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# Effects of Increased Nitrogen Deposition on the Dominance of Hedera helix in the Pacific Northwest

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Effects of Increased Nitrogen Deposition on the Dominance

of *Hedera helix* in the Pacific Northwest

by

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**ABSTRACT**

Invasive species have the potential to cause severe ecological and economic harm by altering ecosystem processes such as nutrient cycling, disturbance regimes, and hydrology; and by causing loss of biodiversity and possible extinction of native species. Though *Hedera helix* is one of the most prolific invaders of the Pacific Northwest, little empirical evidence exists on the mechanisms behind its invasion. Increased nitrogen deposition has been shown to promote the dominance of invasive species, and nitrogen deposition rates have steadily increased in most of the Western United States in recent years due to population increases. The purpose of this study was to determine if increased rates of nitrogen deposition are contributing to the success of *H. helix* in the Pacific Northwest. Plots were established in Lesser Park in Portland, Oregon and received monthly treatments of ammonium nitrate for one year. Growth, measured as change in percent cover, was compared between treated and untreated plots for both *H. helix* and native species. Additionally, a greenhouse experiment was employed wherein *H. helix* was grown in shared pots with two native species, *Fragaria vesca* and *Polystichum munitum*, and three treatment levels were compared; none, low, and high nitrogen. Relative growth rate was compared between treatments for each species after twelve weeks of treatment.

Results from the greenhouse experiment were highly variable and no general conclusions could be drawn about the effects of increased nitrogen deposition on competition between *H. helix* and native species. In the field, treatment did not have a significant effect on growth but species did have a significant effect, as *H. helix* had a

greater increase in percent cover than native species regardless of treatment. However, a marginally significant interaction was found between species and treatment. Native species cover actually increased in plots that received nitrogen addition and decreased in control plots, while *H. helix* had a slightly higher increase in cover in control plots. Though the growth of *H. helix* was significantly higher than the growth of native species in control plots, nitrogen addition caused the growth rates to converge towards more similar means in treated plots. Results indicate that increased nitrogen deposition may actually have a positive effect on the growth of native species by reducing the invasive potential of *H. helix*.

# ACKNOWLEDGEMENTS

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**INTRODUCTION**

As the human population has grown, activities such as agriculture, industry, and commerce have increased, which can all have major impacts on ecosystems. Impacts include transformation of the landscape, alteration of major geochemical cycles, and biotic additions and losses (Vitousek et al. 1997a). Additionally, these activities can have synergistic effects, such as in the case of increased nitrogen deposition and the introduction of invasive species. Invasive species are defined in Executive Order 13112 (1999) as "an alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health." Invasive species are one of the biggest threats to biodiversity, second only to habitat loss (Wilcove et al. 1998), and can alter ecosystem processes such as nutrient cycling, natural fire regimes, and hydrology (Mack et al. 2000).

Increased nitrogen deposition and the subsequent increase in soil nitrogen have been identified as key drivers in the dominance of invasive species and decrease in biodiversity (Vitousek et al. 1997b). Nitrogen deposition has dramatically increased in the last 200 years due to human activity (Phoenix et al. 2006), and models predict that by the year 2100 nitrogen deposition will be the third largest threat to biodiversity after land use and climate change, with invasive species ranking fourth (Sala et al. 2000).

This study aims to examine the possible relationship between increased nitrogen deposition and the dominance of a widespread invader, *Hedera helix*, through both a field study in Portland, Oregon, and a greenhouse study. *H. helix* has significantly altered the landscape of the Pacific Northwest, and successful identification of the mechanisms behind this invasion is essential for preservation of our endangered ecosystem.

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**BACKGROUND**

**Nitrogen Deposition Rates**

Global nitrogen emissions are estimated to be almost four times higher than before the industrial and agricultural revolutions (Phoenix et al. 2006), and it is predicted there will be a nearly 10-fold increase by 2050 (Galloway et al. 2004). In some biodiversity hotspots nitrogen deposition is 50% greater than it was globally just 20 years ago (Phoenix et al. 2006). However, many developed areas that once suffered from increasing deposition rates have now stabilized emission rates and deposition is declining, whereas developing regions will see a large increase in deposition in coming years due to growing populations and industry (Phoenix et al. 2006).

Nitrogen deposition rates have steadily increased in most of the Western United States in recent years due to population increases (Fenn et al. 2003). $NO_x$ is the largest contributor and typically accounts for 50 to 75% of total atmospheric nitrogen deposition in the U.S. (Greaver et al. 2012), mostly resulting from automobile exhaust and industrial and power plant emissions. $NH_3$ emissions predominantly originate from fertilized crops, animal feeding operations, and more recently, automobiles (Greaver et al. 2012). Unlike $NO_x$ emissions, however, $NH_3$ emissions are not regulated by the EPA. It is projected that $NO_x$ emissions in the Western U.S. will decrease by 28% by 2018 due to stricter emission controls and improved technology, and $NH_3$ emissions will increase by 16% (Fenn et al. 2003), which should ultimately result in an overall reduction of nitrogen deposition.

Deposition rates in the Portland area from 2002-2008 have been estimated using the Community Multiscale Air Quality (CMAQ) model v4.7 (Schwede et al. 2009),

which was developed by the U.S. EPA Atmospheric Modeling Division. Total nitrogen is measured as wet and dry reduced ($NH_4^+$) and oxidized ($NO_3^-$) species. Nitrogen deposition rates in 2002 were estimated at 9.22 kg N ha$^{-1}$, and fluctuate the following years with an overall increase to 10.02 kg N ha$^{-1}$ by 2008. The global average deposition rate in the mid-1990's of 3.5 kg N ha$^{-1}$yr$^{-1}$ (Phoenix et al. 2006) suggests that deposition rates have dramatically increased here in the last twenty years. Though models predict that nitrogen deposition rates in much of the U.S. will slightly decline in the future, it is important to study how these increased rates are impacting our ecosystems.

**Impacts of Nitrogen Deposition on Plant Communities**

Plants need large quantities of nitrogen to photosynthesize, grow, and reproduce (Gurevitch et al. 2006); and it has often been considered the most limiting nutrient in temperate terrestrial ecosystems (Vitousek and Howarth 1991). An increase in anthropogenic nitrogen deposition can therefore have many implications for naturally limited ecosystems and the plant communities that inhabit them. A whole suite of changes, both direct and indirect, may occur in plant tissues and soil that can affect a particular plant species or the plant community as a whole.

One well-documented effect of increased nitrogen deposition is the acidification of soil, which can lead to decreased buffering capacity, the depletion of base cations needed by plants, and increases in toxic metals (Bobbink et al. 1998, Bowman et al. 2008, Gilliam 2006, Phoenix et al. 2012). Bowman et al. (2008) found that extractable $Mg^{2+}$, $Ca^{2+}$, $K^+$, and $Mn^{2+}$ all decreased significantly with nitrogen addition, resulting in less

nutrients available for plants as well as less cations available to act as soil buffers. Acidification also results in an increase in toxic metals $Al^{3+}$ and $Fe^{3+}$ as they replace depleted base cations (Bowman et al. 2008, Phoenix et al. 2012). Aluminum can inhibit root growth and uptake of $Ca^{2+}$, and coupled with base cation loss can lead to foliar injury, increased susceptibility to temperature stress, and a decrease in aboveground biomass (Bowman et al. 2008).

