QUANTIFYING INTERSPECIFIC COMPETITION EFFECTS OF HERB ROBERT (*GERANIUM ROBERTIANUM*) ON THREE NATIVE WESTERN WASHINGTON FORBS

by

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ABSTRACT

Quantifying interspecific competition effects of Herb Robert (*Geranium robertianum*) on three native western Washington forbs

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Invasive species pose a threat to ecosystems and biodiversity around the world. While many hypotheses have been offered to explain why some species successfully invade, they tend to be broad and inadequate at explaining invasiveness for any one species. Reductionist studies can reveal patterns that are species- and habitat-specific that improve our understanding of invasiveness at a local level. *Geranium robertianum* L. is an annual/biennial Eurasian herb that has invaded western Washington and continues to spread across the Pacific Northwest coast. Its ability to invade intact forest understory plant communities makes this invasive species somewhat distinctive among invasive plants and concerning for native biodiversity. In order to determine the interspecific competition effect of *G. robertianum*, a greenhouse study was conducted between *G. robertianum* and three native forbs: *Geum macrophyllum*, *Tellima grandiflora*, and *Dicentra formosa*, all herbaceous perennials. Each native species was grown with and without *G. robertianum*. The plants grown with *G. robertianum* had two separate soil treatments in addition to one group without soil treatments. Activated charcoal was added to assess the potential allelopathic interference of *G. robertianum* and native mineral soil was added to assess the effect the soil microbial community has on the competitive ability of the native plants. After 12 weeks, plants were harvested, dried, separated into above- and belowground components, and weighed. *G. macrophyllum* experienced the highest level of interspecific competition by *G. robertianum* but the effect was ameliorated by the native soil treatment. None of the treatments significantly affected the growth of *T. grandiflora*, which appears to be a robust competitor to *G. robertianum*. The charcoal treatment produced a generally negative effect for all species, indicating that allelopathic interference is not a primary invasive mechanism for *G. robertianum*. However, patterns in above- and belowground biomass for *T. grandiflora* and two of the *G. robertianum* groups showed some allelopathic interference but lacked statistical significance. This suggests that allelopathy is not the primary invasive strategy used by *G. robertianum* but potentially contributes a weak effect in conjunction with other above- and belowground invasive mechanisms.

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Introduction

The global redistribution of non-native plant species by humans is an ever-increasing dilemma that can have profound consequences for ecosystems and biodiversity (Kueffer, 2017). The costs can be great, both in terms of ecological and economic damage. Often cited as the second leading cause of biodiversity loss and species extinctions, invasive species ultimately become drivers that reshape biotic communities and alter ecosystems (Wilcove et al., 1998; Catford et al., 2009; Jose et al., 2013). There are a handful of invasive plant species that cause a disproportionate amount of harm and therefore generate ample amounts of research. This leaves a paucity of research on species that are perhaps not yet widespread in their new range or the magnitude of their impacts is unknown. Invasive plants species that disrupt, damage, or otherwise harm agriculture are often give the greatest amount of attention and funding, as the economic impacts tend to be more quantifiable and are not always synonymous to the ecological impacts (Pyšek & Richardson, 2006).

As invasion success is likely context- and species-dependent and due to a wide variety of factors and mechanisms, a robust and comprehensive theoretical framework is necessary to advance our understanding of what makes invasive species so successful (Parker et al., 1999; Catford et al., 2009). While there is likely no "holy grail" mechanism or equivalent hypothesis for successful invasion, small-scale studies can improve our understanding of which mechanism and factors contribute to success for any given species in an ecosystem-specific context (Davis et al., 2000; Catford et al., 2009). The aim of this study is just that – a reductionist examination of *Geranium robertianum* (herb Robert), a successful invader in western Washington. This plant is not problematic for agriculture and has not cost millions of dollars in attempts to eradicate. So why does it matter? It could be argued that its impacts to biodiversity justify exploring this question and it is with this lens that this thesis evaluates the effects of *G. robertianum* on the growth of native species found in forest understory plant communities. Pacific Northwest forested habitats are generally resistant to invasive plant species, but they are readily invaded by *G. robertianum,* where it is able to flourish and dominate the plant community.

There are several characteristics that are attributed to the success of *G. robertianum* as an invasive species: self-pollination, abundant seed production, not palatable to herbivores, and high ecological tolerance – able to grow in both full shade and full sun (Bertin, 2001; Barndt, 2008). Additionally, the role of allelopathy – the release of chemical compounds potentially harmful to other plants – is still under consideration as a primary mechanism for invasion success. The intent of this thesis is to address these mechanisms and gain a better understanding of whether allelopathy is indeed a primary mechanism utilized by *G. robertianum*. Specifically, the following research questions were posed: What are the effects of *G. robertianum* on the growth of native species? Is there evidence of allelopathic interference?

These questions were addressed using several different experimental treatments, specifically, activated charcoal and native mineral soil added to an artificial potting mix. Activated charcoal is efficient at absorbing biochemical compounds and is often used in experiments assessing allelopathy (Del Fabbro et al., 2014). The addition of activated charcoal to the potting mix as a treatment group assesses the potential allelopathic effect of *G. robertianum* on the growth of the native species. Similarly, the relationship between plants and the soil microbial community is integral to growth and development. The addition of native mineral soil to the potting mix as a treatment group assesses whether the native species were given a competitive advantage over the invasive species. The native species grown in the presence of *G.*

robertianum without the addition of either soil amendment serve as a reference to gauge the effect of interspecific competition by this successful invasive species.

Literature Review

Introduction

Throughout this literature review, I will synthesize the relevant academic literature and prevailing themes in invasion biology. The first section of this literature review will provide an overview of invasive species and the discipline of invasion biology. Specifically, this section will describe what an invasive species is and their impacts on ecosystems, as well as a brief overview of the history of invasion biology. The second section will provide a holistic framework for invasion hypotheses that attempts to capture all the factors and mechanisms that contribute to successful invasions. The invasion hypotheses that are most relevant to the success of *Geranium robertianum* as an invasive species will be discussed more in depth. Finally, the last section will provide an overview of *Geranium robertianum* – its distribution, biology, and ecology. The literature on *G. robertianum* is limited and this review is based on a handful of comprehensive sources. Research on the role of this species as an invader is limited as well, particularly in western North America where it continues to spread. The aim of my research is to contribute quantitative data to a relatively small body of knowledge on this invasive species.

Invasion Biology

Invasive Species

Most nonnative species are introduced by humans either intentionally or unintentionally (Simberloff, 2013; Jose et al., 2013). Intentional introductions tend to include species used for agriculture or horticulture; unintentional introductions ironically can result from the intentional ones – garden escapees, contaminated soil, shipping. However, not all nonnative species are considered invasive – under optimum conditions some may develop traits that enable them to outcompete and eventually dominate recipient communities, thus becoming an invasive species

(Jose et al., 2013). The definition of *invasive* also differs depending on who is referring to the species in question. Policymakers generally consider a species invasive if it causes negative impacts to the environment, human health, or the economy. In his extensive treatise on invasive species, Simberloff (2013) argues this is in stark contrast to biologists who consider a species' evolutionary history in determining invasiveness: where did it evolve and was its transportation aided by humans? Once established, an invasive species can be conveyed and spread by human activities and disturbances, especially as our modern globalized economy facilitates the large-scale redistribution of plant species (Kueffer, 2017).

There are a few invasive species that have caused a disproportionate amount of ecological alteration and extinctions: feral hogs, rats, and predatory snakes are common examples, especially in isolated island ecosystems (Gurevitch & Padilla, 2004). Some researchers argue that most invasive plant species are likely to alter plant community composition or cause displacement, rather than extinctions (Sax & Gaines, 2003; Gurevitch & Padilla, 2004). Several widely known and studied invasive plant species impact North American ecosystems, costing millions of dollars annually in control efforts: English ivy (*Hedera helix*), kudzu (*Pueraria spp.*), garlic mustard (*Alliaria petiolata*), cheatgrass (*Bromus tectorum*), and knapweeds (*Centaurea spp.*). Knapweed alone occupies more than 7 million acres, negatively impacting grazing for livestock and wildlife (Callaway & Ridenour, 2004). The impacts to recipient ecosystems by these invasive plant species, and others, include alterations to nutrient cycling, natural succession patterns, soil chemistry, water availability, herbivory patterns, and natural fire regimes (Jose et al., 2013; Simberloff, 2013).

Despite Charles Eton's 1958 seminal work on invasive species, little attention was paid to invasives species by the scientific community in the mid-1900s. However, by the 1980s, with increasing awareness of problems associated with invasive species, invasion biology emerged as an independent discipline (Simberloff, 2013). Researchers continually build and modify frameworks for understanding the many aspects of invasives: what allows invasives to be so successful? What are their impacts? How do we manage them? Researchers continue to discuss the impacts of invasive species, often without a solid or specific definition of "impact" (Parker et al., 1999). Authors tend to use the term equally for different plants with varying degrees of effects on recipient ecosystems. Parker et al. (1999) suggests quantifying impact by looking at range, abundance, and the per-biomass effect of the invader. However, the issue of quantifying the impacts of non-natives/invasives is further confounded by the presence of multiple invasives, as well as other environmental stressors, such as anthropogenic disturbances and climate change. Additionally, the interactions of multiple invasives – invasional meltdown – may impact ecosystems on a larger scale than what would be expected had their impacts been measured individually (Simberloff & Von Holle, 1999; Von Holle, 2011). Biotic and abiotic factors – pollinators, seed-dispersers, herbivory pressures, soil types, and fire regimes – in conjunction with anthropogenic stressors can influence the degree of impact (Catford et al., 2009).

As Parker et al. (1999) point out in their attempt at formalizing a framework for invasive species, the problem with too many generalizations around impacts is more than an academic problem: it is ultimately a problem for management efforts. Distinguishing the effects of invasives as minor or major is necessary in order to prioritize management efforts. A common metric used by managers for assessing the impact of invasives species is to assess their economic cost in terms of damage or eradication efforts (Parker et al., 1999). This method can be limited and myopic in nature, focusing primarily on human needs, favoring short-term outcomes over long-term consequences and ignoring ecosystem function as a factor. Quantifying the value of ecosystem function remains problematic, as it also requires placing a human-based measure of value on a non-human system. Barney et al. (2013) argue that the focus should come from a conservation and ecosystem protection perspective. While the economic consequences of invasives should not be ignored or underrated, neither should the ecological consequences.

Invasion Hypotheses

Dozens of hypotheses have been presented to explain, at least partially, why some species are so successful in recipient ecosystems. Many of these hypotheses focus on either the attributes of the invader or attributes of the recipient community, while struggling to incorporate the two (Pyšek & Richardson, 2006; Crystal-Ornelas & Lockwood, 2020). The simplest and broadest of them hypothesizes that successful invaders possess attributes that give them an edge over native communities, that they are overall competitively superior (Lewis & Jerde, 2012). This broad hypothesis performs more as a definition of an invasive species versus a theory to describe *why.* Because successful invasion is so context-dependent, the vast majority of invasive species are likely successful due to different combinations of biotic and abiotic factors and thus, are not easily quantifiable under one broad invasion hypothesis (Catford et al., 2009). Indeed, it likely requires multiple small-scale hypotheses to begin to describe a successful invasion versus the "holy grail" approach to explaining and predicting invasiveness which has resulted in the dozens of disparate hypotheses (Catford et al., 2009; Crystal-Ornelas & Lockwood, 2020).

Catford et al. (2009) describe a broad, overarching framework for successful invasion that is structured around plant reproduction, or propagule pressure, and abiotic and biotic characteristics of the recipient plant community and invading species (PAB). This framework cleverly unifies all of the disparate hypotheses into a top-down approach that enables reductionist, or small-scale, experiments to hone in on the number of potential mechanisms contributing to successful invasion (Pyšek & Richardson, 2006; Catford et al., 2009). The three components represent the most fundamental characteristics necessary for the success of invaders in novel ecosystems: (P) successful and sufficient propagation across temporal and spatial scales; (A) hospitable abiotic environmental characteristics of an invaded site; and (B) species-specific and ecology-evolutionary interactions across biotic communities (Figure 1). An important component of this framework is the inclusion of anthropogenic interference as a key driver of invasions.

