 0.34192 1 121.42 1.8393 0.17755

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

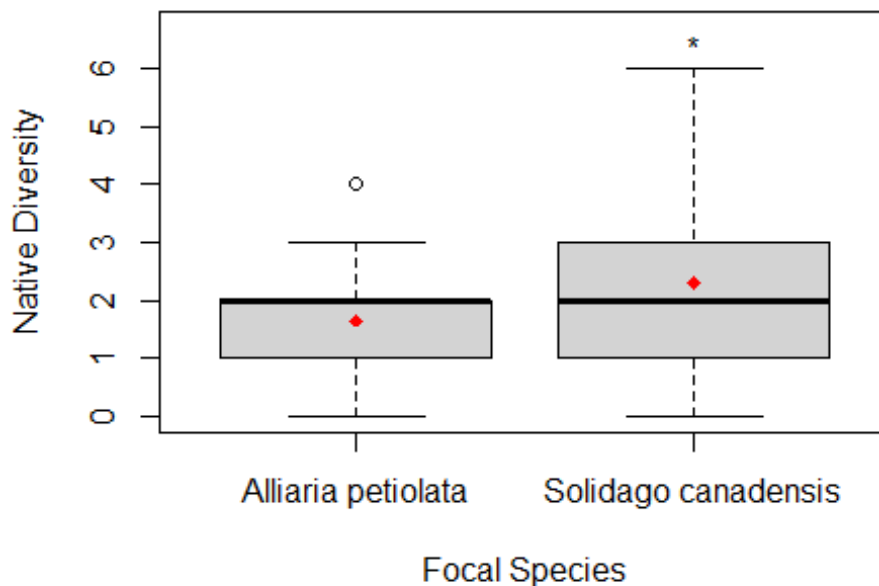
Testing assumptions
plot(lm.nated)

```



```
#Does not appear overdispersed

Native Species Diversity Plot
boxplot(natived~as.factor(focalspecies), data=data, col = "lightgray",
xlab="Focal Species", ylab = "Native Diversity", ylim=c(0,6.7), names=c
("Alliaria petiolata", "Solidago canadensis"))
text(2,6.5, "*")
nated_means = tapply(data$natived, data$focalspecies, mean, na.rm=TRUE)
points(nated_means, col="red", pch=18)
```



### Non-native Diversity Analysis

```
lm.nond <- lmer(sqrt.nond ~ focalspecies * focal + (1|location), data=data)
summary(lm.nond); anova(lm.nond)

Linear mixed model fit by REML. t-tests use Satterthwaite's method [
lmerModLmerTest]
Formula: sqrt.nond ~ focalspecies * focal + (1 | location)
Data: data
##
REML criterion at convergence: 180.9
##
Scaled residuals:
Min 1Q Median 3Q Max
-2.1864 -0.5219 -0.2430 0.6392 3.1382
##
```

```

Random effects:
Groups Name Variance Std.Dev.
location (Intercept) 0.05323 0.2307
Residual 0.18677 0.4322
Number of obs: 129, groups: location, 5
##
Fixed effects:
Estimate Std. Error df t value
(Intercept) 1.199342 0.144650 11.077830 8.291
focalspeciesgoldenrod 0.665537 0.143778 124.999886 4.629
focal -0.007918 0.003395 124.845609 -2.332
focalspeciesgoldenrod:focal 0.003830 0.004381 124.702288 0.874
Pr(>|t|)
(Intercept) 4.43e-06 ***
focalspeciesgoldenrod 9.07e-06 ***
focal 0.0213 *
focalspeciesgoldenrod:focal 0.3836

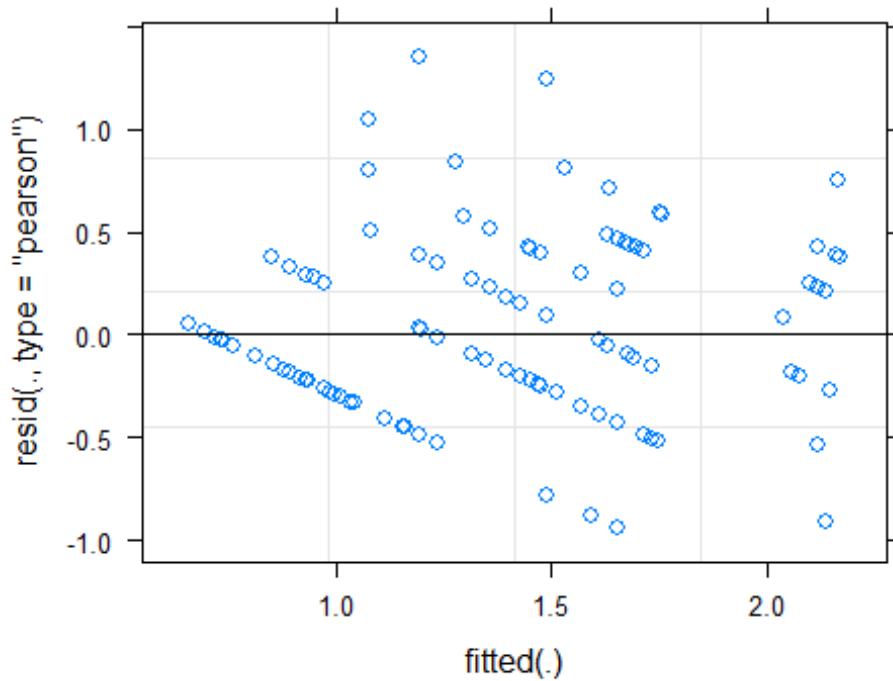
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspc focal
fclspcsgldn -0.493
focal -0.584 0.562
fclspcsgld: 0.459 -0.759 -0.795

Type III Analysis of Variance Table with Satterthwaite's method
Sum Sq Mean Sq NumDF DenDF F value Pr(>F)
focalspecies 4.0019 4.0019 1 125.00 21.4268 9.067e-06 ***
focal 1.4954 1.4954 1 123.87 8.0064 0.00544 **
focalspecies:focal 0.1428 0.1428 1 124.70 0.7644 0.38364