Increased nitrogen deposition can also lead to increased sensitivity to secondary biotic and abiotic stresses. Plants may show greater susceptibility to pathogens and herbivory and increased damage from extreme climatic events such as drought and freezing (Bobbink et al. 1998, Gilliam 2006). These stresses may also interact; for example nitrogen storage in plant tissue can lead to a greater incidence of herbivory, which may increase the likelihood of pathogens (Gilliam 2006).

Elevated nitrogen levels can also directly affect plant community composition and biodiversity. Long-term increased nitrogen deposition will lead to an accumulation of nitrogen compounds in the soil and a gradual increase of nitrate and ammonium available to plants (Bobbink et al. 1998). Nitrophilous species will fare better under these conditions and will have a competitive advantage over species less adapted to nitrogen enriched soil (Bobbink et al. 1998, Phoenix et al. 2006). Fast growing species also have an advantage over slower growing species that cannot uptake and utilize the soil nitrogen as quickly (Funk and Vitousek 2007, Phoenix et al. 2006). Additionally, due to increased soil acidity species that are more acid-tolerant will have an advantage (Bobbink et al.

1998). Therefore, increased nitrogen deposition will likely shift community composition towards fast-growing, nitrophilous, and acid-tolerant species.

**Nitrogen Deposition and the Dominance of Invasive Species**

This shift towards an environment that favors such species may also aid in the facilitation of invasion by non-native species. Invasive species may originate from an environment with acidic soil or high levels of nitrogen, or both. Therefore, as soils become less hospitable for native species not adapted to those conditions, invasive species are able to thrive and gain a competitive advantage. Additionally, adaptability (Prentis et al. 2008) and phenotypic plasticity (Daehler 2003, Davidson et al. 2011) are traits common to invasive species that allow them to thrive in many different habitats. Even if the native habitat of an invasive species was not nitrogen rich or highly acidic, it may just be better able to adapt to changing conditions. Another possible mechanism for successful invasion is a superior response to a resource (Shea and Chesson 2002), so as nitrogen levels increase superior resource competitors will gain an advantage.

Further exacerbating the problem, a feedback loop may exist wherein added nitrogen can facilitate an invasion, and the invasion can lead to changes in nutrient cycling and further increases in soil nitrogen (Ehrenfeld 2003). This in turn can help spread the invasion or secure the establishment of the invasive species. Invasive species can alter nutrient cycling by changing the soil microbial community associated with different plant species, changing the physical properties of the soil, or changing the plant functional types (Ehernfeld 2003). This last possibility can have a particularly strong

impact on nutrient cycling if the invasive species is a N-fixer or if it replaces N-fixing species.

Several studies have found a positive correlation between increased levels of nitrogen addition and the dominance of invasive species. In a field study in the Mojave Desert, invasive grasses and forbs increased in biomass while native species decreased in biomass after nitrogen addition (Brooks 2003). The authors speculated that the decrease in native biomass was likely due to increased competition for light and other resources as a result of greater invasive biomass. More commonly studies have found that invasive species increase in biomass with increasing nitrogen levels while native species either show no response or experience significantly less of an increase in biomass than invasive species (Littschwager et al. 2010, Lowe et al. 2003, Rickey and Anderson 2004). Conversely, nitrogen addition to plants common to the coastal sage scrub of California in a greenhouse experiment resulted in a greater relative yield of native shrub species than invasive grass and forb species, though all species increased in biomass by a factor of 1.5 to 2.5 (Padgett and Allen 1999). However, it was noted that field observations showed native shrubs responding unfavorably to elevated levels of nitrogen, and it is possible that the short duration of this experiment (3 months) did not allow for simulation of long-term results.

Many nitrogen addition experiments have focused on grasslands, deserts, and shrublands (Brooks 2003, Padgett and Allen 1999); all ecosystems that are known to be nitrogen limited. Nitrogen has often been considered the most limiting nutrient in temperate terrestrial ecosystems (Vitousek and Howarth 1991) but it is unclear how

limiting it may still be in the Pacific Northwest due to the large increase in deposition the last couple decades (Phoenix et al. 2006), especially in urban centers such as Portland. Additionally, grasses are the most commonly studied invasive species tested in nitrogen addition experiments (Brooks 2003, Lowe et al. 2003, Rickey and Anderson 2004), with far fewer focusing on herbaceous and woody plants. This represents a gap in our understanding of how increased nitrogen deposition may affect herbaceous or woody invasive species in forested ecosystems.

### *Hedera helix*

A widespread and particularly invasive species in Oregon is English ivy, which has the potential to form dense understory monocultures and crowd out native vegetation, as well as climb and eventually kill trees (Okerman 2000, Soll 2005). Though invasive ivy found in the Pacific Northwest is commonly referred to as English ivy, or *Hedera helix*, recent genetic testing of ivy populations in British Columbia, Washington, and northern Oregon found that the majority of populations sampled were *H. hibernica* (Atlantic or Irish ivy) (Clarke et al. 2006). However, this classification has not yet been recognized by North American taxonomic sources (Waggy 2010) and the accepted range of *H. hibernica* in the U.S. is currently limited to North and South Carolina (USDA 2012a). Additionally, there is debate over whether *H. hibernica* is a subspecies of *H. helix* or its own distinct species (Metcalfe 2005). Therefore, invasive ivy found in the Pacific Northwest commonly identified as English ivy will hereafter be referred to as *H. helix*.

*H. helix* belongs to the Araliaceae (ginseng) family and is native to Eurasia. It was brought over to the U.S. by early colonial settlers as an ornamental (ODA 2012), and its presence is documented in the U.S. as early as the late 1700s (Wells 2000). *H. helix* has since been widely sold as an ornamental, but the propagation, transport, and sale were banned by the Oregon Department of Agriculture in June 2010. However, many homeowners still continue to incorporate it into their gardens and landscapes. Its occurrence is reported in 33 states and Washington D.C., and it is considered invasive in 18 of those states as well as Washington D.C. (EDDMapS 2012). Though distributed throughout much of the U.S., it is considered most invasive in the Pacific Northwest (Waggy 2010), and in Oregon is considered most invasive west of the Cascades (ODA 2012). Even in some areas of its native European habitat it is considered a weed due to its aggressive growth (Metcalfe 2005) and ability to affect richness and composition of valued communities (Marrs 2010). However, Morisawa (1999) suggested that it is only a pest in disturbed habitats of its native region.