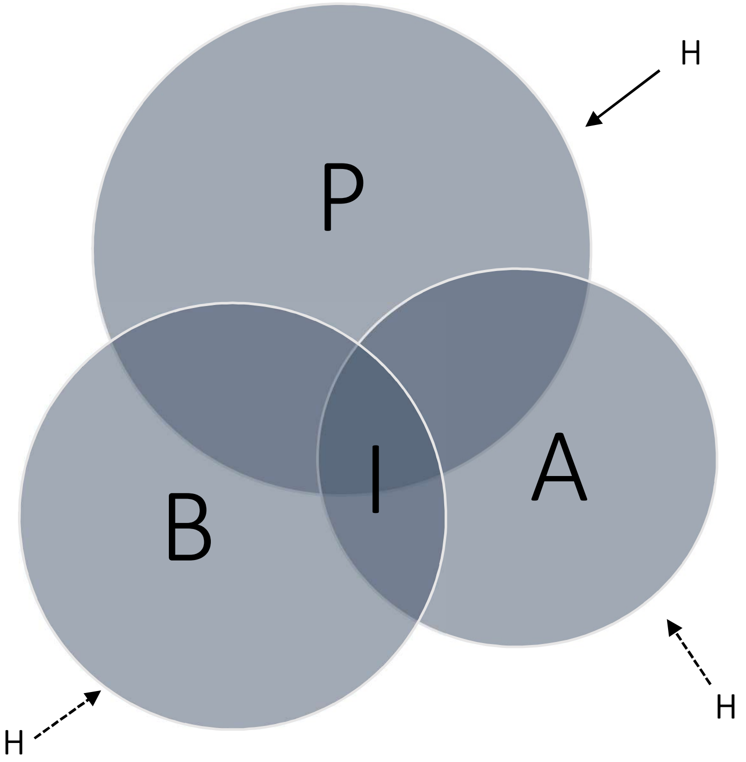

Figure 1. Schematic diagram illustrating the relationship between propagule pressure (P), abiotic characteristics (A), and biotic characteristics (B). Degree of interference by humans (H), solid line greater interference than dashed line. Larger circles represent greater extent of influence. This interaction drives invasion (I). Adapted from Catford et al., 2009.

Of the dozens of invasion hypotheses situated in the PAB framework, 12 standout as plausible mechanisms contributing to the success of *Geranium robertianum* as an invasive species. Of these theories, many of them focus primarily on biotic factors of successful invasion.

Due to the complexity of biological and ecological systems, many of these theories possess similarities and overlap. The 6 most relevant to this thesis are described below (Table 1).

Table 1. Primary invasion hypotheses relevant to the success of *Geranium robertianum* as an invasive species in western Washington.

Hypothesis	**Human Interference**	**Propagule Pressure**	**Abiotic Factors**	**Biotic Factors**
Propagule pressure*	++	++		+
Sampling	+	+		++
Ideal weed*	+	+		++
Enemy Release				++
Enemy of my enemy				++
Biotic indirect effects*			+	++
Invasional meltdown*			+	++
Adaptation*			++	+
Novel weapons*				++
Habitat filtering			++	+
Disturbance	+		++	+
Naturalization	+		++	++

Symbology: ++ major driver of invasion; + secondary driver influencing invasion; * theories discussed in more depth in this thesis. Adapted from Catford et al. 2009.

Propagule pressure, specifically the number of propagules reaching a new location, serves as a key factor in the success of invasive species. Propagule pressure acts like a filter for invasion – without sufficient propagation at sufficient frequency, establishment cannot occur. So important to successful invasion, many invasion hypotheses automatically consider it to be a requirement of invasion versus simply a driver (Catford et al., 2009). In order to serve as a driver, the plant must already have a large amount of its resources allocated to reproductive output (Leishman & Harris, 2011). Reproductive output can effectively be increased when a plant is released into a novel ecosystem and freed from natural enemies. This concept overlaps with the Enemy Release theory, which refers to the reallocation of resources that were previously used for defense to increased growth and reproduction (Catford et al, 2009; Leishman & Harris, 2011). A study by Mason et al. (2008) found that, on average, invasive species produced more seeds than native species – upwards of seven times more seeds per individual per year. While the authors did not determine a mechanism responsible for the difference in seed production, it does point to the possible reallocation of resources as proposed by the Enemy Release theory. An additional concept related to propagule pressure pertains to the role of seed banks for successful, long-term invasion. Seed banks enable plants to spread the risk of germination and establishment over time periods greater than a single growing season, assisting in the stabilization of population dynamics and aiding in recovery after disturbances. Many successful North American invaders, including purple loosestrife (*Lythrum salicaria*) and garlic mustard (*Alliaria petiolata*), have long-lived seeds that can dominate soil seed banks (Leishman & Harris, 2011; Jose & Holzmueller, 2013). This serves both as a mechanism to outcompete native species, but also makes long-term control of invasives incredibly challenging.

Many of the characteristics that contribute to successful invasion overlap under the Ideal Weed hypothesis. This early hypothesis focuses on the traits of plants only and has limited success in predicting invasiveness (Pyšek & Richardson, 2006). The plant traits associated with invasiveness – high and early seed production, high phenotypic plasticity, rapid growth, and small seed size – are all biotic drivers of successful invasion. However, this hypothesis ignores the abiotic factors of successful invasion, making Ideal Weed an incomplete, albeit important, hypothesis. Similarly focusing on biotic factors, the Biotic Indirect Effects hypothesis looks specifically at interactions that cascade through plant communities – the effects one species has on a second, which in turn effects a third (Callaway et al., 2004; Catford et al., 2009). These interactions are more holistic than the Ideal Weed hypothesis, expanding the biotic factors beyond a single species. If expanded to include related interactions with soil biota, these two hypotheses cover a wide breadth of biotic factors that contribute to successful invasion.

Interactions between the invader and recipient community can influence overall invasive success, where strong competitive interactions, such as beneficial mutualistic relationships and novel pathogens can reduce the likelihood of successful invasion (Lewis & Jerde, 2012). Where strong competitive interactions are lacking, an overall reduction in species richness can be a consequence of invasion. This in turn increases the likelihood of further invasions. This mutualistic and facilitative interaction between successive invaders is the basis for the Invasional Meltdown hypothesis (Von Holle, 2011). These interactions can occur over a range of trophic levels with the potential to lead to synergistic impacts on the recipient community. The distinction between this hypothesis and the similar Biotic indirect effects hypothesis is that the impacts of invasive species can be greater than the sum of their individual impacts (Von Holle, 2011; Braga et al., 2018). A somewhat recent and alarming example of invasional meltdown is the introduction and subsequent spread of the spotted lanternfly (*Lycorma delicatula*), a planthopper native to northern China, whose preferred host is the highly invasive *Ailanthus altissima* (‘tree of heaven’). First introduced to the United States in the mid-1800s as an ornamental specimen, *A. altissima* has since spread to nearly all 50 US states and is considered one of the worst invasive plant species in North America (USDA NRCS Plant Database, 2021). The establishment of this invasive plant species is facilitating the spread of *L. delicatula*, which was first detected in Pennsylvania in 2014 and has the potential to cause substantial damage to agriculture, particularly grapes (*Vitis spp*.) and apples (*Malus spp*.) (Dara et al, 2015; WA NWCB, 2021).

It was previously proposed by Darwin (1859) that introduced plant species would be less likely to establish themselves in places with pre-existing congeneric native species due to similar resource requirements (Duncan & Williams, 2002). However, a study focusing on invasive species in New Zealand found the opposite to be true – the naturalization rate was much higher among introduced genera with pre-existing native species. Modeling based on plant families with the highest number of non-native species revealed that genus was a highly significant predictor of invasive probability for an introduced species (Duncan & Williams, 2002). The Adaptation hypothesis is based on this concept and suggests that invasive species may be successful in a new range given shared competitive abilities and traits to native congeneric species (Catford et al., 2009). While this study is based on concepts that can be related to island endemism and thus places at especially high risk of invasion, it makes an important case for considering congeneric species as pre-adapted invaders.

Del Fabbro et al. (2013) and others argue that allelopathy is one of several mechanisms that contribute to the success of invasive species (Callaway & Ridenour, 2004; Thorpe et al., 2009; Callaway, 2011). Allelopathic chemical compounds are secondary metabolites and, therefore, not required by the plant for metabolism – growth, development, or reproduction (Inderjit, 2011). First described in 1937, allelopathy is not a new concept. However, it was Callaway and Ridenour (2004) who first made the connection between allelopathy and invasive species, developing what they termed the 'Novel Weapons Hypothesis' in an attempt to explain why some invasive species are so successful in recipient ecosystems. It is theorized that allelopathic compound-producing plants can quickly dominate plant communities that possess no adaptation or tolerance to the compounds, effectively suppressing the germination and growth of native plants (Callaway & Ridenour, 2004; Del Fabbro et al., 2013).

Overall, the evidence for this hypothesis is largely based on greenhouse and laboratory studies. How applicable or relevant greenhouse allelopathic studies are to real-world scenarios has been debated. An allelopathic study on knotweed (*Fallopia* x *bohemica*) found that allelopathic potential and impact was directly related to the physical and chemical properties of the soil; essentially the discrepancy was between artificial soil mixes that are often used in lab and greenhouse experiments and native soil (Parepa & Bossdorf, 2016). The higher pore space and permeability of artificial soil mixes may contribute to higher levels of allelopathic effect compared to native soils. Furthermore, unless native soils that are used for lab or greenhouse studies is sterilized, the presence of soil biota could contribute to erroneous study results.

However, field evidence of allelopathy does exist. Thorpe et al. (2009) produced strong evidence for the hypothesis in their field study of *Centaurea stoebe* (spotted knapweed), an aggressive Eurasian invader of North American rangelands. Allelopathic effects were assessed in experimental *in situ* plots in both native and invaded ranges. Interestingly, they demonstrated that secondary metabolites produced by *C. stoebe* reduced growth of native plant species in Montana, but had no effect on plants in its native range in Romania (Thorpe et al., 2009). However, they do point out that soil chemistry likely plays an important role in the retention and activity of the secondary metabolites produced by *C. stoebe*, which requires further study. In a laboratory study, Inderjit et al. (2009) demonstrated similar results using *Nicotiana attenuata* (wild tobacco) seedlings. A portion of the *N. attenuata* seedlings were genetically modified to be 'silent' – unable to synthesize or release specific secondary metabolites. It was demonstrated in this experiment that the release of secondary metabolites in unsilenced seedlings did have an influence on neighboring seedlings, whereas the silenced seedlings did not.

The Novel Weapons hypothesis is not without criticism and skepticism. It is important to point out that allelopathy is a conditional and species-specific effect and not a mechanism used by all successful invasive plant species (Inderjit et al., 2009; Thorpe et al., 2009). Additionally, soil chemistry is an increasingly an important component for this mechanism that is still under-studied. How secondary metabolites respond to specific soil chemistries and regional climate is still being debated. Researchers have pointed out that one of the secondary metabolites of interest, (±)-catechin, degrades more quickly in wet soils compared to dry soils, putting into doubt its role as an allelopathic compound that explains the success of *Centaurea stoebe* (Blair et al., 2006). However, other research showed the (±)-catechin may be benefiting *C. stoebe* in terms of competition for nutrients, specifically phosphorous: *C. stoebe* contained twice the phosphorous levels in comparison to neighboring native species (Thorpe et al., 2006). This difference was six times greater in areas with very low soil phosphorus availability. A greenhouse study on the same species demonstrated that it could also obtain up to 15% of its carbon from a native grass (*Festuca idahoensis*) through mycorrhizal networks (Carey et al., 2004).

Despite somewhat conflicting conclusions in these studies, they demonstrate the complexity of the direct and indirect effects plants have on soil chemistry and microbial communities (Weidenhamer & Callaway, 2010). It is estimated that mycorrhizae are responsible for up to 75% of all phosphorus taken up annually by terrestrial plants worldwide (van der Heijden et al., 2008). Because of the reliance plants have on mycorrhizal fungi symbiosis to collect and deliver phosphorous and other nutrients, disruption to these networks by invasive plants and allelopathic compounds can significantly transform ecosystem structure and function (Reinhart & Callaway, 2006; Hagan & Shibu, 2013). Over time these changes can create positive feedback loops that impart greater benefit to the invader. *Alliaria petiolata* (Garlic mustard), an aggressive invader of eastern North American forests, was found to reduce mycorrhizal fungal densities and impede the growth of native tree seedlings despite not forming mycorrhizal associations itself (Hagan & Shibu, 2013). Interestingly, a study on forest soils invaded by *A. petiolata* found that nutrient levels were consistently and significantly higher than non-invaded soils. The difference appears not to be due to allelopathic compounds, but instead to increased rates of decomposition of native trees, fundamentally altering nutrient cycles (Rodgers et al., 2008).

Overall, this presents a complicated duality where invasive species can alter soil microbial communities both directly and indirectly, becoming drivers of coevolutionary trajectories that permanently alter plant community composition (Callaway et al., 2004). Given the complex interactions between abiotic and biotic factors, along with anthropogenic interference, it is very likely that no single hypothesis could explain the invasion process. An integrated approach to test multiple hypotheses and factors to identify the primary causes of invasion should be considered on a species by species basis when considering management decisions (Catford et al., 2009; Batish et al., 2013).