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

#Testing assumptions
plot(lm.nond)

```



*#does not appear overdispersed*

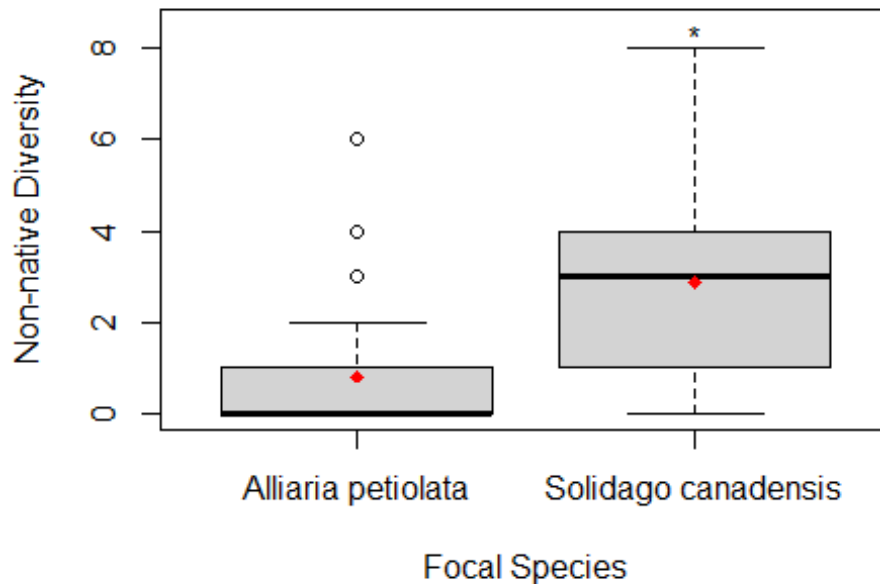
*#Non-native diversity plot*

```
boxplot(nond~as.factor(focalspecies), data=data, col="lightgray", xlab="Focal Species", ylab = "Non-native Diversity", ylim=c(0,8.5), names=c("Alliaria petiolata", "Solidago canadensis"))
```

```
text(2,8.4, "*")
```

```
nond_means = tapply(data$nond, data$focalspecies, mean, na.rm=TRUE)
```

```
points(nond_means, col="red", pch=18)
```



### Native:non-native Diversity Analysis

```
rel_div <- cbind(data$natived, data$nondiv)
glmm_reldiv <- glmer(rel_div ~ focalspecies + (1|location), family="binomial", data=data)
summary(glmm_reldiv); anova(glmm_reldiv)

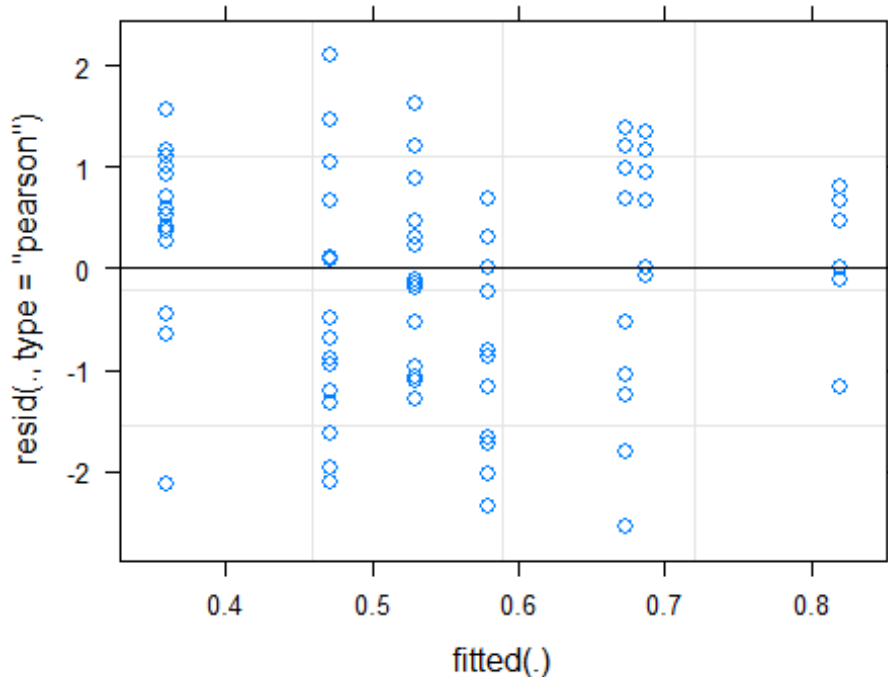
Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial (logit)
Formula: rel_div ~ focalspecies + (1 | location)
Data: data
##
AIC BIC logLik deviance df.resid
328.0 336.6 -161.0 322.0 126
##
Scaled residuals:
Min 1Q Median 3Q Max
-2.5563 -0.6797 0.3170 0.9553 2.1159
##
Random effects:
Groups Name Variance Std.Dev.
location (Intercept) 0.2075 0.4556
Number of obs: 129, groups: location, 5
##
Fixed effects:
Estimate Std. Error z value Pr(>|z|)
```

```
(Intercept) 0.8765 0.2747 3.191 0.001417 **
focalspeciesgoldenrod -0.8972 0.2441 -3.676 0.000237 ***

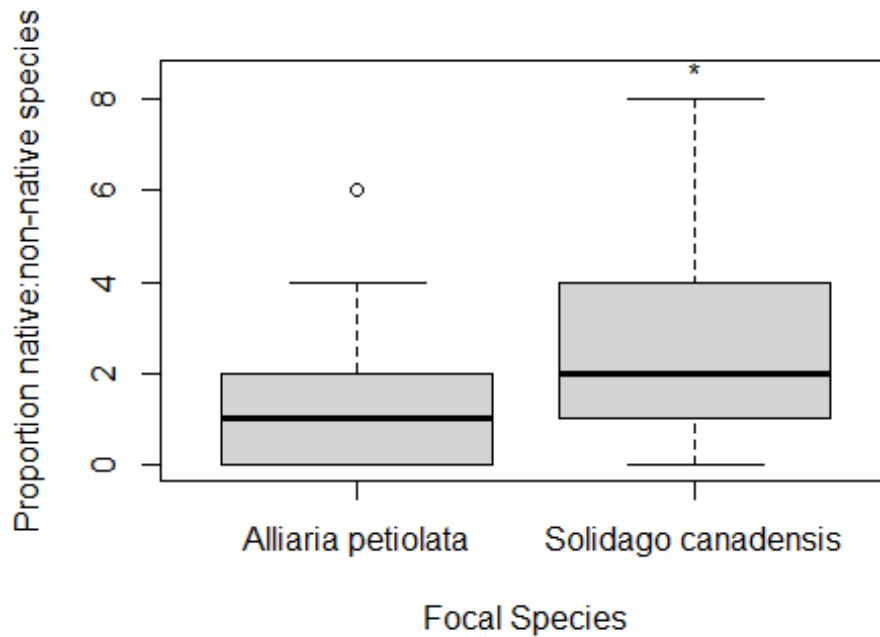
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr)
fclspcsgldn -0.429

Analysis of Variance Table
Df Sum Sq Mean Sq F value
focalspecies 1 13.997 13.997 13.997