*H. helix* is a woody evergreen vine with long trailing stems (ODA 2012) and two distinct growth phases, the juvenile phase and the adult phase (Soll 2005, Waggy 2010). Leaves are dark green, alternate, and waxy, and typically juvenile leaves have 3 to 5 lobes while adult leaves are unlobed and ovate to rhombic (Soll 2005, Swearingen and Diedrich 2009). As a juvenile, *H. helix* has small root-like structures which exude an adhesive substance that allow it to climb surfaces (Soll 2005). As an adult, it has erect, woody non-climbing stems that are more akin to a shrub than a vine (Okerman 2000). Vegetative reproduction occurs through stem or root fragments and sexual reproduction

through seed dispersal by birds, though *H. helix* is more commonly spread vegetatively (Waggy 2010). Mature forms of *H. helix* are better adapted to high light levels and have better photosynthetic capacity (Okerman 2000, Waggy 2010), so juvenile forms typically form dense ground cover and as they climb trees they transition into the adult phase (Waggy 2010). The juvenile stage often lasts for 10 years or more (Swearingen and Diedrich 2009, Waggy 2010).

*H. helix* grows along the ground creating ivy deserts by outcompeting native species, and climbs up into tree canopies where it can engulf branches and cause loss of tree vigor and eventual death (Soll 2005). Older vines can reach up to 1 foot in diameter (Swearingen and Diedrich 2009, Waggy 2010), and the added weight of the vines can steadily weaken trees and make infested trees much more susceptible to blow-over (Soll 2005). It is not uncommon for *H. helix* to climb as high as 90 ft (Okerman 2000, Waggy 2010), and it has been known to reach heights of 300 ft and climb as fast as 30 ft per year (Soll 2005). Native grasses, herbs, and trees can all be displaced by *H. helix* due to intense competition for light and nutrients, which results in a decrease in native vegetation and a loss of wildlife habitat (Okerman 2000). Consequently, sites infested with *H. helix* can lead to a lower diversity of birds, mammals, and amphibians (Soll 2005). For example, though berries of *H. helix* may be eaten by native birds, they have been shown to attract mostly non-native starlings (Soll 2005).

*H. helix* thrives in deciduous or mixed conifer-deciduous forests, and typically prefers shade, damp soils, and a cool environment (Okerman 2000). It is adaptable to different light levels so it also grows well in tree canopies, and does well in both basic

and acidic soil (Okerman 2000, Waggy 2010). *H. helix* can thrive in most environments under 3,000 feet in elevation and is widespread in urban and disturbed forests (Waggy 2010). Indeed, there are hundreds of different cultivars of *H. helix*, and many are adapted to different types of environments (Okerman 2000).

Manual, mechanical, and chemical removal may be employed to manage *H. helix*, though currently there are no known biological control agents (Swearingen and Diedrich 2009). ODA (2012) estimates that removal of *H. helix* from Oregon parks costs $2000 per acre, while the Nature Conservancy estimates mechanical and manual removal costs $2000 to $8000 per acre (Soll 2005). Chemical control can cost $100 to $500 per acre, though chemical removal may not be as effective as manual removal due to the waxy layer on leaves inhibiting herbicide absorption in leaves (Soll 2005). Though Portland land managers have been aggressively fighting to control *H. helix* for decades, it continues to persist and spread in our urban and natural environments. Therefore, it is important to identify the mechanisms behind *H. helix* invasions so we are better able to fight this prolific invader.

**Mechanisms of Invasion**

Three overall factors contribute to a successful invasion: propagule pressure, the invasive characteristics of the new species, and the invasibility of the site (Davis et al. 2000). Propagule pressure is the number of individuals entering a new environment, and will be more successful the greater number of propagules, the more release events, and the healthier the propagules (Lockwood et al. 2007). Propagule pressure will help a

population become established, but it is the characteristics of the introduced species and the environment that determine if this new species will spread and become invasive. Due to the devastating impacts invasive species can have on ecosystems, several studies have investigated what traits make for a successful invader (Daehler 2003, Ren and Zhang 2009) as well as what traits make a community more invasible (Davis et al. 2000, Hobbs and Huenneke 1992).

Commonly studied mechanisms that allow plants to become invasive include adaptability and plasticity, allelopathy, and superior resource competition. Often plants possess more than one of these characteristics, and it may be the interaction of these that allows the plant to successfully invade. Additionally, the same plant may invade different environments using different mechanisms (Ren and Zhang 2009). Though many studies have examined the characteristics of *H. helix*, few have empirically tested if these characteristics are actual mechanisms behind its invasiveness.

Rapid adaptation and phenotypic plasticity are often cited as characteristics of successful invaders (Bossdorf et al. 2005, Prentis 2008, Daehler 2003). Invasive species may evolve by genetic drift in founder populations, inter or intraspecific hybridization, or adaptive evolution due to changes in selection pressures (Bossdorf et al. 2005). Phenotypic plasticity, the ability of a genotype to alter its phenotypic expression dependant on environmental conditions (Bradshaw 1965), could alternately allow an invader to tolerate a variety of different environments, or disturbed environments where conditions are constantly in flux (Daehler 2003). As previously noted, *H. helix* has hundreds of different cultivars adapted to different types of environments (Okerman

2000), and it has been shown to thrive in sun and shade as well as basic and acidic soil (Okerman 2000, Waggy 2010). This could either be due to rapid adaptation or phenotypic plasticity, but whatever the mechanism it is likely one of the reasons *H. helix* is such a successful invader.

Allelopathy, the release of a toxic chemical by plants, has been proposed as the mechanism behind the success of some invasive species (Bais et al. 2003, Callaway and Ridenour 2004). This mechanism can be described by the novel weapons hypothesis, which states that non-native plants may exude toxic chemical substances that native plants have never encountered and have not developed a defense against (Callaway and Ridenour 2004). Without a defense mechanism, native populations will decline and invasive populations will benefit from greater resource availability (Shea and Chesson 2002). Very few studies have tested if *H. helix* possesses any allelopathic abilities, though Biggerstaff and Beck (2007) found a marginally significant reduction in germination of a native seed when *H. helix* was present, suggesting possible allelopathic effects. Bonanomi et al. (2006) found that the decomposing litter of *H. helix* exhibited allelopathic effects, though interestingly they found phytotoxicity in 88% of a functionally diverse group of species.

Superior response to resources is another possible mechanism for successful invasion. Invasive species often possess life history traits such as high growth rates, early reproduction, and a high number of offspring, which will give them a competitive advantage in nutrient-rich environments (Funk and Vitousek 2007). Additionally, if an environment is limited by a particular resource, the invader may attain a competitive

advantage by acquiring the resource at a faster rate and/or more efficiently converting it into a gain (Shea and Chesson 2002). Invasive species may also be better at conserving resources in a nutrient-limited environment which will maximize resource use efficiency, or carbon assimilation per unit of resource (Funk and Vitousek 2007). A pair-wise comparison of phylogenetically related native and invasive species found that invasive species showed higher rates of carbon assimilation than native species due to higher light-use efficiency and nitrogen-use efficiency (Funk and Vitousek 2007). The rapid growth rate of *H. helix* may be a result of superior resource competition. This could be advantageous in both nutrient-poor and nutrient-rich environments if it is able to acquire and effectively utilize resources at a faster rate than native species, though higher resource availability would allow faster growth and a greater competitive advantage.