***Geranium robertianum*, L. (Herb Robert)**

Distribution

Occurring widely in its native Eurasia, *Geranium robertianum* L. (common name Herb Robert), can be found in northern Africa northward to Great Britain and Scandinavia and eastward to Russia and central Asia (Tofts, 2004). Introduced populations are found in Japan, Chile, New Zealand, Australia, South Africa, and North America. Whether or not it is native to North America is unclear. Washington and Oregon states list it as a Class B noxious weed, whereas East Coast and Midwest populations are considered naturalized, with Indiana, Maryland, and Rhode Island listing *G. robertianum* as Threatened, Endangered, and of Special Concern, respectively (USDA NRCS; WA NWCB).

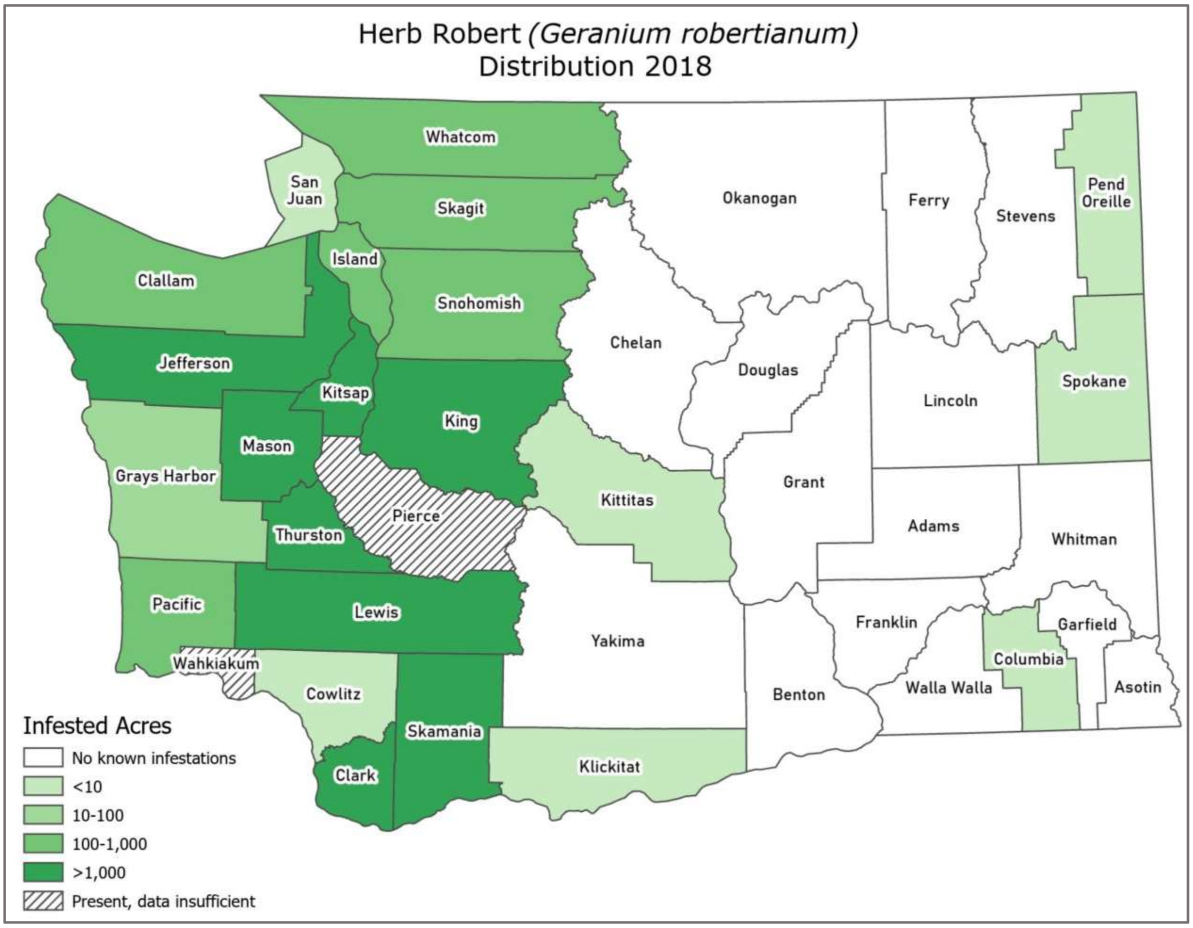

Figure 2. Distribution of *Geranium robertianum* (Herb Robert) in Washington State. 2018 data from Washington State Noxious Weed Control Board, map by author.

First recorded in Washington state in 1911 in Klickitat county, it was listed as an invasive species in 1998 and is now present in at least 23 counties (Figure 2; WA NWCB). Of the 17 species from the genus *Geranium* found in Washington state, 12 are introduced, including *G. robertianum*, leaving only 5 species considered native to the region (Hitchcock & Cronquist, 2018; Giblin & Legler, 2021).

Description

Plants range from 10 – 50 cm in size, stems branching from the base, further pairs of branches forking from the previous base. Forking of branches continues, creating a dense branching system that eventually ends at a dormant bud and an inflorescence develops (Tofts, 2004). Leaves are bright green with red margins, rarely entirely red, 3.5 – 10 cm wide, ternately to palmately divided, with somewhat hairy pinnately-lobed leaflets with rounded margins (Tofts 2004; Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). Leaves are fragile and produce a pungent odor when crushed, described by Tofts (2004) as a "strong, disagreeable smell." Flowers are pale to bright pink, sometimes with white stripes, and generally paired in axillary cymules that arise from the axils of the uppermost leaves. Flowers are radially symmetric, perfect (containing male and female parts) and complete with five sepals, five petals, ten stamens in two whorls, and five carpels; seeds are brown, smooth, and 1-2 mm long with a sticky string attached (Tofts, 2004; Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). The majority of its biomass is distributed within the stems and leaves, with lesser amounts in its shallow, fibrous root system (Boerner, 1990).

Figure 3. Photograph of *Geranium robertianum*. Note the deeply dissected leaves and hairy stems and flowers. Photo credit: King County Noxious Weed Control Program.

Habitat

Geranium robertianum possesses a wide ecological amplitude, occurring in biomes that range from Mediterranean to boreal and continental conditions (Tofts, 2004). While Herb Robert is tolerant of varying amounts of light, found in environments that range from full light to 1/370th ambient light, it most often occurs in moist, shaded woodland habitats (Tofts, 2004; Bertin, 2001). Light conditions were found to impact biomass production and flowering, where moderate ambient light conditions produced more biomass and had a higher percentage of blooms than plants grown in full light (Tofts, 2004). *G. robertianum* is equally tolerant of a range of soil conditions, even growing as an epiphyte on trees and cracks in rocks and walls. North American habitat has been variably described as anything from moist, rocky, or deciduous woods to ravines, gravelly shores, and clearings along roads and trails (Gleason & Cronquist, 1991; Hitchcock & Cronquist, 2018). In the British Isles, most *G. robertianum* populations are encountered on moderately nitrogen-rich soils (Tofts, 2001). Poorer soils tend to produce plants with red leaves, which indicates phosphate or nitrogen deficiency. Soils in Pacific Northwest forests tend to be nitrogen-poor, suggesting that *G. robertianum* is able to outcompete native species that are adapted to low-nitrogen conditions.

Research on populations in Massachusetts by Bertin (2001) found a correlation between climatic conditions and second-year survivorship of biennial individuals. In Maryland and Rhode Island, where populations are less secure, hot, dry summers and exceptionally cold winters without snow cover reduced overall winter survival. In comparison, West Coast climatic conditions, where the summers are cooler and the winters milder, may lead to greater second-year survivorship. This difference is supported by similar observations in Britain when compared to seedling survival in Poland, which was significantly lower in the colder climate (Tofts, 2001).

Phenology and Reproduction

Within its native habitat of Eurasia, and particularly in Great Britain, *Geranium robertianum* exhibits striking differences between populations and subspecies (Tofts, 2004; Baker, 1956). The subspecies *robertianum*, present in Washington state, generally presents as a spring and fall annual, occasionally as a biennial. Plants that germinate in fall overwinter as dense rosettes of leaves, elongating (bolting) in early spring and fruiting in early to mid-summer (WA NWCB, 1997). Morphological differences between populations continue with second-year growth, including growth patterns – erect or prostrate – and degree of hairiness (Tofts, 2004). Similar to the variations in morphology, *G. robertianum* also exhibits variations in recruitment patterns. Researchers in Poland identified three seasonal peaks of seedling emergence (late spring, mid-summer, and late summer to early fall), which was similar to the recruitment patterns in Massachusetts populations (Falińska & Pirožnikow, 1983; Bertin, 2001). They noted in both Polish and Northeastern U.S. populations that late season seedlings had the poorest rates of overwinter survival with only 5-6% and 0-23% seedlings surviving into spring, respectively. Observations of overwinter survival in Great Britain contrasted with the findings in Poland and Massachusetts: dense aggregates of plants with retained cotyledons persisted through winter (Tofts, 2001). Again, this suggests that milder climates result in better overwinter survival and more robust population sizes.

Populations in Great Britain flower from April to September and set fruit from June to November; populations in the Northeastern U.S. have a similar phenology (Bertin, 2001; Tofts, 2004). Flowering can occur throughout the year in sufficiently mild climates. Pollinator data for populations in Great Britain include visits from flies, butterflies, and bees (Tofts, 2004). In Washington state populations pollination is apparently unspecialized (WA NWCB, 1997). However, most authors consider the plant to be primarily self-pollinated due to its relatively short flowering period of approximately 48 hours and ability to set seed in greenhouse conditions lacking pollinators (Bertin, 2001; Tofts, 2004). After approximately three weeks of ripening, five seeds develop within the capsule, which are then ballistically ejected up to seven yards away from the plant in response to the drying of the capsule (WA NWCB, 1997; Bertin, 2001; Tofts, 2004). Minute threads attached to the seeds further aid dispersal via wind, insects, and herbivores.

Seed set quantity in natural conditions varies by location, with plants in Poland producing 100 – 200 seeds per plant and plants in the British Isles producing 10 – 310 seeds (Tofts, 2004). Research by the Washington State Noxious Weed Control Board found seed production to be relatively low under full canopy with low light conditions, however under 50-60% canopy cover with higher light levels, plants produced approximately 3100 seeds/m^2 (WA NWCB, 1997). Some disagreement exists on germination requirements. Scarification requirements have been reported by some authors, whereas others have reported the seeds germinating freely soon after dispersal (Tofts, 2004, WA NWCB, 1997). Germination rates varied by both age of seed and by seasonal cohort. In general, peak germination rates occurred with 1-year old seeds from the spring cohort, with summer and fall cohort seeds having much lower germination rates (Tofts, 2004; Falińska & Pirožnikow, 1983). The study by Bertin (2001) generally agreed with the 1983 study, reporting that most seeds germinated in the first and second years and none by the seventh year (Bertin, 2001). Based on these two studies, it would appear as though the seed bank for *G. robertianum* is not particularly long lived in the soil.

Physiology

Geranium robertianum is reported to generally be infected with arbuscular mycorrhiza fungi. Depending on the species of mycorrhiza and soil nutrient levels, mycorrhizal associations in *G. robertianum* may result in increased biomass or nutrient-uptake compared to uninfected plants (Boerner, 1990; Tofts, 2004). Inoculation with the arbuscular mycorrhiza *Paraglomus occultum* (syn. *Glomus occultum*) was found to increase phosphorous uptake efficiency even in low phosphorous conditions (Boerner, 1990).

G. robertianum is reported as containing no alkaloids. However, it possesses three types of secretory trichomes: Types I and II secrete terpenoids and phenols, and Type III accumulate anthocyanins in the apical cells – cells capable of dividing and forming new cells – and secrete flavonoids (Tofts, 2004). Studies on flavonoids have identified several types excreted by the plant, but contain no discussion as to their function (Ivancheva & Petrova, 2000). Barndt (2008) examined the allelopathic potential of *G. robertianum* through leaf leachate and decomposing leaves. Difficulty with germination of test species proved problematic for the study and results were not clear, leaving the allelopathic potential of *G. robertianum* still in question.

Materials & Methods

Species Selection

Intact forest understory, specifically the *Alnus rubra/Polystichum munitum* (Red alder/Sword fern) plant association, influenced species selection, as it is both a common plant association throughout the Puget Trough ecoregion and the invasion by *Geranium robertianum* is of increasing concern there (Figure 4; Chappell, 2004).