#Testing assumptions
plot(glmm_reldiv)
```



```
#Relative native:non-native plant diversity plot
#plot(rel_div ~ focalspecies * focal + (1|location), family="binomial",
data=data, xlab="Focal Species", ylab = "Rlative Native:Non-Native Dive
rsity")
boxplot(rel_div~as.factor(focalspecies), data=data, col="lightgray", xl
ab="Focal Species", ylab = "Proportion native:non-native species", ylim
=c(0,8.5), names=c("Alliaria petiolata", "Solidago canadensis"))
text(2,8.7,"*")
```



#### Conclusions from the diversity analyses

- Goldenrod plots had significantly higher species diversity than garlic mustard plots
- Higher total species diversity in goldenrod plots was driven by higher diversity of both native and non-native plants
- Higher proportion of native:non-native plants in garlic mustard plots

## Appendix 2 - Recruitment Experiment Analyses

Maria O'Sullivan

July 23, 2018

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### Total Diversity Analysis

```
totaldiversity.model <- glmer(totaldiversity ~ focalspecies + (1|site),
family = "poisson", data = data, na.action=na.fail)
summary(totaldiversity.model)
```

```
Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: poisson (log)
Formula: totaldiversity ~ focalspecies + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
189.3 199.8 -89.7 179.3 55
##
Scaled residuals:
Min 1Q Median 3Q Max
-1.31236 -0.70032 -0.03662 0.38342 2.13711
##
Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.03648 0.191
Number of obs: 60, groups: site, 5
##
Fixed effects:
```

```

Estimate Std. Error z value Pr(>|z|)
(Intercept) 0.67488 0.20271 3.329 0.00087 ***
focalspeciesdamesrocket 0.06453 0.25357 0.254 0.79913
focalspeciesgarlicmustard -1.00331 0.35171 -2.853 0.00433 **
focalspeciesgoldenrod -0.14311 0.26736 -0.535 0.59245

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspcsd fclspcsgr
fclspcsdmsr -0.646
fclspcsgrlc -0.465 0.372
fclspcsgldn -0.612 0.490 0.353

Anova(totdiversity.model, type = "II")

Analysis of Deviance Table (Type II Wald chisquare tests)
##
Response: totaldiversity
Chisq Df Pr(>Chisq)
focalspecies 10.156 3 0.01728 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(totdiversity.model, mcp(focalspecies="Tukey")))

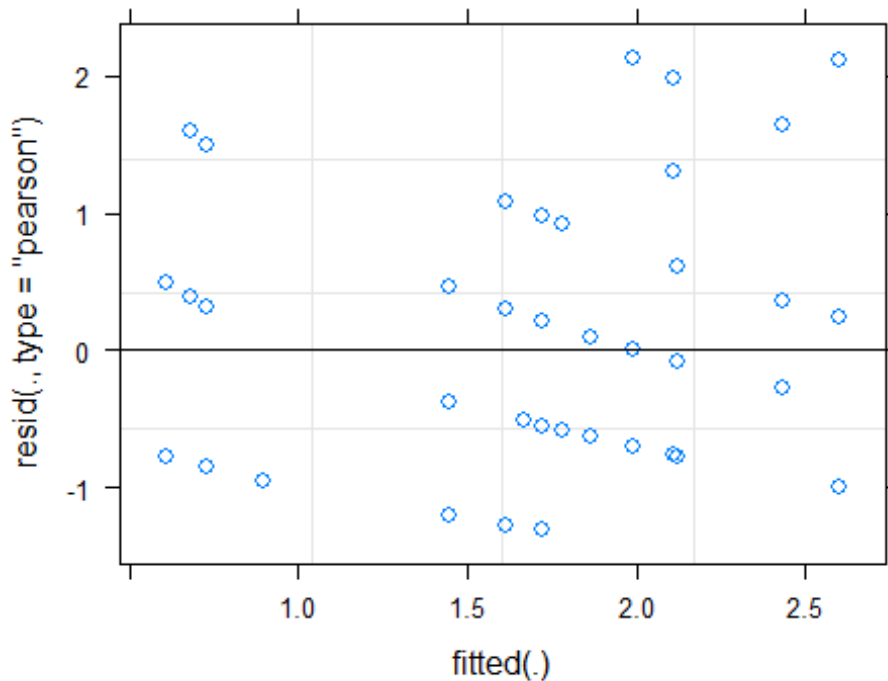
##
Simultaneous Tests for General Linear Hypotheses
##
Multiple Comparisons of Means: Tukey Contrasts
##
##
Fit: glmer(formula = totaldiversity ~ focalspecies + (1 | site), data = data,
family = "poisson", na.action = na.fail)
##
Linear Hypotheses:
Estimate Std. Error z value Pr(>|z|)
)
damesrocket - control == 0 0.06453 0.25357 0.254 0.994
1
garlicmustard - control == 0 -1.00331 0.35171 -2.853 0.021
8 *
goldenrod - control == 0 -0.14311 0.26736 -0.535 0.949
5
garlicmustard - damesrocket == 0 -1.06784 0.34874 -3.062 0.011
6 *
goldenrod - damesrocket == 0 -0.20764 0.26345 -0.788 0.857
5
goldenrod - garlicmustard == 0 0.86020 0.35889 2.397 0.075
8 .

```

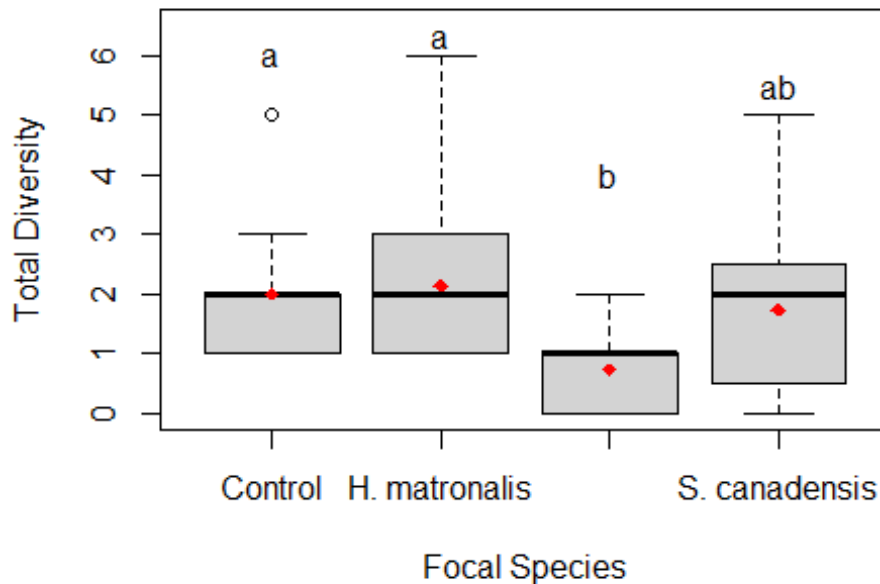
```