Resource availability is essential for establishment of an introduced species, so an increase in the amount of unused resources can enhance the invasibility of an ecosystem (Daehler 2003, Davis et al. 2000, Shea and Chesson 2002). Unused resources allow invasion from species that may not be superior resource competitors, and creates an easier path of invasion for those that are. An increase in resource availability may occur due to a decline in resource use from native populations or from an increase in resources above the needs of native populations or faster than they can sequester them (Davis et al. 2000). Increased nitrogen deposition can therefore make a plant community more susceptible to invasion if nitrogen levels exceed the requirements of present populations or if populations cannot sequester nitrogen at the enhanced deposition rate. An ecosystem experiencing an increase in resource availability will be especially susceptible to invasion

by a superior resource competitor that is able to sequester those resources at a faster rate than native species. It may be this combination of an increase in nitrogen deposition and superior resource competition from *H. helix* that is facilitating invasion in the Pacific Northwest.

### *H. helix* and Nitrogen Deposition

*H. helix* is ubiquitous in the Portland landscape and can be seen along roadsides and in most natural areas. The Oregon Department of Transportation (ODOT) once planted *H. helix* along highways for aesthetics and erosion control but stopped these practices about a decade ago. However, *H. helix* still remains prevalent along roadsides even outside of the scope of ODOT plantings. This could be due to the rapid spread of *H. helix* from planted areas, or roadways could also act as transportation corridors with automobiles increasing propagule pressure. Another possibility is that nitrogen deposition is higher along roadsides due to nitrogen emissions from automobiles, and this increased nitrogen deposition facilitates invasion. The propagule pressure created by already established populations or transportation corridors could also act in concert with nitrogen deposition, with the former facilitating the spread and establishment of *H. helix* and the latter increasing its competitive advantage.

Though *H.helix* is reported to do well in a range of soil acidity, the Plant Conservation Alliance suggests it prefers slightly acidic conditions with a pH of 6.5 (Swearingen and Diedrich 2009), and it was reported in the United Kingdom to favor soil with a pH of 6.0 or above (Waggy 2010). Therefore, soil that has become more acidic

due to increased nitrogen deposition may create ideal growing conditions for *H. helix*, giving it a competitive advantage over less acid-tolerant native species. Additionally, a literature review of *H. helix* in the United Kingdom found it grows best in moist fertile soils or very fertile soils that are fairly dry to slightly damp (Metcalfe 2005). As the Pacific Northwest has a generally wet climate except for the dry summer months, added nitrogen may promote ideal soil conditions for *H. helix* to thrive. Finally, high nitrogen deposition creates nutrient-rich soil, which may give a fast grower and possible superior resource competitor such as *H. helix* a competitive advantage. Therefore, acidic fertile soil and the high growth rate of *H. helix* may all contribute to its success under high nitrogen conditions.

Though a small number of publications have asserted that *H. helix* is a nitrogen indicator (Binggeli 2005, Yang 2012), little empirical evidence was found to support this claim. Lameire (2000) found that *H. helix* cover increased over a 20 year period in a deciduous forest where nitrogen deposition also increased, but correlation does not imply causation. Vidra et al. (2006) also found a positive correlation in an urban riparian forest between exotic vine richness and soil fertility, with *H. helix* included as a vine. Many nutrients were analyzed but nitrogen was not due to difficulty in obtaining accurate measurements. To date no studies have explored the possible relationship between increased nitrogen levels and the dominance of *H. helix* in the Pacific Northwest.

Successful identification of the conditions under which *H. helix* thrives is the first step in advancing our knowledge on how to control existing populations and prevent further spread. If it is found that increased nitrogen deposition does lead to the dominance

of *H. helix*, new management strategies can be implemented such as focusing prevention and eradication efforts in high nitrogen areas like roadsides, or soil nitrogen reduction through practices such as soil C addition or topsoil removal.

**THIS STUDY**

**Goal and Hypotheses**

The goal of this study was to determine if increased levels of nitrogen deposition affect the growth of *Hedera helix* and its ability to compete with native species. This was achieved through both a field study and a greenhouse study. Plots were established in the field to observe changes in percent cover of *H. helix* and native species after a year of added nitrogen deposition. In the greenhouse *H. helix* shared pots with two native species, *Fragaria vesca* and *Polystichum munitum,* to determine differences in relative growth rates among three levels of nitrogen treatments after twelve weeks.

I hypothesized that *H. helix* would have a positive response to added nitrogen by showing a greater increase in percent cover in treated plots than control plots in the field, and showing a greater increase in relative growth rate in high nitrogen treatments in the greenhouse. I also hypothesized that *H. helix* would be able to more quickly uptake the added nitrogen than native species or more efficiently convert the added nitrogen into a gain, so native species would show no change or a significantly smaller increase in cover or relative growth rate.

**METHODS**

**Field experiment**

*Site description*

Experimental plots were established in Lesser Park located in southwest Portland, Oregon. Portland is located at the northern end of the Willamette Valley at the confluence of the Willamette and Columbia Rivers, and has a temperate climate characterized by warm, dry summers and mild, wet winters. The Portland metropolitan area had a population of 2.26 million as of July 2011 (U.S. Census Bureau), but despite being Oregon's most populated city maintains over 11,000 acres of parks and natural areas (Portland Parks and Recreation 2012).

Lesser Park covers 8.39 acres and is located directly next to Portland Community College Sylvania in a highly populated residential area. It was selected due to the prevalence of *H. helix* and because Portland Parks had no plans for mitigation throughout the course of the study. It is a mixed evergreen-deciduous forest with a very gentle west-facing slope. Trees common to the park include *Acer macrophyllum*, *Pseudotsuga menziesii*, *Corylus cornuta*, and *Acer circinatum*. Common groundcover includes native species such as *Polystichum munitum*, *Rubus ursinus*, and *Berberis nervosa*, though it is also heavily infested with invasive species such as *H. helix*, *Rubus discolor*, and *Lamiastrum galeobdolon*. Soil in the park is mostly silt loam and slightly acidic with an average pH of 6.1.

*Nitrogen treatments*

Sixteen 3 x 3 m plots were established within the park, with the two closest plots located 4 m apart. Plots that were chosen had an initial *H. helix* cover ranging from 15% to 55% so that there would be enough *H. helix* established to respond to the nitrogen deposition, but not so much that a response could not be observed. Half of the plots were randomly assigned as control and the other half as treated.

Treated plots received nitrogen in the form of ammonium nitrate ($NH_4NO_3$) at a rate of 3 g N m$^{-2}$yr$^{-1}$, a rate three times greater than the background atmospheric nitrogen deposition rate in Portland as of 2008 (10 kg N ha$^{-1}$yr$^{-1}$ = 1 g N m$^{-2}$yr$^{-1}$) (EPA 2012). Fertilization began August 15, 2011 and occurred monthly in twelve equal applications of 0.25 g N m$^{-2}$, with the final treatment occurring July 15, 2012. Ammonium nitrate was dissolved in 1.25 L of water and sprayed evenly across treated plots. A volume of 1.25 L was chosen so that there would be enough water to spray the plots evenly and to prevent foliar burning (Jim McKay 2011, personal communication). Nitrogen was applied in the form of wet deposition to facilitate even application of a small quantity of dissolved ammonium nitrate pellets. Also, as Portland is typically under drought conditions in the summer, the water helped ensure that the ammonium nitrate would be absorbed by leaves or into the soil for summer treatments. Control plots were sprayed with 1.25 L of water without ammonium nitrate.