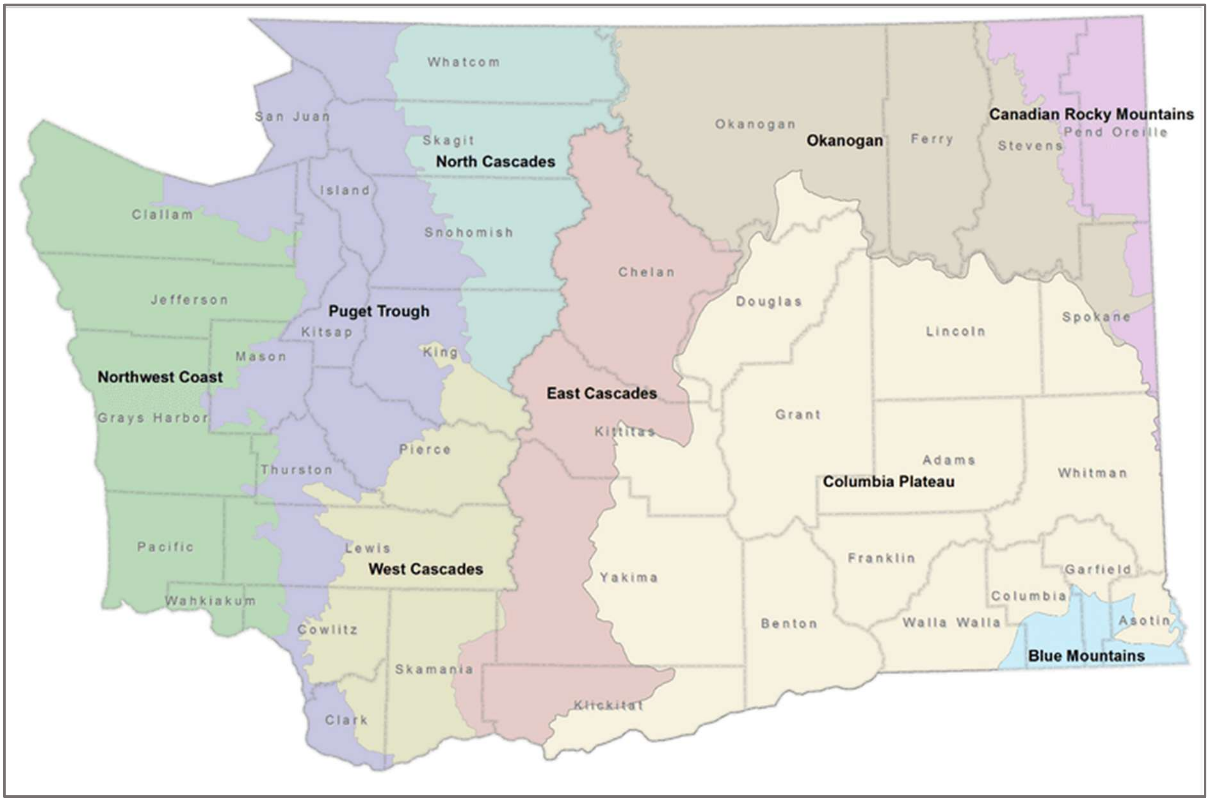

Figure 4. Ecoregions of Washington State. Plant association chosen for this study based on the *Alnus rubra/Polystichum munitum* plant association commonly found in the Puget Trough ecoregion (purple). Map by Washington State Dept. of Natural Resources.

Plugs were chosen over seed due to time constraints involved in stratifying seed and the wide use of plant plugs over seed in restoration projects. Three different native species of herbaceous angiosperms, all from different plant families, were chosen to represent different growth forms and phenologies: *Geum macrophyllum* Willd., Rosaceae; *Tellima grandiflora* (Pursh) Douglas ex Lindl., Saxifragaceae; and *Dicentra formosa* (Haw.) Walp, Papaveraceae (Figure 5). All three species were sourced from the Puget Sound region: 40 *G. macrophyllum* (10 cc plugs) from South Sound Native Plants, Thurston County; 40 *T. grandiflora* (8 cc plugs), seed sourced from the SE Olympic lowlands and grown at Washington Corrections Center for Women, Pierce County; and 35 *D. formosa* (4 inch square pots) of unknown plant stock from Plantas Nativas nursery, Whatcom County. *G. robertianum* plants were collected between January and February 2021 from three different locations in Thurston County. Plants varied in size, but all were established rosettes. *G. robertianum* plants were placed in trays of artificial soil mix for up to a week prior to replanting with the native species.

Geum macrophyllum Willd. is a widely distributed herbaceous perennial in the Rosaceae family found in western North America from Alaska to Baja California and east across Canada to the northeast Atlantic coast (Figure 5; Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). In Washington state it is found on both sides of the Cascade crest. Classified as a facultative wetland species, it is common in moist woodlands, frequently found along wet meadows and streambanks from sea level to subalpine zones (Hitchcock & Cronquist, 2018; Fertig, 2021; Giblin & Legler, 2021). Plants grow from short rhizomes with a fibrous root system and are tolerant of a variety of soil types, including moderately calcareous soils. Flowering occurs between April and August (Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). Cymose inflorescences of 3 – 16 solid yellow flowers have a strong UV signature and attract a range of pollinators, particularly small flies (USDA NRCS, 2021). Fruits are aggregated achenes with hooks which may aid in seed dispersal by animals. *G. macrophyllum* is not known to be toxic or allelopathic (USDA NRCS, 2021).

Tellima grandiflora (Pursh) Douglas ex Lindl. is a monotypic herbaceous perennial in the Saxifragaceae family primarily found west of the Cascade crest in Washington state, its range extending along the west coast of North America from Alaska to California and east to western Montana (Figure 5; Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). A common forb in *Alnus rubra*/*Polystichum munitum* forest associations in the Puget Trough, it grows in rich, organic soil from sea level to mid-elevations in moist woodlands, forest edges, and along streambanks (Chappell, 2004). Semi-evergreen during mild winters, plants grow from short rhizomes with fibrous roots, blooming from April to July. It produces long, spike-like racemose inflorescences of 10 – 35 greenish-white to sometimes pink flowers. The plant self-seeds readily, producing 100 – 150 seeds per plant (Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). *T. grandiflora* is noted as species useful for restoration by the Washington Native Plant Society, as it can create thick patches and outcompete invasive species in disturbed and shady areas (WNPS, 2021).

Figure 5. Photographs of native species used in experimental design, left to right: *Geum macrophyllum*, *Tellima grandiflora*, and *Dicentra formosa*. Photos by author.

Dicentra formosa (Haw.) Walp. is an herbaceous perennial in the Papaveraceae family found primarily in low to mid-elevation moist woodlands west of the Cascades, from British Columbia south to California (Figure 5; Hitchcock & Cronquist, 2018; Giblin & Legler, 2021). It grows from relatively shallow, brittle rhizomes. Tolerant of varied soil textures and full shade, it does poorly in calcareous soils and drought conditions (USDA NRCS, 2021). Dormant in winter, it emerges in mid-spring and flowers between March and July, producing panicles of 2 – 30 distinct pink-colored, heart-shaped flowers. It can go dormant during the dry summer season, emerging and flowering again in late summer to early fall. Seeds are borne in elongated capsules with elaiosomes, fleshy structures containing fats. Seed dispersal is aided by ants, which are attracted to the elaiosomes, and seeds require cold stratification for germination. The plant is considered moderately toxic to herbivores but is not known to be allelopathic (USDA NRCS, 2021).

Experimental Design

Growing media for all treatments was an artificial soil mix composed of a 2:1:1 mixture of peat moss, perlite, and coarse sand sourced from a local hardware store. Components were measured by volume and mixed in 40 L batches. Ten plants of each species were randomly selected for each of four treatment groups (Table 2). The first treatment group served as the control group and consisted of a single native species grown in the absence of *G. robertianum* in the artificial soil mix with no other soil amendments (n = 30). The second treatment group consisted of a single native species grown in the presence of *G. robertianum* with no soil amendments (n = 30). The third treatment group consisted of a single native species grown in the presence of *G. robertianum* with the addition of 20 g granulated activated horticultural charcoal (20 g/pot, n = 30; Rio Hamza Trading Co., Corbin, KY). The final treatment group consisted of a single native species grown in the presence of *G. robertianum* with the addition of native mineral soil (200 g/pot, n = 30). The native mineral soil was collected from two locations adjacent to Schneider Creek, Thurston County, in undisturbed *Alnus rubra/Polystichum munitum* plant association forest, then mixed for uniformity before being added to each treatment pot. Altogether there were 4 different treatments for 3 different species group with each combination replicated 10 times, for a total of 4 x 3 x 10 = 120 pots.

G. macrophyllum and *T. grandiflora* plants were uniform in size and development within their species group. *D. formosa* varied the most in terms of rhizome size and development per pot. Plants varied from singular rhizomes with minimal root development to extensive rhizome and root development that filled out the entire nursery pot. Plants with exceptionally large rhizomes were divided to accommodate the need for an additional five plants to bring the count up to 40.

Table 2. Summary of treatment groups by species with shorthand.

Species	Shorthand	Treatment 1 Control	Treatment 2 Invaded	Treatment 3 Carbon	Treatment 4 Soil
Geum macrophyllum	GEMA	GM	GMGR*	GMGRC	GMGRS
Tellima grandiflora	TEGR	TG	TGGR	TGGRC	TGGRS
Dicentra formosa	DIFO	DF	DFGR	DFGRC	DFGRS
Geranium robertianum w/GEMA	GERO	-	GRGM	GRGMC	GRGMS
Geranium robertianum w/TEGR	GERO	-	GRTG	GRTGC	GRTGS
Geranium robertianum w/DIFO	GERO	-	GRDF	GRDFC	GRDFS

***G**eum **M**acrophyllum grown with **G**eranium **R**obertianum; C = activated charcoal treatment (**C**arbon group); S = native soil treatment (**S**oil group)

For each treatment group, plant plugs were transferred to 1-gallon plastic nursery pots filled with the artificial soil mix and any additional soil treatments (native mineral soil or charcoal). Plugs were either planted in the center of the pot or equidistant to *G. robertianum*, depending on the treatment group. Pots were placed by treatment group on nursery racks in a utility room adjacent to a large, SE facing window with full natural light. Due to low-light levels in winter, light was supplemented with 2-bulb, 4-foot light fixtures fitted with 6500K fluorescent light bulbs (Philips F40T12/Daylight) positioned at equal heights above the pots. Lights were controlled by a timer and left on for 12 hours per day from 8 AM to 8 PM. Pots were rotated weekly to minimize any possible effects of shelf level or proximity to the window. The temperature of the room was kept between 17 – 20° C (62 – 68° F) for the duration of the experiment. Water was added as needed to maintain consistent moisture levels across all species and treatment groups. Water was sourced from a domestic community well with chlorine levels kept at or below 1 ppm and left in open containers at room temperature for 24 hours before using.

At approximately three weeks into the experiment, aphids infested all of the *G. robertianum,* but did not infest the native species. In order to limit the impact of the infestation, all *G. robertianum* was treated with insecticidal soap (Natria© Insecticidal Soap, 1% potassium salts of fatty acids), regardless of level of infestation, and any overspray onto the native species was immediately rinsed with water. This was repeated 10 days after the first treatment. Aphids did return, but at significantly lower numbers. At eight weeks (56 days), all pots were fertilized with 200 mL of diluted Alaska fish fertilizer (5-1-1 NPK, 15 mL per 1-gallon of water). At 12 weeks (84 days), all plants were harvested and aboveground and belowground parts separated. Due to the nature of commercially available plant plugs often consisting of more than one seedling, many of the plugs for *T. grandiflora* and *G. macrophyllum* contained multiple plants. The number of plants present and their relative size in each pot for these species was recorded during harvesting. For *D. formosa*, all aboveground biomass was collected, but only a subset of 5 replicates per treatment group were selected for belowground biomass collection. Each of these 5 replicates were chosen using a random number generator. All above- and belowground samples were then oven-dried in separate paper bags at 80° C (176° F) for 24 hours and weighed with an analytical scale (RADWAG AS 82/820.R2). Aboveground and belowground biomass data were also collected for *G. robertianum*.

Figure 6. Photographs of the full setup of all species and treatment groups.

Data analysis

A one-way analysis of variance (ANOVA) was used to analyze the effects of each treatment on the biomass of the native species and *G. robertianum* using R Statistical Software (RStudio version 2021.9.0+351). Aboveground, belowground, and total biomass of the native species and *G. robertianum* were all analyzed individually (see Tables 1 & 2 in Appendix A). For *G. macrophyllum* and *T. grandiflora*, each response variable was also analyzed on a per-plant basis where there was more than one plant per plot. One-way ANOVAs were used to evaluation treatment effects on the following response variables: aboveground biomass, belowground biomass, ratio of aboveground to belowground biomass for each species, and ratio of native total biomass to *G. robertianum* total biomass. Due to the high variability in existing rhizome development across the 40 plants *D. formosa* plants, a ratio for aboveground to belowground growth was not calculated for this species.