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

#checking assumptions
plot(totaldiversity.model)
```



```
#Total Diversity Plot
plot(data$totaldiversity~data$focalspecies, col="lightgray", ylim=c(0,6.5),
 xlab="Focal Species", ylab = "Total Diversity", names=c("Control",
"H. matronalis", "A. petiolata", "S. canadensis"))
text(1,6, "a")
text(2, 6.3, "a")
text(3, 4, "b")
text(4, 5.5, "ab")
tdiv_means = tapply(data$totaldiversity, data$focalspecies, mean, na.rm
=TRUE)
points(tdiv_means, col="red", pch=18)
```



### Conclusions from the total diversity analyses

- Garlic mustard pots had significantly lower diversity than control pots and pots with dame's rocket
- Garlic mustard pots did not vary sig from GR pots, although lower  $p = 0.07586$
- All other comparisons were not significant

### Native Diversity Analysis

```
natediversity.model <- glmer(natediversity ~ focalspecies + (1|site)
), family = "poisson", data = data, na.action=na.omit)
summary(natediversity.model)

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: poisson (log)
Formula: natediversity ~ focalspecies + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
118.4 128.1 -54.2 108.4 46
##
Scaled residuals:
Min 1Q Median 3Q Max
-1.04237 -0.71334 -0.08002 0.55290 1.97215
```

```

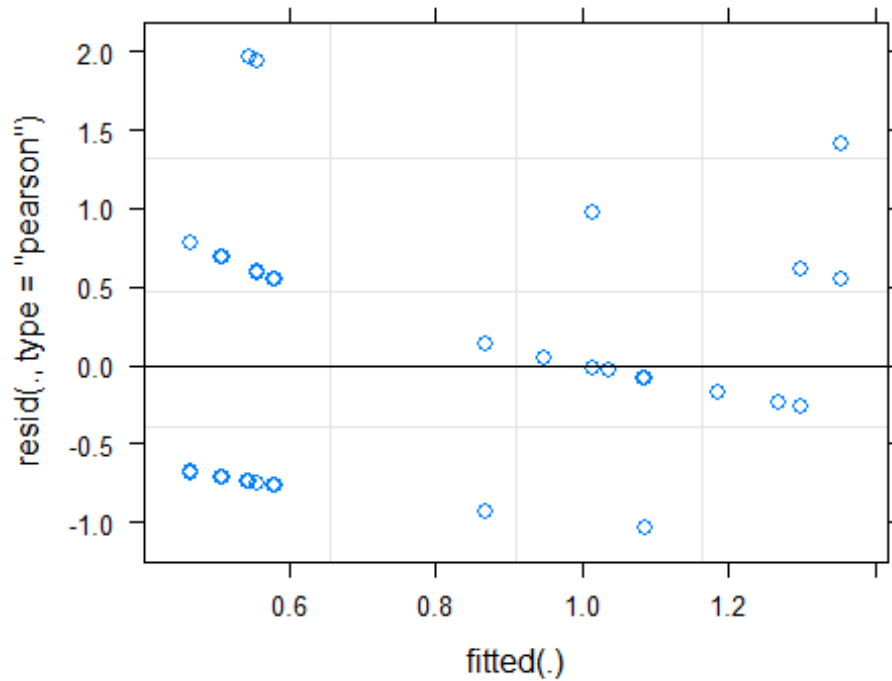
##
Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.02957 0.172
Number of obs: 51, groups: site, 5
##
Fixed effects:
Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.01465 0.27314 -0.054 0.957
focalspeciesdamesrocket 0.22371 0.36548 0.612 0.540
focalspeciesgarlicmustard -0.62480 0.45761 -1.365 0.172
focalspeciesgoldenrod -0.62176 0.48455 -1.283 0.199
##
Correlation of Fixed Effects:
(Intr) fclspcsd fclspcsgr
fclspcsdmsr -0.667
fclspcsgrlc -0.527 0.396
fclspcsgldn -0.488 0.375 0.303

Anova(nativediversity.model, type = "II")

Analysis of Deviance Table (Type II Wald chisquare tests)
##
Response: nativediversity
Chisq Df Pr(>Chisq)
focalspecies 5.2268 3 0.1559

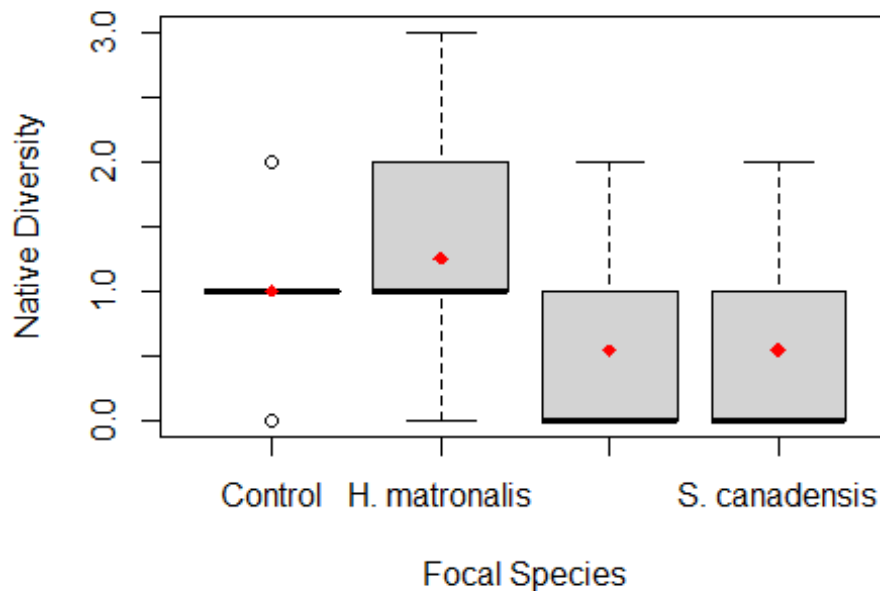
#Checking assumptions
plot(nativediversity.model)

```



```
#Native Diversity Plot
```

```
plot(data$nativediversity~data$focalspecies, col="lightgray",xlab="Focal Species", ylab = "Native Diversity", names=c("Control", "H. matronalis", "A. petiolata", "S. canadensis"))
natdiv_means = tapply(data$nativediversity, data$focalspecies, mean, na.rm=TRUE)
points(natdiv_means, col="red", pch=18)
```



### Conclusions from native diversity analysis

- Anova results are not significant

### Non-native Diversity Analysis

```
nondiversity.model <- glmer(nondiversity ~ focalspecies + (1|site), family = "poisson", data = data, na.action=na.omit)
summary(nondiversity.model)