*Variables Measured*

In order to determine the difference in growth of *H. helix* and native species between treatments, percent cover was measured at two time points. Initial measurements of percent cover of each species were recorded in the center square meter of each plot on August 16, 2011 and August 18, 2011. Final measurements of percent cover were recorded on August 17, 2012. Only the center square meter of each plot was measured to minimize edge effects, as *H. helix* is a far-reaching vine that has the potential to grow from one plot to another. Growth of each species was calculated as the change in percent cover from the beginning of the experiment to the end. Initial *H. helix* cover in the entire 3 x 3 m plot was also recorded before treatments began.

To explore the relationship between growth and covariates, measurements were also taken of other potential predictors. Soil moisture and canopy cover were measured in each plot in the summer and winter. Summer values were determined by averaging measurements taken in August 2011 and August 2012; and winter values were recorded in February 2011. Canopy cover was measured using a concave spherical densiometer (Model C) in the four cardinal directions and averaged for each plot. Soil moisture was recorded with a HydroSense (12 cm) at three locations in each plot and averaged; in the center and in two opposing corners of the center square meter.

*Statistical Analysis*

Data were analyzed using SPSS 19.0, including correlation analysis and a two-way Analysis of Covariance (ANCOVA). The change in percent cover of all native

species in each plot was combined for statistical analysis. This was necessary because on average each native species was present only in three plots, with several species only present in one. Therefore, for each plot the change in percent cover of all native species found in that plot were added together for one number representing change in percent cover of native species.

The relationship between change in percent cover and all other covariates was tested using the Pearson product-moment correlation coefficient. It was determined that the data met the assumptions of the ANCOVA model, so any covariates found to be correlated with change in percent cover were included in a two-way ANCOVA test. A two-way ANCOVA was used to determine the effects of treatment (nitrogen addition versus no addition) and species (*H. helix* versus native species) on change in percent cover. The Tukey test was used for post-hoc analysis to determine significance among factors.

**Greenhouse experiment**

*Experimental Design*

In order to determine competitive differences between *H. helix* and native species, a greenhouse experiment was carried out for 12 weeks between *H. helix*, *Polystichum munitum*, and *Fragaria vesca*. *P. munitum* (sword fern) is a large evergreen fern native to the west coast and ranges from Alaska down to southern California (Crane 1989). It is dominant in the understory of Pacific Northwest forests and can often be found growing alongside *H. helix*. The preferred habitat of *P. munitum* is moist coniferous forests at low

to middle elevations (Pojar and Mackinnon 2004, Calflora 2012), though it is a very hardy plant that can survive in sun or shade and wet or dry soil (WNPS 2012). *F. vesca* (woodland strawberry) is a deciduous perennial herb that produces runners and has a wide range that includes all of the U.S. except for the southeast and Nevada (USDA 2012b). It can be found in a variety of habitats and in the western U.S. it commonly occurs in wooded areas and can be found in all but the driest forest types (Munger 2006).

All three species were placed in a one gallon pot together and given one of three nitrogen treatments (none: 0 g N m$^{-2}$; low: 3 g N m$^{-2}$; or high: 9 g N m$^{-2}$) with 8 pots per treatment, for a total of 24 pots. Soil was collected from private property in Sandy, Oregon on June 2, 2012, and mixed in a 1:1 ratio with Pro-Gro 5F soil blend on June 4. The 5F blend consists of 33% fine bark, 33% peat moss, and 33% pumice. The 1:1 ratio of native soil to soil blend allowed the potting mixture to be as close to native soil conditions as possible while still allowing for drainage and aeration.

*H. helix* was collected from Balch Creek watershed in Forest Park on June 5 and Lesser Park on June 6. Forest Park is a mixed evergreen-deciduous forest located on the east side of the Tualatin Mountains in northwest Portland, and at over 5,100 acres is the largest forested natural area in the U.S. The *H. helix* was cut in approximately twelve inch segments with two to five leaves on each so that all *H. helix* would have a similar biomass at the start of the experiment.

Upon collection *H. helix* was placed in a Ziploc bag with a wet paper towel and then brought to the greenhouse and immediately transferred into pots. *H. helix* was evenly distributed among each treatment so that for each treatment level half of the plants

originated in Forest Park and the other half in Lesser Park. Two *H. helix* plants of the

same origin were transplanted to each pot and Kangaroots Root Drench was applied to all

pots on June 6 at a concentration of 2 tsp per gallon of water. Kangaroots is an organic

formula containing macro and micronutrients as well as microbes, and is designed to

encourage root mass development. The *H. helix* plants immediately began to wilt and

show transplant shock, so they were thoroughly misted several times a day for the first

ten days. One *H. helix* plant was removed from each pot two weeks after transplant,

leaving the one that looked the healthiest and most likely to survive so that only one plant

remained per plot.

The *P. munitum* and *F. vesca* were purchased from Bosky Dell Natives, located in

West Linn, OR, in 4" pots, and then transplanted into the pots containing *H. helix* on June

8 and 9. Kangaroots Root Drench was once more applied after all plants were

transplanted to the experimental pots. Roots of each plant were thoroughly washed to

remove the original potting soil from the 4" pots and the fresh weight of each plant was

recorded. *Gaultheria shallon* was originally included in the experimental design and was

also transplanted to the gallon pots. The original location of the plants in each pot placed

the *H. helix* in the middle with the three native species placed in a triangular pattern

around the *H. helix*. However, the *G. shallon* did not respond well after transplant and

most plants began to die, so all *G. shallon* were removed from pots before the experiment

began.

Pots were randomly assigned to one of three treatments (no nitrogen, low

nitrogen, or high nitrogen). Nitrogen was again added as ammonium nitrate dissolved in

water, and applied once every two weeks beginning June 26. Pots in the control group, no nitrogen, received only water. The water capacity of the pots was tested and determined to be 100 mL, so ammonium nitrate was added to 100 mL of water for the first two treatments. However, some drainage was observed after these treatments, and though it was minimal, only 80 mL of water was applied for the remaining four treatments. Plants were watered as needed, which was almost every day throughout the duration of the experiment.