Individual treatment groups within a dataset not meeting the assumptions of normality required the entire dataset to be transformed with Log10 transformation. Datasets that could not be transformed to meet the assumptions of normality and homogeneity of variance were analyzed instead with the Kruskal-Wallis test for non-parametric data. Differences among treatment groups were examined with the Dunn’s (Benjamini-Hochberg) post hoc test. For data meeting ANOVA assumptions, differences among treatment groups were examined using Tukey’s HSD post hoc test.

Results

Geum macrophyllum

There was a statistically significant difference between aboveground and belowground biomass by treatment group ($F_{7,72} = 61.04$, $p < 0.001$; Figure 7; Figure 8; Table 4). Additionally, there was a statistically significant treatment effect on all response variables except for the belowground *per plant* analysis (Table 3). The Invaded and Carbon treatment groups exhibited the greatest decrease in both aboveground and total biomasses compared to the Control group ($p < 0.001$ for both) and Soil treatment group ($p < 0.001$ for both) (Table 4). There was no significant treatment effect for the Soil treatment group compared to the Control group ($p = 0.164$). When comparing the ratio of aboveground to belowground biomass across treatment groups, three out of six pairwise comparisons were statistically significant (Table 4). The mean aboveground-belowground ratio (AG:BG) for the entire species group was 2.03 (Table 1, Appendix B). None of the plants died, nor produced flowers.

Figure 7. Photograph of *Geum macrophyllum* Control group (left) and *Geum macrophyllum* Carbon Group (right) after ~10 weeks of growth. The differences between these two treatment groups was statistically significant ($p < 0.001$).

Tellima grandiflora

There were no significant differences between treatment groups for this species. Mean aboveground biomass did not significantly differ from the corresponding belowground biomass for each treatment group (Figure 8). The mean aboveground-belowground ratio (AG:BG) for the entire species group was 0.99 (Table 1, Appendix B). Out of the 40 *T. grandiflora* plants, none died and only three produced flowers, all of which were in the Soil treatment group.

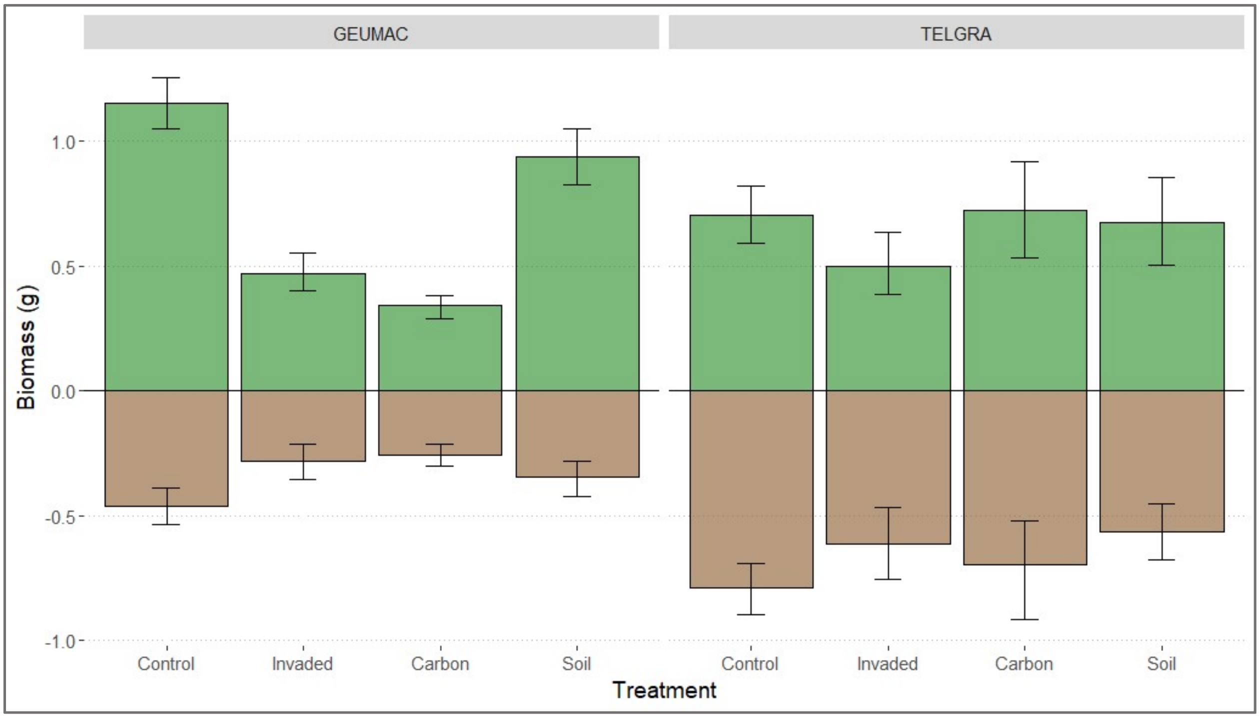

Figure 8. Effect of treatments on native biomass for *Geum macrophyllum* (left) and *Tellima grandiflora* (right); green and brown portions represent aboveground and belowground biomass, respectively (note: numbers on y-axis are *not* negative). Values are means and standard errors for each treatment group.

Dicentra formosa

The aboveground biomass was the only response variable which showed a significant treatment effect for *D. formosa* ($F_{3, 33} = 10.84$, $p < 0.001$; Table 3). This effect was limited to the Soil treatment group, with significantly less aboveground biomass than all other treatment groups (Figure 9; Table 4). All four treatment groups produced flowers, with the Soil group representing the lowest number of blooms (DF: 9 flowers; DFGR: 8; DFGRC: 8; and DFGRS: 3). Three plants (DFGR: 1 and DFGRC: 2) either did not produce aboveground growth or they died back, though the rhizomes were intact and alive. New rhizome growth was conspicuous and noted during harvesting. Not all plants produced new rhizome growth during the course of the experiment. Of the five subsamples taken from each treatment group for belowground biomass, the Control group had two plants with new rhizome growth, all five in the Invaded group had new growth, none of the Carbon group had new growth, and two in the Soil group had new growth.

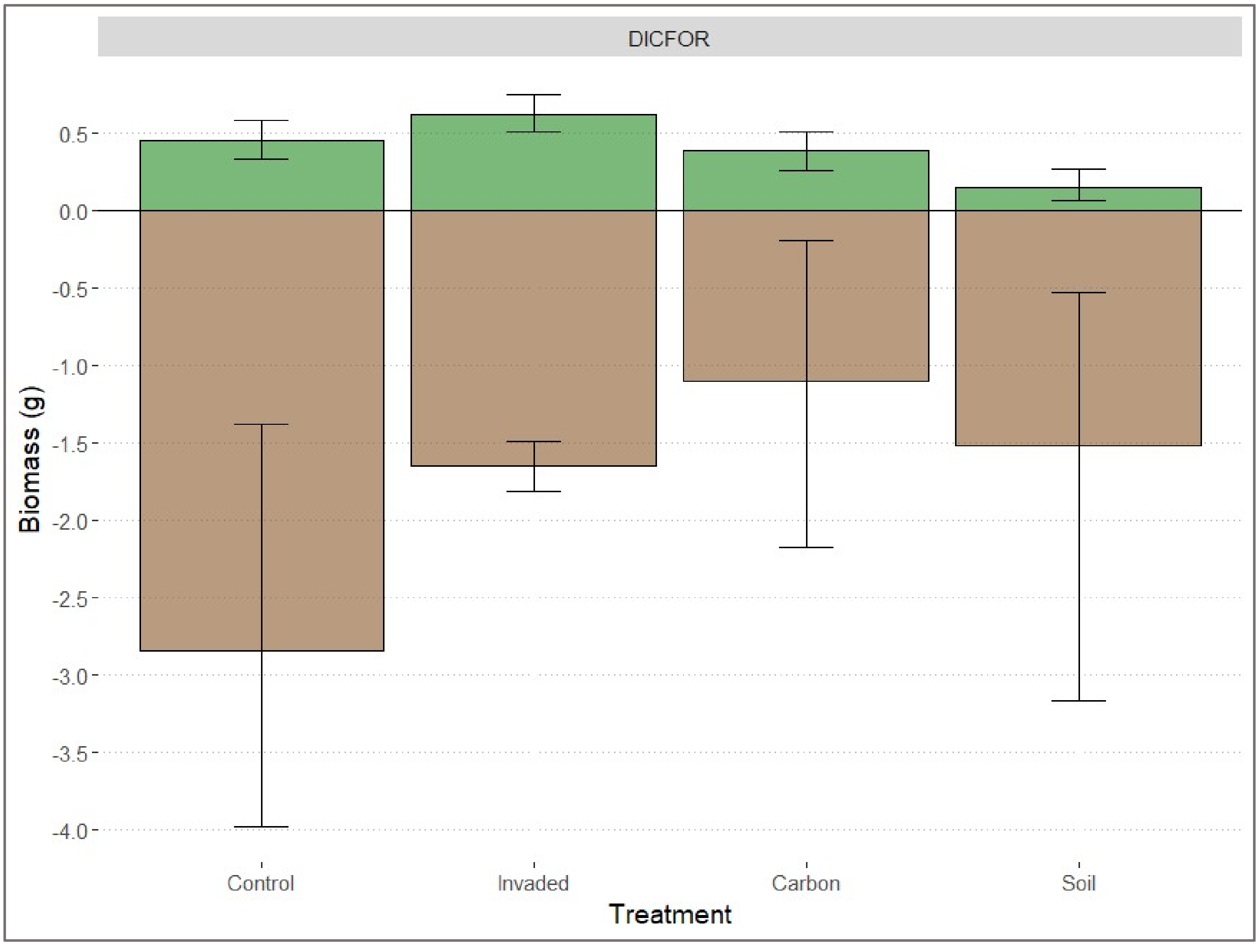

Figure 9. Effect of treatments on biomass of *Dicentra formosa*; green and brown portions represent aboveground and belowground biomass, respectively (note: numbers on y-axis are not negative). Values are means and standard errors for each treatment group.

Table 3. Analysis of variance (ANOVA) results for biomass of native species *Geum macrophyllum*, *Tellima grandiflora*, and *Dicentra formosa* grown with and without *Geranium robertianum* for all treatment groups. Significant p-values ($p < 0.05$) are bolded.

Species & Response Variable	df	SS	*F*	*p*
G. macrophyllum				
Aboveground Biomass*	3	1.908	67.34	**< 0.001**
Aboveground Biomass *per plant**	3	0.486	9.690	**< 0.001**
Belowground Biomass	3	0.251	6.471	**0.001**
Belowground Biomass *per plant***	3	-	-	0.052
Total Biomass	3	6.645	48.7	**< 0.001**
Total Biomass *per plan*t**	3	-	-	**< 0.001**
AG:BG Ratio**	3	-	-	**< 0.001**
AG:BG by Group	7	7.614	61.04	**< 0.001**
T. grandiflora				
Aboveground Biomass*	3	0.176	0.059	0.196
Aboveground Biomass *per plant**	3	0.054	0.224	0.879
Belowground Biomass	3	0.299	1.583	0.210
Belowground Biomass *per plant*	3	0.338	1.267	0.300
Total Biomass	3	0.902	1.344	0.275
Total Biomass *per plant*	3	0.649	0.684	0.568
AG:BG Ratio	3	0.703	2.867	**0.050**
AG:BG by Group	7	0.621	1.336	0.246
D. formosa				
Aboveground Biomass*	3	3.595	10.84	**< 0.001**
Belowground Biomass**	3	-	-	0.268
Total Biomass**	3	-	-	0.300

*Transformed data; **Nonparametric data analyzed with Kruskal-Wallis

Table 4. P-value results from pairwise comparisons of treatment groups; post hoc for ANOVA & Tukey's HSD and Kruskal-Wallis & Dunn's Test. Significant p-values ($p < 0.05$) are bolded. Green highlight indicates nonparametric analysis; blue indicates parametric analysis after transformation.