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: poisson (log)
Formula: nondiversity ~ focalspecies + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
99.7 109.3 -44.8 89.7 46
##
Scaled residuals:
Min 1Q Median 3Q Max
-0.9948 -0.6437 -0.2761 0.3087 3.3020
##
Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.009376 0.09683
Number of obs: 51, groups: site, 5
##
```

```

Fixed effects:
##
Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.004678 0.268776 -0.017 0.9861
focalspeciesdamesrocket -0.874258 0.516786 -1.692 0.0907 .
focalspeciesgarlicmustard -2.563602 1.032840 -2.482 0.0131 *
focalspeciesgoldenrod -0.312970 0.443211 -0.706 0.4801

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspcsd fclspcsgr
fclspcsdmsr -0.487
fclspcsgrlc -0.244 0.125
fclspcsgldn -0.594 0.296 0.148

Anova(nondiversity.model, type = "II")

Analysis of Deviance Table (Type II Wald chisquare tests)
##
Response: nondiversity
Chisq Df Pr(>Chisq)
focalspecies 8.1008 3 0.04397 *

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(nondiversity.model, mcp(focalspecies="Tukey")))

##
Simultaneous Tests for General Linear Hypotheses
##
Multiple Comparisons of Means: Tukey Contrasts
##
##
Fit: glmer(formula = nondiversity ~ focalspecies + (1 | site), data
= data,
family = "poisson", na.action = na.omit)
##
Linear Hypotheses:
##
Estimate Std. Error z value Pr(>|z|)
)
damesrocket - control == 0 -0.8743 0.5168 -1.692 0.308
4
garlicmustard - control == 0 -2.5636 1.0328 -2.482 0.056
1 .
goldenrod - control == 0 -0.3130 0.4432 -0.706 0.887
1
garlicmustard - damesrocket == 0 -1.6893 1.0955 -1.542 0.391
4
goldenrod - damesrocket == 0 0.5613 0.5728 0.980 0.746
3
goldenrod - garlicmustard == 0 2.2506 1.0618 2.120 0.134

```

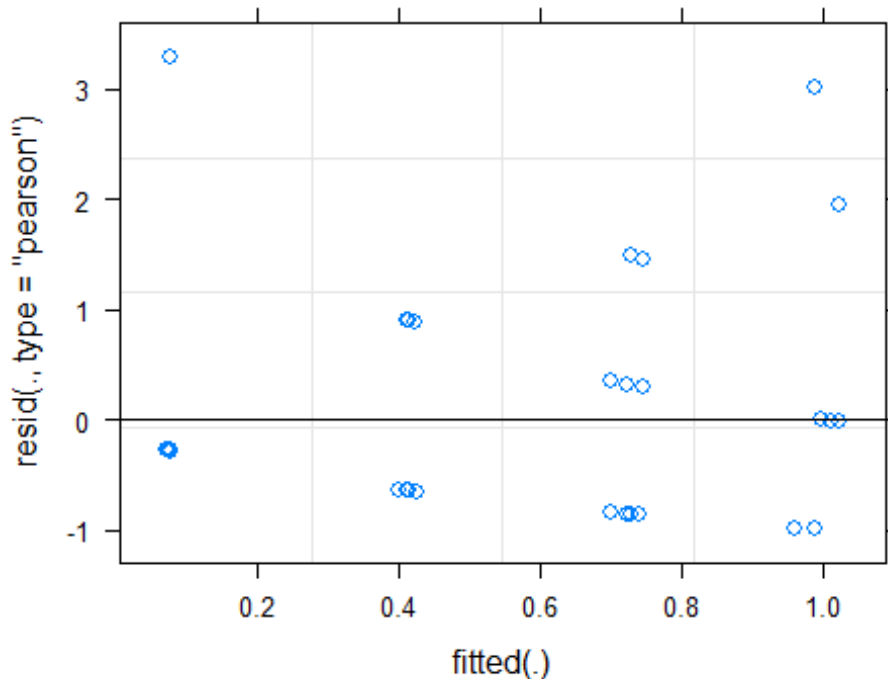
```

3

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

#Checking assumptions
plot(nondiversity.model)

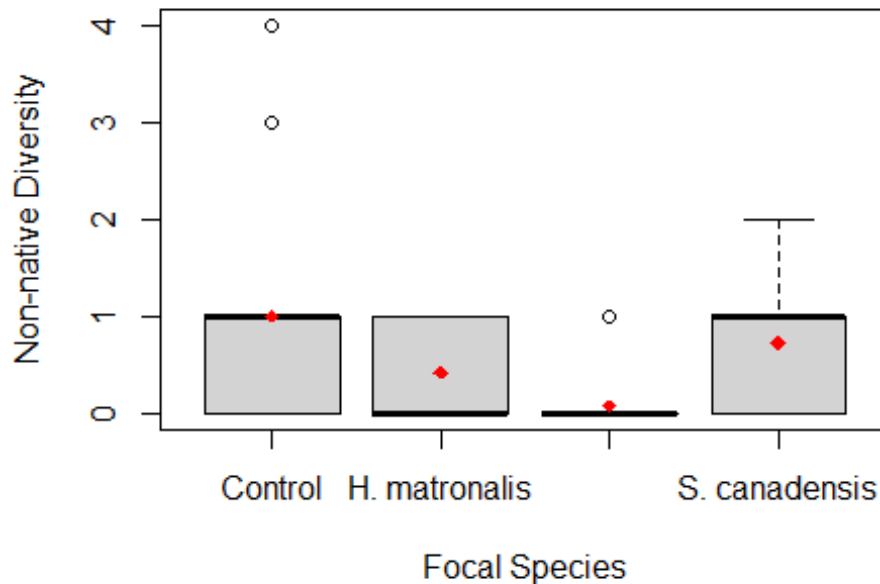
```



```

#Non-native Diversity Plot
plot(data$nondiversity~data$focalspecies, col="lightgrey",xlab="Focal S
pecies", ylab = "Non-native Diversity", names=c("Control", "H. matronal
is", "A. petiolata", "S. canadensis"))
nondiv_means = tapply(data$nondiversity, data$focalspecies, mean, na.rm
=TRUE)
points(nondiv_means, col="red", pch=18)

```



### Conclusions from non-native diversity analysis

*Garlic mustard* had sig lower non-native species diversity than the control pots  
 Tukey test showed no significant difference between any of the pairs

### Proportion Native:Non-native Diversity Analysis

```
#Binding data to get proportion
diversity.bind = cbind(data$nativediversity, data$nondiversity)

diversitybind.model = glmer(diversity.bind ~ focalspecies + (1|site), f
amily = "binomial", data = data, na.action=na.omit)
summary(diversitybind.model)

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: binomial (logit)
Formula: diversity.bind ~ focalspecies + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
68.3 78.0 -29.2 58.3 46
##
Scaled residuals:
Min 1Q Median 3Q Max
-2.0349 -0.1322 0.0000 0.5036 1.1350
##
```