A fungal leaf scorch as well as white flies and aphids were observed on the leaves of *F. vesca* on September 6. AzaMax was applied to all plant material the following day at a rate of ½ tbsp per quart of water, which is in the low to moderate application range. The active ingredient in AzaMax is Azadirachtin, an all-natural botanical insecticide; and acts by way of repellence, anti-feedance, and interference with the molting process. After the AzaMax dried, a spot treatment of Safer Fungicide was applied to *F. vesca* growing in pots at a rate of 2 tbsp per quart of water. Safer Fungicide is an organic fungicide that utilizes sulfur to inhibit the attack of fungal disease. Fungicide was not applied to the runners of *F. vesca* to avoid damage to tender new growth which had already been sprayed with AzaMax. Safer Fungicide was applied again on September 11 at the same rate to all plants because the leaf scorch fungus was still spreading on the *F. vesca*. Additionally, some of the most infected leaves were removed from17 out of 24 of the *F. vesca* plants and placed in bags. It was also observed on this date that a different fungus, *Rhizoctonia solani*, had developed on the *P. munitum* and the *H. helix*.

The planned date of harvest was September 18, but due to the spread of fungus harvesting began on September 13, eight days after the final nitrogen treatment. Plants were harvested September 13 and September 16, with an equal number of plants from each treatment harvested on each day. On each day of harvest plants were removed from soil, the roots thoroughly washed, and then plants were weighed and placed in paper bags. All of the *F. vesca* plants that had leaves removed were harvested on the 13th so that clippings could go into the drying oven without any further decay. The paper bags were placed in a drying oven at 105˚C for 24 hours, after which plants were removed from the bags and weighed. One of the *H. helix* plants in the high nitrogen treatment died soon after the experiment began, so the final sample size for each treatment was (no nitrogen n=8; low n=8; high n=7).

*Statistical Analysis*

Growth of each plant was estimated by calculating the relative growth rate (RGR) from an inferred initial dry mass. Initial dry mass was inferred by calculating a conversion factor for each species. The final dry weight of each plant was divided by the final fresh weight, and these numbers were averaged for a single conversion factor for each species. The average conversion factor for *H. helix* was calculated as 0.309, so all fresh weights of *H. helix* were multiplied by 0.309 to calculate the inferred initial dry mass. The conversion factor for *F. vesca* was calculated as 0.389, and the conversion factor for *P. munitum* was calculated as 0.304. Averages and standard errors for each treatment level are given in Appendix A (Table A1).

The initial fresh weight of each plant was then multiplied by this conversion factor to estimate an initial dry mass. Relative growth rate for each plant was then calculated as RGR = $(\ln(W_2)-\ln(W_1))/(t_2-t_1)$ where $W_1$ and $W_2$ were plant dry weights at times $t_1$ and $t_2$ and time was measured in days (Hunt 1982). Estimated growth was used as opposed to final biomass to determine the effects of added nitrogen because of the large variation in initial mass of each species and the small sample size. For example, the fresh weights of *H. helix* ranged from 3.39 g to 12.43 g; the fresh weights of *F. vesca* ranged from 0.75 g to 3.66 g; and *P. munitum* had the largest spread ranging from 7.44 g to 34.64 g. This may have made detection of a response more difficult, as can be seen by the similar patterns in initial fresh weight and final dry mass (Appendix B, Figures B1 and B2).

Inferring initial dry mass relies on the assumption that water content of plant tissue did not change from the start of the experiment. Plants were thoroughly watered in the weeks leading up to the experiment and for the entire duration so water stress should not have contributed to a change in water content. Some studies have found that water content can change at different nitrogen levels or plant ages. Shimshi (1970) found that nitrogen deficient plants had higher water content due to lower chlorophyll content reducing stomatal openings and transpiration rates. However, Seginer et al. (2004) found that nitrogen deficient plants had a reduced water content. Conversion factors were calculated for each species at each treatment level and ANOVA tests showed no significant difference between treatments (Appendix A, Table A2), so it was assumed that nitrogen treatment did not significantly affect plant water content in this experiment.

Additionally, plants have exhibited lower water contents as they age (Treitel 1949), though it was assumed this would not be a factor in a 12 week experiment.

Growth of all species by treatment was separately tested for normality in SPSS 19.0 using the Shapiro-Wilk test. Data were not found to be normally distributed even after transformation, so the Kruskal-Wallis test was used to compare growth between treatments for each species. These tests were performed separately for each species for a total of three Kruskal-Wallis tests.

## RESULTS

### Field experiment

A total of 32 different species were observed in study plots, with 26 native and 6 non-native species recorded (Table 1). Four of the five non-native species besides *H. helix* were found only in one or two plots and with changes in percent cover at 5% or less after a year of treatment for each plot. The exception to this was *Lamiastrum galeobdolon*, which was only found in one untreated plot but had an increase in percent cover of 45%. The most commonly found species besides *H. helix* was *Rubus ursinus*, present in 15 out of 16 plots.

| Native species | |
|---|---|
| *Acer circinatum* | Vine maple |
| *Acer macrophyllum* | Bigleaf maple seedlings |
| *Alnus rubra* | Red alder |
| *Asarum caudatum* | Wild ginger |
| *Carex leptopoda* | Taperfruit shortscale sedge |
| *Claytonia sibirica* | Siberian miner's lettuce |
| *Corylus cornuta* | Western beaked hazelnut |
| *Galium aparine* | Cleavers |
| *Gaultheria shallon* | Salal |
| *Hydrophyllum tenuipes* | Pacific waterleaf |
| *Lonicera ciliosa* | Orange honeysuckle |
| *Mahonia aquifolium* | Oregon grape |
| *Maianthemum dilatatum* | False lily of the valley |
| *Nemophila parviflora* | Smallflower nemophila |
| *Osmorhiza chilensis* | Mountain sweet-cicely |
| *Polystichum munitum* | Sword fern |
| *Prosartes hookeri* | Hooker's fairybells |
| *Pteridium aquilinum* | Bracken fern |
| *Rosa gymnocarpa* | Baldhip rose |
| *Rubus parviflorus* | Thimbleberry |
| *Rubus ursinus* | Trailing blackberry |
| *Symphoricarpos albus* | Common snowberry |
| *Trillium ovatum* | Western trillium |
| *Vancouveria hexandra* | Inside-out flower |
| *Viola glabella* | Stream violet |
| *Vicia cracca* | Tufted vetch |
| **Non-native species** | |
| *Crataegus monogyna* | European hawthorn |
| *Geranium robertianum* | Herb robert |
| *Geum urbanum* | Wood avens |
| *Hedera helix* | English ivy |
| *Lamiastrum galeobdolon* | Yellow archangel |
| *Rubus armeniacus* | Himalayan blackberry |

**Table 1**. **All species recorded in study plots.**

Change in percent cover of *H. helix* in the sample area was significantly and

positively correlated with change in initial percent cover of *H. helix* in the entire plot (r =

0.51, p = 0.04) (Table 2, Figure 1), indicating that plots that had higher initial cover of *H.*

*helix* experienced a higher growth rate. Control plots had an average initial percent cover

of *H. helix* in the entire plot of 34.38 % (S.E. 3.46%) and treated plots had an average

initial percent cover of *H. helix* in the entire plot of 26.25% (S.E. 3.75%). Figure 2 shows

the spread of data for initial percent cover of *H. helix*. All other covariates measured-

summer moisture, winter moisture, summer canopy, and winter canopy- were not

significantly correlated with change in percent cover of *H. helix* (p > 0.05) (Table 2).