Corresponding *p* Values for:	AG Biomass	AG Biomass *per plant*	BG Biomass	BG Biomass *per plant*	Total Biomass	Total Biomass *per plant*	AG:BG Ratio
Geum macrophyllum (GM)							
GMGR – GM	**< 0.001**	**0.017**	**0.006**	0.175	**< 0.001**	**0.034**	0.099
GMGRC – GM	**< 0.001**	**< 0.001**	**0.002**	0.062	**< 0.001**	**0.001**	**0.003**
GMGRS – GM	0.164	0.867	0.121	0.493	**0.008**	0.516	0.619
GMGRC – GMGR	**0.017**	0.351	0.969	0.503	0.402	0.232	0.188
GMGRS – GMGR	**< 0.001**	0.104	0.582	0.426	**< 0.001**	0.123	**0.039**
GMGRS – GMGRC	**< 0.001**	**0.002**	0.324	0.164	**< 0.001**	**0.007**	**0.001**
Geranium robertianum grown with *G. macrophyllum* (GRGM)							
GRGMC – GRGM	0.067	-	0.660	-	0.083	-	**0.046**
GRGMS – GRGM	0.945	-	0.478	-	0.900	-	0.707
GRGMS – GRGMC	**0.041**	-	0.113	-	**0.040**	-	0.270
Tellima grandiflora (TG)							
TGGR – TG	0.207	0.890	0.392	0.916	0.283	0.805	0.999
TGGRC – TG	0.997	0.931	0.825	0.801	0.983	0.910	0.537
TGGRS – TG	0.957	0.908	0.197	0.240	0.627	0.502	0.090
TGGR – TGGRC	0.287	0.999	0.881	0.994	0.477	0.995	0.488
TGGR – TGGRS	0.452	0.999	0.973	0.588	0.929	0.957	0.076
TGGRS – TGGRC	0.989	0.999	0.651	0.746	0.834	0.878	0.707
Geranium robertianum grown with *T. grandiflora* (GRTG)							
GRTGC – GRTG	0.740	-	0.992	-	0.788	-	0.300
GRTGS – GRTG	0.995	-	0.576	-	0.993	-	0.536
GRTGS – GRTGC	0.688	-	0.651	-	0.848	-	**0.039**
Dicentra formosa (DF)							
DFGR – DF	0.660	-	0.589	-	0.727	-	-
DFGRC – DF	0.969	-	0.368	-	0.466	-	-
DFGRS – DF	**< 0.001**	-	0.447	-	0.403	-	-
DFGRC – DFGR	0.431	-	0.620	-	0.589	-	-
DFGRS – DFGR	**< 0.001**	-	0.668	-	0.668	-	-
DFGRS – DFGRC	**0.006**	-	0.669	-	0.789	-	-
Geranium robertianum grown with *D. formosa* (GRDF)							
GRDFC – GRDF	0.200	-	0.928	-	0.802	-	0.585
GRDFS – GRDF	0.433	-	0.987	-	0.957	-	0.801
GRDFS – GRDFC	0.846	-	0.980	-	0.945	-	0.940

Notes: Native plants grown without *G. robertianum* are the Control Group (GM, TG, DF); plants grown with *G. robertianum* but without additional treatments are the Invaded group (GMGR, TGGR, DFGR); Addition of C or S indicates Carbon treatment and Soil treatment groups, respectively (e.g., TGGRC and TGGRS).

Geranium robertianum

All three groups of *G. robertianum* had greater mean aboveground biomass than corresponding belowground biomass (Figure 10; Table 1, Appendix B). Many of the plants produced flowers and seeds (GRGM: 18/30; GRTG: 29/30; and GRDF: 21/30). After the completion of the experiment, the 20 leftover *D. formosa* pots with intact soil were placed outside. Within several weeks, several of the pots had newly sprouted *G. robertianum* seedlings.

For *G. robertianum* grown with *G. macrophyllum,* the Soil and Invaded treatment groups were nearly equal in terms of mean aboveground biomass. There was a statistically significant treatment effect for the Carbon group when compared to the Soil group (p = 0.041; Table 4), with both the aboveground and belowground biomass significantly lower for the Soil group. Similarly, the aboveground and belowground biomass for the Invaded treatment group was smaller relative to the Carbon treatment group, however the difference was not statistically significant (p = 0.067; Table 4). The ratio of aboveground to belowground biomass for the Carbon treatment group was significantly greater than the Invaded treatment group (p = 0.046), indicating aboveground growth was greater than belowground growth for the Carbon treatment group. Four plants died during the experiment, three in the Invaded treatment group (GMGR) and one in the Soil treatment group (GMGRS).

There were no significant treatment effects for the *G. robertianum* grown with *T. grandiflora*, with the exception of the aboveground-belowground ratio for the Soil treatment group compared to the Carbon treatment group, with larger ratios for the Soil treatment group (p = 0.039). None of the plants died in this species group. Similarly, there were no significant treatment effects for the *G. robertianum* grown with *D. formosa* (Table 5). A total of six plants died in this group: four in the Invaded group, three after flowering, and two in the Soil group.

Table 5. Analysis of variance (ANOVA) results for biomass of *Geranium robertianum* by native species group for all treatment groups (Invaded, Carbon, and Soil). Significant p-values ($p < 0.05$) are bolded.

Species & Response Variable	df	SS	*F*	*p*
G. robertianum w/*G. macrophyllum*				
Aboveground Biomass*	2	0.679	4.528	**0.024**
Belowground Biomass*	2	0.178	2.226	0.134
Total Biomass*	2	0.577	4.396	**0.026**
AG:BG Ratio*	2	0.242	3.519	**0.049**
G. robertianum w/*T. grandiflora*				
Aboveground Biomass	2	0.259	0.416	0.664
Belowground Biomass	2	0.017	0.611	0.550
Total Biomass	2	0.211	0.250	0.781
AG:BG Ratio	2	22.33	3.388	**0.049**
G. robertianum w/*D. formosa*				
Aboveground Biomass	2	1.007	1.716	0.204
Belowground Biomass	2	0.002	0.068	0.935
Total Biomass	2	0.256	0.207	0.817
AG:BG Ratio	2	2.346	0.561	0.592

*Transformed data

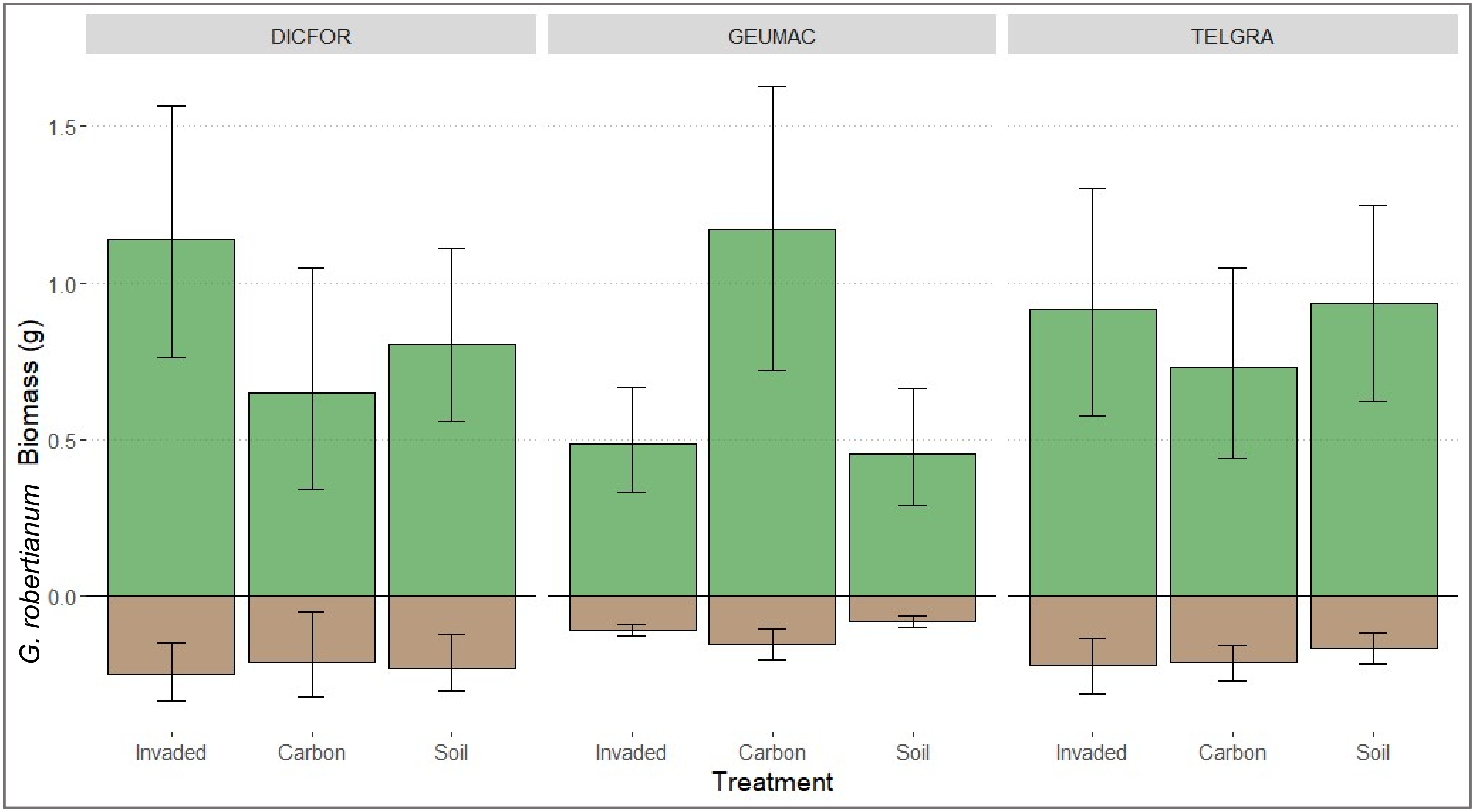

Figure 10. Effect of treatments on *Geranium robertianum* biomass; green and brown portions represent aboveground and belowground biomass, respectively. Values are means and standard errors for each treatment group. Groups are as follows: DICFOR = *G. robertianum* grown with *Dicentra formosa*; GEUMAC = *G. robertianum* grown with *Geum macrophyllum*; TELGRA = *G. robertianum* grown with *Tellima grandiflora.*

Discussion

Examining the direct effects of the invasive *Geranium robertianum* on native species was the intent of this research. Specifically, what are the effects on the growth of the native species and is there any evidence of allelopathic interference from *G. robertianum*. While this plant is somewhat wide spread in western Washington, little is known about its direct effects on native plants or whether its presence has long-term consequences for native plant community composition. This is somewhat concerning as it is capable of invading intact, uninvaded forest understory. Overall, this study revealed interesting patterns when comparing results across species groups. In terms of competitive ability and effects of treatments, *Geum macrophyllum* and *Tellima grandiflora* stood out as highly contrasting species. The results for *Dicentra formosa* are less informative, which is likely due to the high variability in preexisting rhizome size from one replicate to the other. Additionally, due to the rhizomatous growth habit of *D. formosa*, it is possible the 12-week duration of the experiment was insufficient to effectively capture the competitive interactions between these species. The presence of multiple plants per pot for *G. macrophyllum* and *T. grandiflora* had the potential to confound the results, but this was not the case with *T. grandiflora* and was of little impact for *G. macrophyllum*, which will be discussed below.

Geum macrophyllum

This species group exhibited the most dramatic changes in biomass relative to treatments, with all but one response variable indicating statistically significant differences among treatment groups (Table 3). The fact that both aboveground and belowground biomass were reduced in the Invaded and Carbon treatments relative to the Control group suggest that *G. robertianum* was directly reducing the growth of this species. In order to demonstrate an allelopathic effect, the response of the native species (above- and/or belowground biomass) should decrease with exposure to a potential allelopathic invader but should recover with the addition of an activated charcoal treatment (Rúa et al., 2008; Del Fabbro et al., 2014). A lack of this specific response by *G. macrophyllum* suggests that the reduction in total biomass for both the Invaded and Carbon groups is likely due to general interspecific competition for resources versus allelopathic interference from *G. robertianum*. In contrast to the Invaded and Carbon treatment groups, the effect of *G. robertianum* on *G. macrophyllum* in the Soil treatment group appears to have been ameliorated by the addition of native soil, as the total biomass *G. macrophyllu*m in the Soil treatment group was nearly 80% of that of the Control group. This result contrasts with the majority of studies on the effects of invasives on soil microbial communities, which are generally characterized as negative. For example, invasive species are able to exploit the benefits of symbioses while avoiding the mutual cost of the network, or alter microbial communities by increasing the abundance of generalist pathogens (Reinhart & Callaway, 2006; Hagan & Jose, 2013; Lankau, 2013). However, arbuscular mycorrhizas are important mediators of competitive interactions between invasive and native plants and, despite generally lacking host-specificity, specificity in growth responses for individual infected plants does exist (Reinhart & Callaway, 2006). Given the complex interactions of plant-mycorrhizal associations, the results for the Soil treatment group suggests that the soil microbial community is important for *G. macrophyllum* in terms of both growth and competitive ability.