```

Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.2877 0.5364
Number of obs: 51, groups: site, 5
##
Fixed effects:
Estimate Std. Error z value Pr(>|z|)
(Intercept) -0.02603 0.45423 -0.057 0.9543
focalspeciesdamesrocket 1.18945 0.68438 1.738 0.0822 .
focalspeciesgarlicmustard 2.13511 1.20790 1.768 0.0771 .
focalspeciesgoldenrod -0.42723 0.69404 -0.616 0.5382

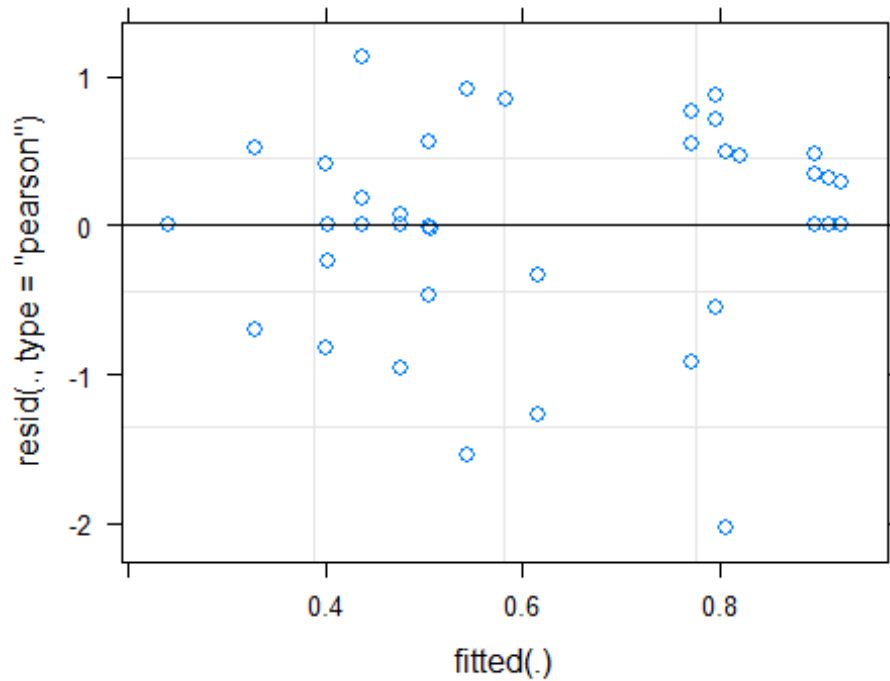
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspcsd fclspcsgr
fclspcsdmsr -0.497
fclspcsgrlc -0.300 0.262
fclspcsgldn -0.426 0.226 0.093

Anova(diversitybind.model)

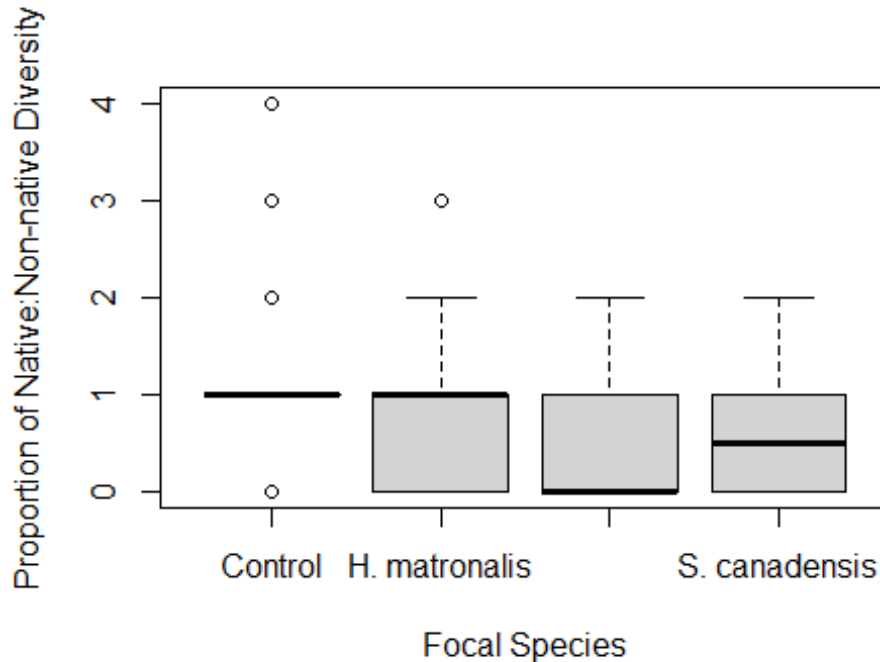
Analysis of Deviance Table (Type II Wald chisquare tests)
##
Response: diversity.bind
Chisq Df Pr(>Chisq)
focalspecies 6.048 3 0.1093

#Checking Assumptions
plot(diversitybind.model)

```



```
#Plotting proportion native:non-native species
plot(diversity.bind~data$focalspecies, col="lightgray", xlab="Focal Species",
 ylab = "Proportion of Native:Non-native Diversity", names=c("Control",
 "H. matronalis", "A. petiolata", "S. canadensis"))
```



### Conclusions from proportion native:non-native diversity analysis

- No significant difference in the proportion of native:non species between any of the pots

### Total Stem Density Analysis

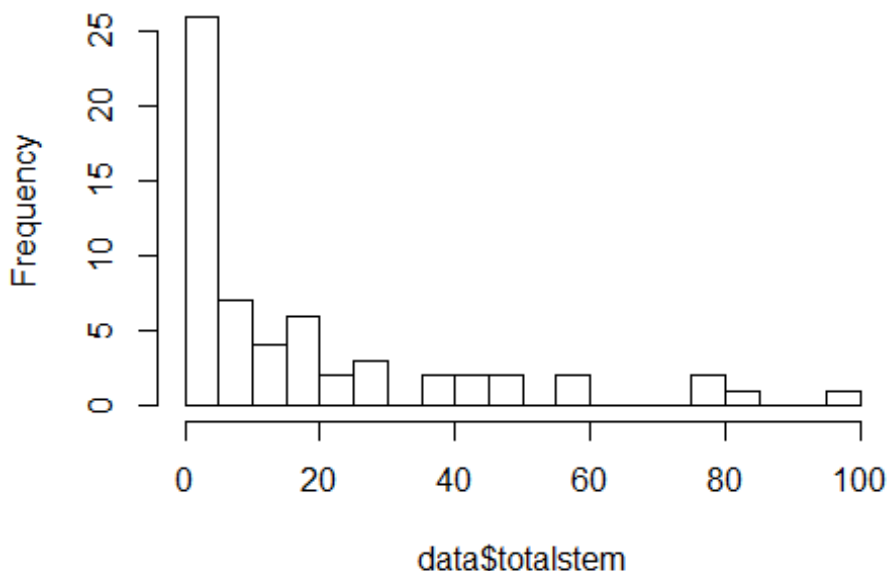
```
totalstem.model <- glmer(totalstem ~ focalspecies + (1|site), family =
"poisson", data = data, na.action=na.omit)
summary(totalstem.model)