| | | Entire plot initial *H. helix* cover | Summer moisture | Winter moisture | Summer canopy | Winter canopy |
|---|---|---|---|---|---|---|
| Change in % cover of *H. helix* | Pearson Correlation | .51[*] | 0.27 | 0.13 | -0.02 | -0.20 |
| | Sig. (2-tailed) | 0.04 | 0.32 | 0.64 | 0.94 | 0.47 |
| | N | 16 | 16 | 16 | 16 | 16 |

**Table 2. Correlation between change in percent cover of *H. helix* and all covariates measured. *p < 0.05**
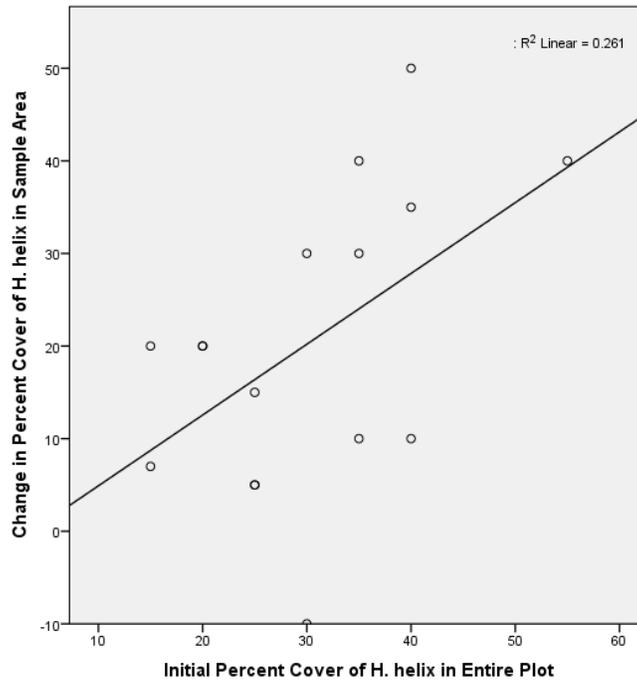
**Figure 1. Positive correlation between growth of *H. helix* in sample area and initial *H. helix* cover in entire plot (p = 0.04).**
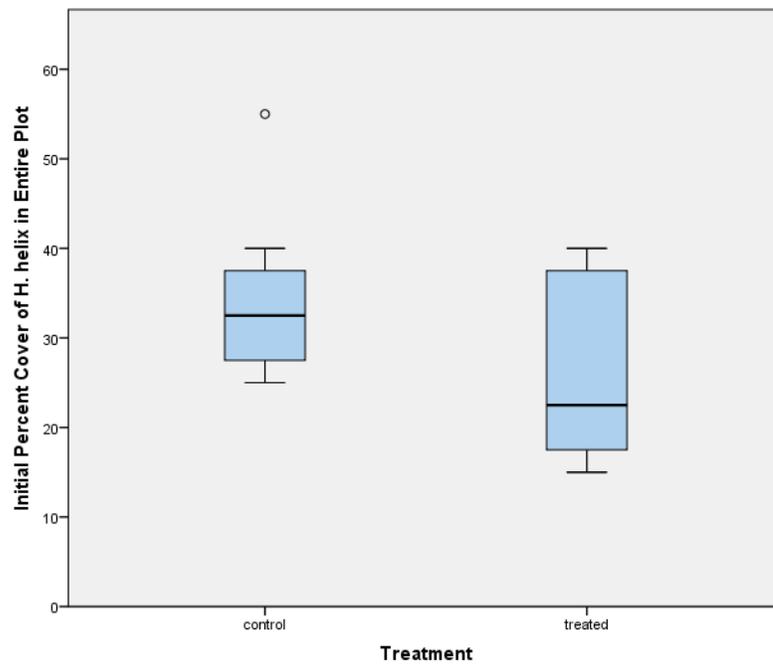


**Figure 2. Spread of data for initial *H. helix* cover in entire plot by treatment. Circle denotes outlier that is between 1.5 and 3 interquartile ranges from the interquartile range.**

No significant relationship was found between change in percent cover of native species and any of the covariates measured ($p > 0.05$) (Table 3).

| | | Entire plot initial *H. helix* cover | Summer moisture | Winter moisture | Summer canopy | Winter canopy |
|---|---|---|---|---|---|---|
| Change in % cover of native species | Pearson Correlation | -0.43 | 0.07 | 0.20 | -0.003 | -0.37 |
| | Sig. (2-tailed) | 0.10 | 0.81 | 0.45 | 0.99 | 0.17 |
| | N | 16 | 16 | 16 | 16 | 16 |

**Table 3. Correlation between change in percent cover of native species and all covariates measured.**

*H. helix* cover increased in all plots except one control plot, with an average increase in control plots of 23.13% (S.E. 6.40%), and an average increase in treated plots of 17.75% (S.E. 5.10%). Native species decreased in cover in control plots with an average decrease of 11.00% (S.E. 5.98%) and increased in treated plots with an average increase of 10.13% (S.E. 10.54%) (Figure 3). Overall, *H. helix* cover decreased in treated plots while native species cover increased in treated plots.
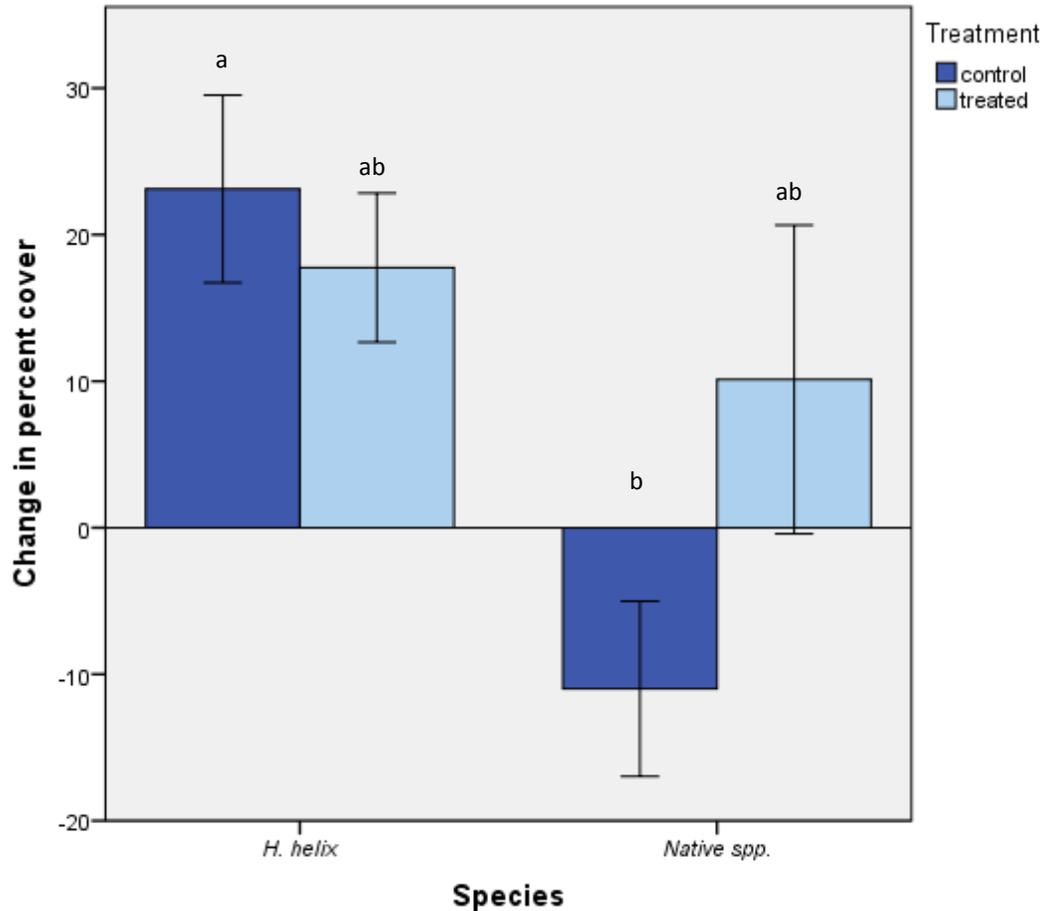
**Figure 3**. **Means ± 1 S.E. for growth of *H. helix* and native species by treatment. Control plots received no nitrogen addition and treated plots received 3 g N m$^{-2}$ yr$^{-1}$.**