Overall, this species had greater mean aboveground biomass than belowground biomass. The mean AG:BG ratio across all treatment groups was 2.03, revealing an emphasis on aboveground growth for this species (Table 1, Appendix B). However, there was a notable shift for the Invaded and Carbon treatment groups – the mean AG:BG ratios were lower than the Control and Soil treatment groups but the effects were not proportional. For example, the aboveground biomass of the Invaded group was ~60% lower than the Control group, whereas belowground biomass was only ~40% lower. It appears as though *G. macrophyllum* altered resource allocation and conservation in response to invasion. The changes in AG:BG ratios and statistical significance between treatment effects is only moderately reduced when the number of plants per pot are taken into consideration. While growing several individuals of the same species with a different species will produce both interspecific and intraspecific competition effects, it is theorized that individuals from the same species will have higher levels of competition due to sharing similar resource needs (Mangla et al., 2011). The results for the Control and Invaded groups contradict this theory, however. Since all four treatment groups contained more than one individual of *G. macrophyllum*, the significant difference between the Control and Invaded groups points to higher levels of interspecific competition, not intraspecific, as the driver behind decreased biomass for *G. macrophyllum.*

Tellima grandiflora

Based on the hypothesis that the native species would exhibit some effect from the presence of *G. robertianum*, the strongest effect exhibited by *T. grandiflora* was by the belowground biomass for the Soil treatment group relative to the Control group, but this was still a rather large *p*-value of 0.197. All treatment groups had belowground biomass that very closely mirrored its corresponding aboveground biomass, with mean AG:BG ratios ranging from 0.82 to 1.12 (species group mean of 0.99). This suggests that whatever effect a treatment or the presence of *G. robertianum* had on the plants, it more or less affected aboveground and belowground growth equally. It is difficult to determine if or how much allelopathy impacted growth versus other modes of interspecific competition, such as resource availability. The total biomass of the Invaded group did slightly decrease compared to the Control, which appears to have been improved by the activated charcoal treatment in the Carbon group. This is the pattern expected for allelopathic interference, however there was no statistically significant differences among the treatment groups. While also lacking statistical significance, *T. grandiflora* responded positively to the native soil treatment compared to the Invaded group in terms of aboveground growth. This is somewhat similar to *G. macrophyllum* where the positive effect was highest for aboveground growth, implicating soil biota as an important factor in resource acquisition for these native species. An additional qualitative observation for the Soil treatment group that highlights the importance of the soil microbial community was that the only plants that flowered (out of all 40 plants in the species group) were in this treatment group, despite all plants being comparable in size and health at the start of the experiment.

Focusing on two different modes of allelopathy, Brandt (2008) used both *G. robertianum* leaf litter and leaf leachate to investigate whether either had a negative impact on the growth of *T. grandiflora*. Decomposing leaf litter had no significant impact on aboveground or belowground biomass, whereas leaf leachate reduced both. However, it was noted that the leaf leachate was very acidic (pH = 4.0), so it is difficult to determine whether it was allelopathy or acidity that impacted growth. Brandt also commented that other modes of allelopathy should be explored for *G. robertianum*, including the possibility of root exudates being the primary mode of its allelopathic potential. While the study design for this thesis did not extract or measure root exudates, plants would have been exposed to any exudates released by *G. robertianum*.

Although the growth of *T. grandiflora* appeared to be overall robust, the presence of *G. robertianum* may affect this species in other ways not examined in this study, such as flowering and seed set. *T. grandiflora* was specifically noted by the Washington Native Plant Society as an ideal species for restoration, owing to its ability to grow well in disturbed and shady areas and outcompete invasive weeds (WNPS, 2021). Even when accounting for the discrepancies in the number of seedlings per pot (AG, BG, and Total Biomass *per plant*) and intraspecific competition, the effects of *G. robertianum* and the treatments were still insignificant, suggesting that *T. grandiflora* is an effective competitor against invasion.

While there is a paucity of studies examining the competitive ability of native plants found in Puget lowland forests, many studies on shrub-steppe ecosystems of western North America have examined various methods to out-compete highly invasive *Bromus tectorum* (cheatgrass). A 2014 study explored whether native "weedy" forbs could effectively compete with *B. tectorum* and enhance the growth of native perennial grasses, finding three of the seven test species were highly effective at suppressing the growth and seed production of *B. tectorum* (Leger et al., 2014). Applying the invasion hypothesis of biotic resistance – competitive resistance of a recipient community that impedes invaders – this example, along with the resilience of *T. grandiflora*, highlights the need for more studies on native species that are potentially resistant to invasion and able to enhance restoration efforts.

Dicentra formosa

The most prominent finding for this species was the significant decrease in aboveground biomass for the Soil treatment group when compared to the other treatment groups (Figure 9). Given the high variability in rhizome size across all groups during planting, it would be easy to conflate this variability with the treatments and the presence of *G. robertianum*. However, the belowground biomass for the Soil treatment group was not proportionately small and did not significantly differ from the other groups. An additional outcome of the Soil treatment group was the reduced number of flowering individuals compared to the other groups – only 3 out of 10 plants flowered. There are a few possible explanations for the reduced aboveground biomass for this group: 1) the lack of inflorescences and associated foliage reduced the overall aboveground biomass; 2) the particular plants selected for this group contained some of the extra-large plants that were divided to reach the required 40, with the plants potentially focusing energy on belowground growth in response to division; or 3) the presence of *G. robertianum* impacted flowering and growth. Considering the other two groups with *G. robertianum* had similar flowering rates to the Control group, it is less likely that the presence of *G. robertianum* would have impacted flowering. However, due to the complexity of belowground interactions with the soil microbial community, including promoting antagonists, the possibility that *G. robertianum* negatively impacted growth and flowering cannot be completely ruled out (Reinhart & Callaway, 2006; Stinson et al., 2006).

Another notable outcome for this species group was the lack of new rhizome growth for the Carbon treatment group. During harvesting, new rhizome growth was conspicuous – pale white in color and easily broken – whereas existing rhizomes were darker in color and more resistant to breakage. Activated carbon is frequently used in allelopathic studies due to its ability to adsorb biochemical compounds (Rúa et al., 2008; Del Fabbro et al., 2014), but it can also interfere with soil nutrient availability, water retention, soil pH, and mycorrhizal development, negatively impacting plant growth (Wurst et al., 2010). Weißhuhn and Prati (2008) found activated charcoal substantially altered substrate chemistry by increasing available phosphate and decreasing the ratio of organic carbon to total nitrogen. The results of these studies indicate that even if activated charcoal is found to reduce allelopathic chemicals, its ability to alter soil chemistry has the potential to confound results and erroneously attribute greater negative effect to allelopathy than is warranted (Weißhuhn & Prati, 2008; Wurst et al., 2010). Considering the Carbon treatment group was the only group that failed to develop new rhizomes, it suggests that the activated charcoal had a negative effect on the soil chemistry.

Geranium robertianum

The native soil treatment had an interesting effect on the *G. robertianum* grown with *Geum macrophyllum* and *Tellima grandiflora.* Aboveground biomass of the Soil treatment group for *G. robertianum* from both species groups was nearly equal to the aboveground biomass of the Invaded groups, but belowground biomass decreased by ~25% for both. In contrast, both native species had positive responses for aboveground biomass in the Soil treatment group, increasing by ~100% and ~36% relative to the Invaded groups, respectively. The plants grown with *Dicentra formosa* had somewhat different results – instead of aboveground biomass of the Soil treatment group being nearly equal to the Invaded group, it decreased by ~30%. Belowground biomass also decreased but only by ~6%. These results both agree and conflict with studies on plant-soil biota feedbacks, where the invader is generally theorized to be the one that receives the most benefits. One study on *Centaurea stoebe* (spotted knapweed) found positive plant-soil biota feedbacks (increased biomass) when grown in North American soil with a native grass, but not when grown in soil from its native region of Europe (Callaway et al., 2004). When grown in sterilized soil, plant-soil biota feedbacks were eliminated and the biomass of the native grass increased. These results show that *C. stoebe* benefits from being removed from co-evolutionary pathogens in its native range, potentially gaining mutualistic associations and outcompeting the native grass. The conflicting results for *G. robertianum* across native species demonstrate how competitive relationships and soil microbial community interactions are species-specific and difficult to predict even within the same plant community (Weißhuhn & Prati, 2008; Weidenhamer & Callaway, 2010).

In a mycorrhizal study on *G. robertianum*, it was noted that the plant is capable of forming mycorrhizal associations and that these associations were particularly helpful in low nutrient soils (Boerner, 1990). Plants inoculated with arbuscular mycorrhizal fungi had higher uptake of phosphorous and nitrogen compared to plants that did not receive inoculation. This increase in nutrient uptake may be due to either the mycorrhiza facilitating greater root mass and length, thereby allowing for greater uptake rates, or the mycorrhiza directly assisting in the increased nutrient uptake, or possibly both (Boerner, 1990, Reinhart & Callaway, 2006). Non-native species that are capable of making novel mycorrhizal associations can enhance their success, tipping the scales from being non-native to invasive. This interaction is two-way, with soil microbial communities affecting and responding to changes in plant community composition (Lankau, 2013). New mycorrhizal associations can facilitate a feedback system that alters existing plant-soil biota interactions, such as increasing the abundance of pathogens or changing the abundance of mutualists, which can be beneficial or inhibitory to native species (Reinhart & Callaway, 2006; Lankau, 2013). The lack of a significant negative effect of the native soil treatment on *G. robertianum* suggests that novel mycorrhizal associations and an absence of co-evolutionary inhibitory soil pathogens may be mechanisms contributing to its success as an invader.

The plants grown with *G. macrophyllum* had a significant increase in aboveground biomass for the Carbon treatment group, whereas aboveground biomass for *G. macrophyllum* was lowest for this is treatment group. This is contrary to what would be expected for a competitive effect driven by allelopathic interference, where you would anticipate an increase in biomass for the native, not the invader. Unexpectedly, the results were opposite for the plants grown with *T. grandiflora* and *D. formosa*, both of which had a reduction in aboveground biomass relative to the Invaded groups. The patterns for plants grown with *T. grandiflora* and *D. formosa* are what you would expect to see if allelopathic chemicals were being inhibited by an activated charcoal treatment, however, none of the differences were statistically significant. Overall, these patterns suggest that the Carbon treatment was not particularly detrimental to growth for *G. robertianum*, nor did they indicate any strong signs of allelopathic interference.

One further notable pattern for *G. robertianum* that may offer an explanation of invasiveness was the amount of its aboveground biomass relative to belowground biomass, which stands out compared to both *T. grandiflora* and *G. macrophyllum*. When considering the phenology and life cycle of each of these species, it does not seem unusual that the native species, which are perennials, dedicate more energy and resources towards belowground biomass than the annual-biennial *G. robertianum*. The mean AG:BG ratio for *G. robertianum* across all species and treatment groups was 4.66, whereas *G. macrophyllum* and *T. grandiflora* only have mean ratios of 2.03 and 0.99, respectively (Table 1, Appendix B). A comparative study of perennials and annuals in Argentina found that root traits of annuals were associated with enhanced resource acquisition via low-density roots with high nitrogen concentration, whereas the perennials demonstrated enhanced root persistence with the presence of thick, dense root systems (Roumet et al., 2006). This study demonstrates resource acquisition and distribution are crucial differences between these plant life cycles. The high AG:BG ratio for *G. robertianum* suggests that an annual life cycle with energy and resources directed towards abundant aboveground growth and proportionally abundant seed quantity are significant contributors to the invasiveness of this species (Table 1, Appendix B; Figure 1, Appendix C). The AG:BG ratios for

G. macrophyllum and *T. grandiflora* are consistent with this notion, as both species directed far more resources to belowground growth compared to *G. robertianum*. Likewise, due to its rhizomatous growth habit, *D. formosa* has a similar pattern, directing more resources to belowground growth compared to *G. robertianum*.

Limitations and Changes

Given the opportunity to reflect on this thesis process, there are several things that I would have done differently. This list is not exhaustive, but merely points out the more obvious aspects that could have been improved upon. Given the interesting plant interactions I was looking at it would have been incredibly useful to have a full factorial study design that included not just the native grown in monoculture but also with the same treatments as the other groups (activated carbon and native mineral soil). Growing *G. robertianum* by itself with the same treatments would also have been informative. To avoid confounding the results with aspects of intraspecific competition, I should have thinned the plugs down to just a few seedlings at the start of the experiment and finally down to a single, dominant seedling once the plugs were well established in the pots. In a similar vein, the rhizomatous growth habit of *Dicentra formosa* made for a challenging set up and interpretation of the results. A standardized rhizome length would have been better to start with and make for more informative results at the end. Using a native species with a more fibrous root structure more similar to *T. grandiflora* and *G. macrophyllum* for the third native in the experimental design may have been more informative. Additionally, I should have been more mindful of phenology, as many of the *G. robertianum* plants had gone to seed and were entering senescence and beginning to die back before harvesting began.

Conclusion

Overall, in evaluating whether any of the treatments were effective at improving competitiveness of the native species, the results were quite mixed. The only native species to respond positively to the activated charcoal treatment was *T. grandiflora*, though the difference was not statistically significant. The carbon treatment for *G. macrophyllum* had the exact opposite outcome expected when looking for allelopathic interference from an invasive species: the invasive biomass increased significantly while the native biomass decreased. Either *G. robertianum* benefited from the presence of the activated charcoal or benefited from the inhibition of *G. macrophyllum* by the activated charcoal. Given the decreased biomass of *G. robertianum* in the Carbon treatment groups grown with *D. formosa* and *T. grandiflora*, it is more likely that *G. robertianum* benefited not from the presence of activated charcoal, but from the inhibitory effect on *G. macrophyllum*. Unlike the activated charcoal treatment, the native soil treatment appeared to be generally neutral to beneficial for all species, with the exception of *D. formosa*. The robust response of *G. macrophyllum* to the soil treatment suggests that it may be a more resilient to invasion than was demonstrated by its performance in the Invaded and Carbon treatment groups.