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: poisson (log)
Formula: totalstem ~ focalspecies + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
1089.2 1099.7 -539.6 1079.2 55
##
Scaled residuals:
Min 1Q Median 3Q Max
-5.126 -2.607 -1.200 1.779 15.783
##
Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.5735 0.7573
```

```
Number of obs: 60, groups: site, 5
##
Fixed effects:
##
Estimate Std. Error z value Pr(>|z|)
(Intercept) 2.91696 0.34379 8.485 < 2e-16 ***
focalspeciesdamesrocket 0.22427 0.07121 3.149 0.00164 **
focalspeciesgarlicmustard -0.68191 0.09161 -7.443 9.81e-14 ***
focalspeciesgoldenrod -1.08180 0.10552 -10.253 < 2e-16 ***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) fclspcsd fclspcsgr
fclspcsdmsr -0.115
fclspcsgrlc -0.089 0.432
fclspcsgldn -0.078 0.375 0.292
hist(data$totalstem, breaks=20)
```

### Histogram of data\$totalstem



```
Anova(totalstem.model, type = "II")
Analysis of Deviance Table (Type II Wald chisquare tests)
##
Response: totalstem
Chisq Df Pr(>Chisq)
focalspecies 225.23 3 < 2.2e-16 ***
```

```

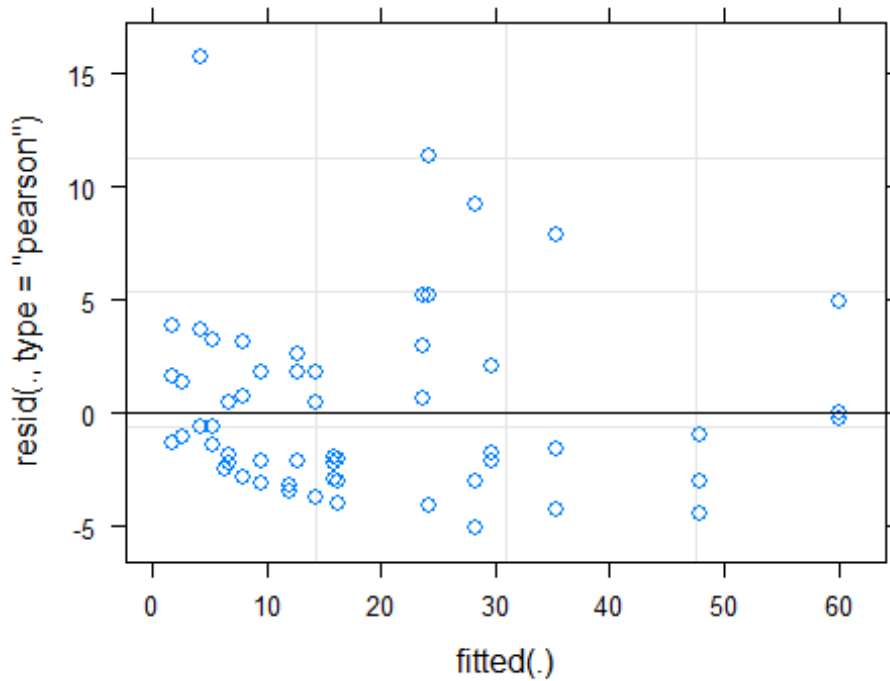
Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

summary(glht(totalstem.model, mcp(focalspecies="Tukey")))

##
Simultaneous Tests for General Linear Hypotheses
##
Multiple Comparisons of Means: Tukey Contrasts
##
##
Fit: glmer(formula = totalstem ~ focalspecies + (1 | site), data = d
ata,
family = "poisson", na.action = na.omit)
##
Linear Hypotheses:
##
Estimate Std. Error z value Pr(>|z|
)
damesrocket - control == 0 0.22427 0.07121 3.149 0.0091
1 **
garlicmustard - control == 0 -0.68191 0.09161 -7.443 < 0.00
1 ***
goldenrod - control == 0 -1.08180 0.10552 -10.253 < 0.00
1 ***
garlicmustard - damesrocket == 0 -0.90618 0.08847 -10.243 < 0.00
1 ***
goldenrod - damesrocket == 0 -1.30608 0.10280 -12.705 < 0.00
1 ***
goldenrod - garlicmustard == 0 -0.39990 0.11785 -3.393 0.0037
4 **

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
(Adjusted p values reported -- single-step method)

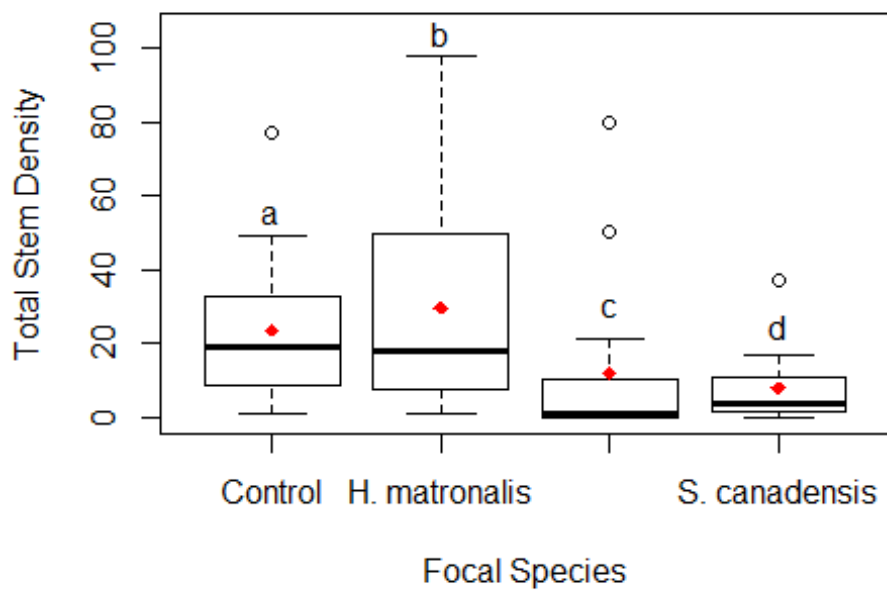
#Checking Assumptions
plot(totalstem.model)
```



```

#Total Stem Density Plot
plot(data$totalstem~data$focalspecies, ylim=c(0,105), xlab="Focal Species",
ylab = "Total Stem Density", names=c("Control", "H. matronalis", "
A. petiolata", "S. canadensis"))
text(1,55, "a")
text(2,104, "b")
text(3,30,"c")
text(4,25, "d")
totalstem_means = tapply(data$totalstem, data$focalspecies, mean, na.rm
=TRUE)
points(totalstem_means, col="red", pch=18)

```



#### Conclusions from Total Stem Density Analysis

- All focal species have sig lower stem density than the control pot
- Garlic mustard pots had sig lower stem density than all other treatments
- Goldenrod pots had sig lower stem density than dame's rocket pots

## Appendix 3 - Germination Experiment Analysis

Maria O'Sullivan

July 23, 2018

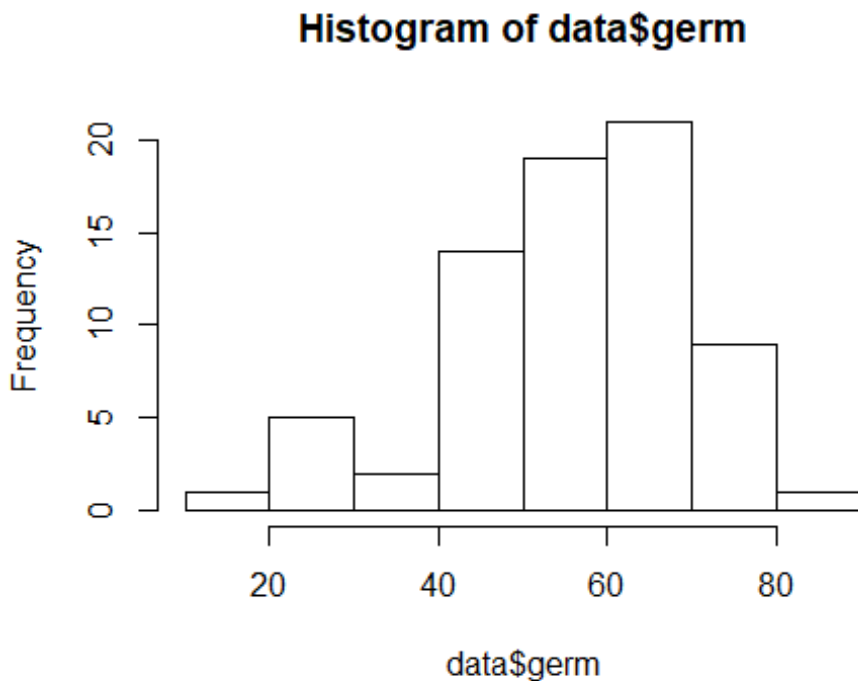
Note: 3 petri dishes were not counted for the analysis due to high fungal activity 2 control and 1 DR (FC1, FC2, HDR1)

R Version 3.5.0

RStudio Version 1.1.423

RMarkdown Version 1.10 lme4 package Version 1.1.15

```
#Testing Normality
hist(data$germ)
```



```
shapiro.test(data$germ)

Shapiro-Wilk normality test

data: data$germ
W = 0.95183, p-value = 0.007819

#Not normally distributed

#Generalised linear mixed effects model
```

```

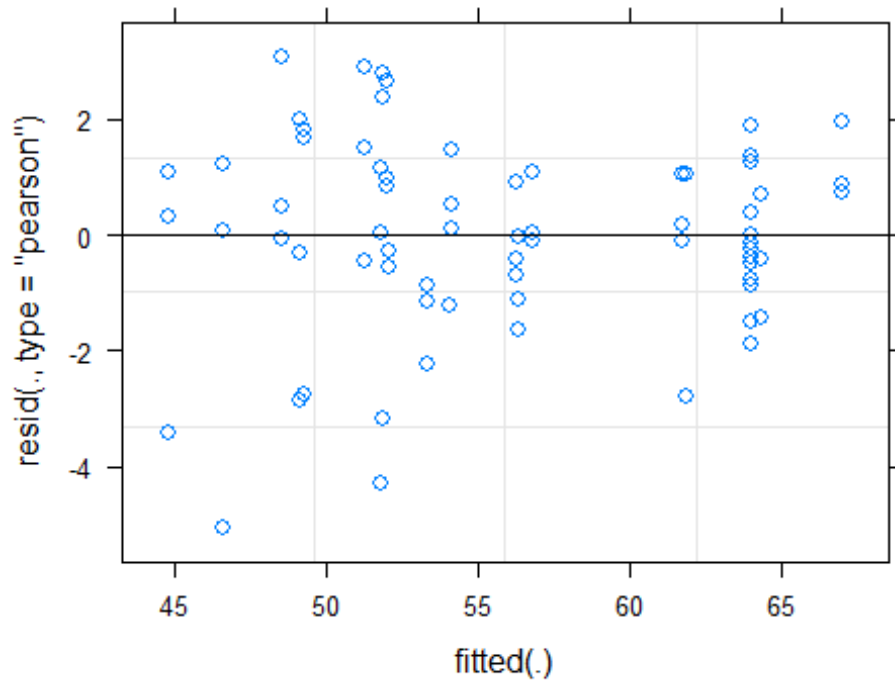
glmm_germ <- glmer(germ ~ species + (1|site), family="poisson", data=da
ta)
summary(glmm_germ); anova(glmm_germ)

Generalized linear mixed model fit by maximum likelihood (Laplace
Approximation) [glmerMod]
Family: poisson (log)
Formula: germ ~ species + (1 | site)
Data: data
##
AIC BIC logLik deviance df.resid
661.7 675.4 -324.8 649.7 66
##
Scaled residuals:
Min 1Q Median 3Q Max
-5.0689 -0.7102 0.0134 1.0775 3.0880
##
Random effects:
Groups Name Variance Std.Dev.
site (Intercept) 0.01075 0.1037
Number of obs: 72, groups: site, 7
##
Fixed effects:
Estimate Std. Error z value Pr(>|z|)
(Intercept) 4.00006 0.05822 68.704 <2e-16 ***
speciesdamesrocket -0.03923 0.05349 -0.734 0.463
speciesgarlicmustard -0.04223 0.05515 -0.766 0.444
speciesgoldenrod 0.03978 0.05134 0.775 0.438
speciessoilcontrol 0.15834 0.12322 1.285 0.199

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
Correlation of Fixed Effects:
(Intr) spcsdm spcsgr spcsgl
spcsdmsrckt -0.468
spcsgrlcmst -0.515 0.492
spcsgldnrd -0.486 0.530 0.517
spcsslcntrl -0.473 0.221 0.244 0.230
##
Analysis of Variance Table
Df Sum Sq Mean Sq F value
species 4 5.5242 1.3811 1.381

#Testing assumptions
plot(glmm_germ)

```



*#Post hoc not needed - no sig effect of treatments*