While there was a clear pattern of interspecific competition effects on the native species by *G. robertianum*, there is no strong evidence of allelopathic interference. The patterns you would expect to see with allelopathic interference are present for several of the groups, however they all lacked statistical significance. Considering the patterns are present, it is possible that a study with more than 10 replicates per treatment group could reveal greater significance. Also, it is possible that allelopathy is only partially responsible for the invasive success of *G.*

robertianum and that its effects are weak, but not entirely insignificant when considered as a part of a complement of invasive mechanisms. In criticism of the Novel Weapons hypothesis, it has been pointed out that allelopathy does not neatly explain the invasiveness of many invasive species (Del Fabbro et al., 2014). The presence of patterns demonstrating allelopathic interference for a very successful invasive species but lacking statistical significance substantiates this claim. Moreover, allelopathic chemicals are not necessary for growth or reproduction and are therefore costly to produce, possibly only produced when sufficient resources are available, suggesting that use of allelopathic chemicals by *G. robertianum* is possibly dependent on life cycle and habitat quality (Parepa & Bossdorf, 2016).

In terms of invasion hypotheses relevant to *G. robertianum*, there does not appear to by a "holy grail" hypothesis or mechanism that adequately explains its invasive success. The abundant aboveground growth and proportionally abundant seed quantity support both the Propagule pressure and Ideal Weed hypotheses as explanations for its success as an invader. Propagule pressure is considered by some as a prerequisite for invasiveness and has been demonstrated to be the primary determinant of habitat invisibility (Van Holle & Simberloff, 2005). High propagule pressure may enable invaders to become established through saturation of the seed bank, which quickly overwhelms any biotic resistance to invasion (Van Holle & Simberloff, 2005; Catford et al., 2009). The broad ecological tolerances exhibited by *G. robertianum* suggest it possesses high phenotypic plasticity, which also falls under the Ideal Weed hypothesis. Evidence for allelopathy and the Novel Weapons hypothesis is weak and does not appear to be the most relevant hypothesis to explain its invasiveness, but is potentially a minor contributing factor. It is worth pointing out, however, that allelopathic interactions can be species-specific and that long-term changes to soil chemistry facilitated by invasives is not well understood. Given that it is widely recognized that plants can influence the structure and composition of the soil microbial community, there is the potential that *G. robertianum* is influencing native soil biota, triggering feedback loops that are either beneficial to itself or detrimental to natives (Del Fabbro & Prati, 2015). As this invasive species shows no signs of slowing its spread in western Washington, it is clear that more research is needed, particularly in terms of impacts to the soil microbial community. Similarly, as invasive species are an unfortunate byproduct of the modern world and rates of introductions show no signs of slowing down, more studies of native plants and their interactions with established invasive species can increase our knowledge of which natives are the most resistant to invasion, which may help inform management and restoration decisions in the future.

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Appendices

Appendix A

Table 1. Data analysis summary for native species by response variable.

Data Analysis Summary for *Geum macrophyllum*					
Response Variable	Meets assumptions of normality?	Homogeneity of Variance?	Transformation Used	Test Used	Post Hoc Used
Aboveground Biomass	Yes	Yes*	Log10	ANOVA	Tukey's HSD
Aboveground Biomass *per plant*	Yes*	Yes*	Log10	ANOVA	Tukey's HSD
Belowground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Belowground Biomass *per plant*	No	Yes	-	Kruskal-Wallis	Dunn's Test
Total Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Total Biomass *per plant*	No	Yes	-	Kruskal-Wallis	Dunn's Test
AG:BG Ratio	No	Yes	-	Kruskal-Wallis	Dunn's Test
Data Analysis Summary for *Tellima grandiflora*					
Aboveground Biomass	Yes*	Yes	Log10	ANOVA	Tukey's HSD
Aboveground Biomass *per plant*	Yes*	Yes	Log10	ANOVA	Tukey's HSD
Belowground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Belowground Biomass *per plant*	Yes	Yes	-	ANOVA	Tukey's HSD
Total Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Total Biomass *per plant*	Yes	Yes	-	ANOVA	Tukey's HSD
AG:BG Ratio	Yes	Yes	-	ANOVA	Tukey's HSD
Data Analysis Summary for *Dicentra formosa*					
Aboveground Biomass	Yes*	Yes	Log10	ANOVA	Tukey's HSD
Belowground Biomass	No	Yes	-	Kruskal-Wallis	Dunn's Test
Total Biomass	No	Yes	-	Kruskal-Wallis	Dunn's Test

*After transformation

Table 2. Data analysis summary for *Geranium robertianum* by response variable.

Response Variable	Meets assumptions of normality?	Homogeneity of Variance?	Transformation Used	Test Used	Post Hoc Used
Data Analysis Summary for *Geranium robertianum* grown with *G. macrophyllum*					
Aboveground Biomass	Yes*	Yes*	Log10	ANOVA	Tukey's HSD
Belowground Biomass	Yes	Yes*	Log10	ANOVA	Tukey's HSD
Total Biomass	Yes*	Yes*	Log10	ANOVA	Tukey's HSD
AG:BG Ratio	Yes*	Yes*	Log10	ANOVA	Tukey's HSD
Data Analysis Summary for *Geranium robertianum* grown with *T. grandiflora*					
Aboveground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Belowground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Total Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
AG:BG Ratio	Yes	Yes	-	ANOVA	Tukey's HSD
Data Analysis Summary for *Geranium robertianum* grown with *D. formosa*					
Aboveground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Belowground Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
Total Biomass	Yes	Yes	-	ANOVA	Tukey's HSD
AG:BG Ratio	Yes	Yes	-	ANOVA	Tukey's HSD

*After transformation

Appendix B

Table 1. Mean aboveground (AG), belowground (BG), total biomass, and aboveground to belowground ratio (AG:BG) for each treatment group by species; includes percent of total biomass for aboveground and belowground biomass. GERO = *Geranium robertianum*; GEMA = *Geum macrophyllum*; TEGR = *Tellima grandiflora*; DIFO = *Dicentra formosa*.

Species & Treatment Groups	Mean AG Biomass	Mean BG Biomass	Mean Total Biomass	AG:BG Ratio*	AG % of Total	BG % of Total
G. macrophyllum						
Control	1.150 g	0.464 g	1.614 g	2.48	71%	29%
Invaded	0.468 g	0.283 g	0.751 g	1.65	62%	38%
Carbon	0.340 g	0.260 g	0.600 g	1.31	57%	43%
Soil	0.937 g	0.348 g	1.285 g	2.69	73%	27%
T. grandiflora						
Control	0.702 g	0.791 g	1.493 g	0.89	47%	53%
Invaded	0.498 g	0.611 g	1.110 g	0.82	45%	55%
Carbon	0.722 g	0.694 g	1.416 g	1.04	51%	49%
Soil	0.675 g	0.563 g	1.238 g	1.20	55%	45%
D. formosa						
Control	0.465 g	2.842 g	3.306 g	0.16	-	-
Invaded	0.498 g	1.649 g	2.147 g	0.30	-	-
Carbon	0.284 g	1.097 g	1.381 g	0.26	-	-
Soil	0.193 g	1.521 g	1.714 g	0.13	-	-
GERO w/GEMA						
Invaded	0.485 g	0.110 g	0.595 g	4.41	82%	18%
Carbon	1.170 g	0.152 g	1.322 g	7.70	89%	11%
Soil	0.451 g	0.082 g	0.534 g	5.50	84%	16%
GERO w/TEGR						
Invaded	0.914 g	0.220 g	1.134 g	4.15	81%	19%
Carbon	0.730 g	0.213 g	0.942 g	3.43	77%	23%
Soil	0.935 g	0.166 g	1.101 g	5.63	85%	15%
GERO w/DIFO						
Invaded	1.136 g	0.247 g	1.383 g	4.60	82%	18%
Carbon	0.650 g	0.214 g	0.864 g	3.04	75%	25%
Soil	0.805 g	0.233 g	1.038 g	3.45	78%	22%

*Mean AG:BG ratio by group: GEMA = 2.03; TEGR = 0.99; DIFO = 0.21; GERO w/GEMA = 5.87; GERO w/TEGR = 4.40; GERO w/DIFO = 3.70; *all* GERO groups = 4.66.

Appendix C

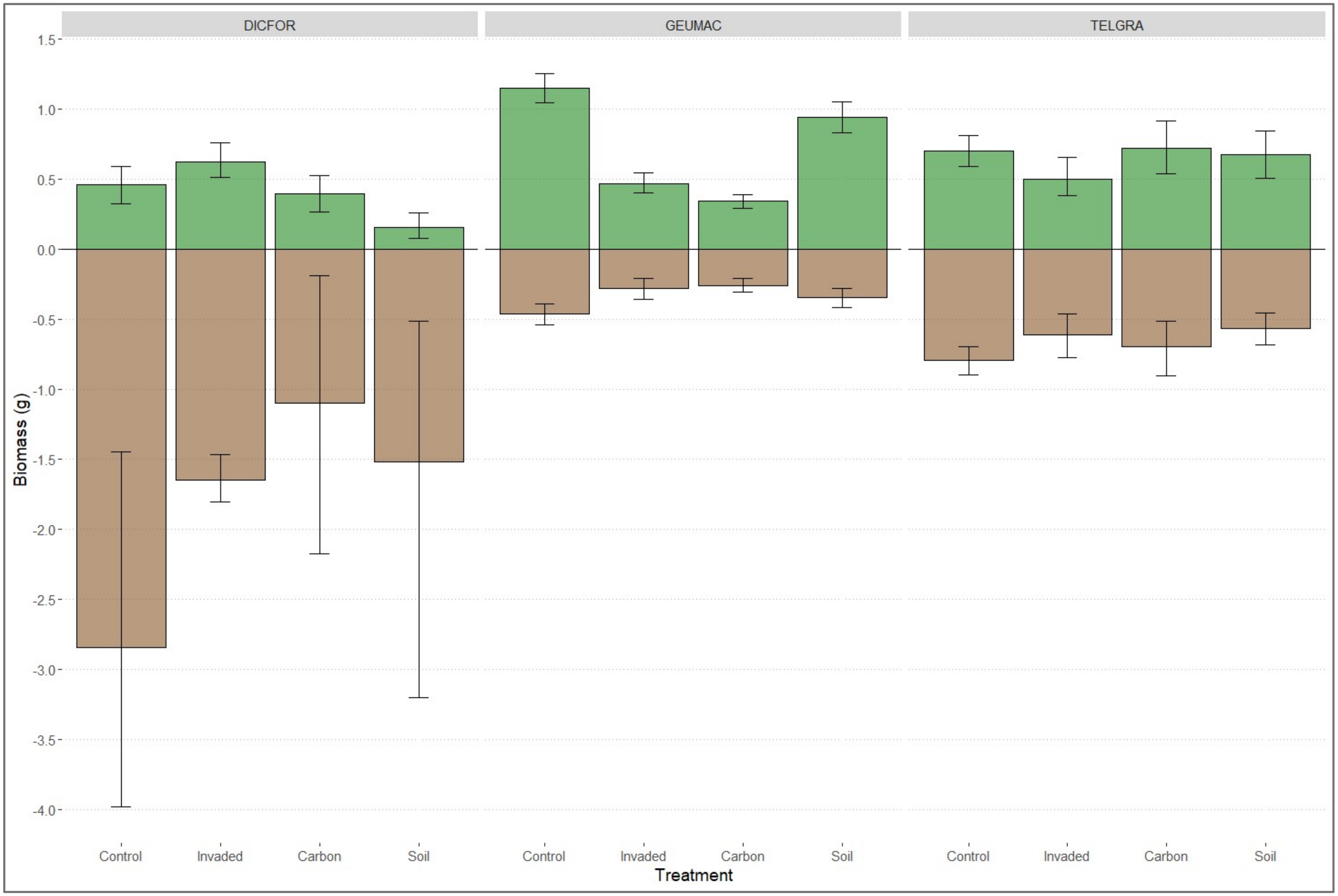

Figure 1. Effect of treatments on biomass of all three native species; green and brown portions represent aboveground and belowground biomass, respectively (note: numbers on y-axis are not negative). Values are means and standard errors for each treatment group. DICFOR = *Dicentra formosa*; GEUMAC = G*eum macrophyllum*; TELGRA = *Tellima grandiflora*