As initial cover of *H. helix* was found to be correlated with change in percent cover of *H. helix*, this covariate was included in the ANCOVA model. Results showed that treatment had no significant effect on change in percent cover ($F = 0.98$, $p = 0.33$), while species did have a significant effect on change in percent cover ($F = 7.87$, $p = 0.01$). Additionally, a marginally significant interaction was found between species and treatment ($F = 3.17$, $p = 0.09$). The Tukey test revealed that change in percent cover of *H. helix* was significantly different from change in percent cover of native species in control

plots, but the addition of nitrogen treatments caused both groups to converge towards more similar means (Fig. 3). No significant relationship was found between initial cover of *H. helix* and change in percent cover while controlling for species and treatment (F = 0.002, p = 0.97).

**Greenhouse experiment**

None of the species tested differed significantly between treatments (*H. helix* H = 1.52, p = 0.47; *F. vesca* H = 0.59, p = 0.74; *P. munitum* H = 0.60, p = 0.74), which was likely due to the large variance in the data  (Figures 4-6). *H. helix* showed a slight but steady increase in RGR as nitrogen levels increased (Table 4, Figure 4). *P. munitum* and *F. vesca* showed no pattern of increasing or decreasing RGR with nitrogen addition. The median RGRs of both species increased in the low nitrogen treatment but decreased in the high nitrogen treatment (Figures 5 and 6). *P. munitum* data were the most variable overall, with an extreme outlier occurring in the nigh nitrogen treatment (Figure 6).
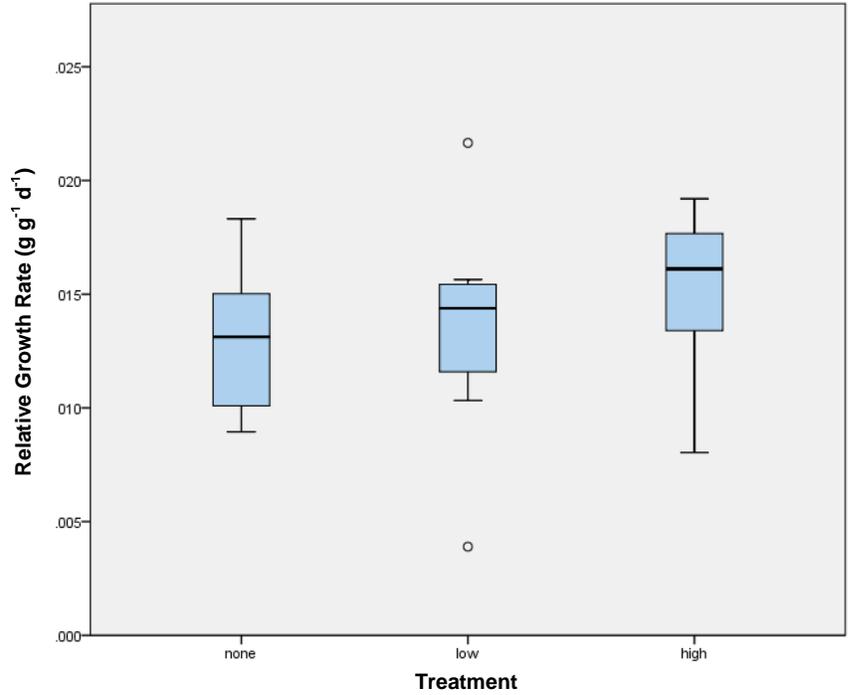
**Figure 4. Spread of data for relative growth rate of *H. helix* by treatment. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$. Circles denote outliers that are between 1.5 and 3 interquartile ranges from the interquartile range.**
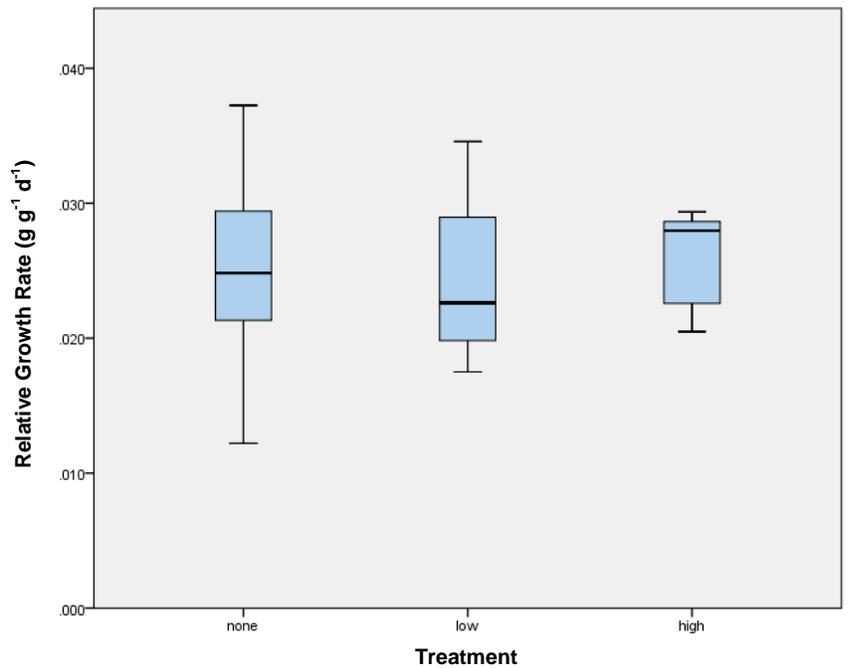


**Figure 5. Spread of data for relative growth rate of *F. vesca* by treatment. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$**
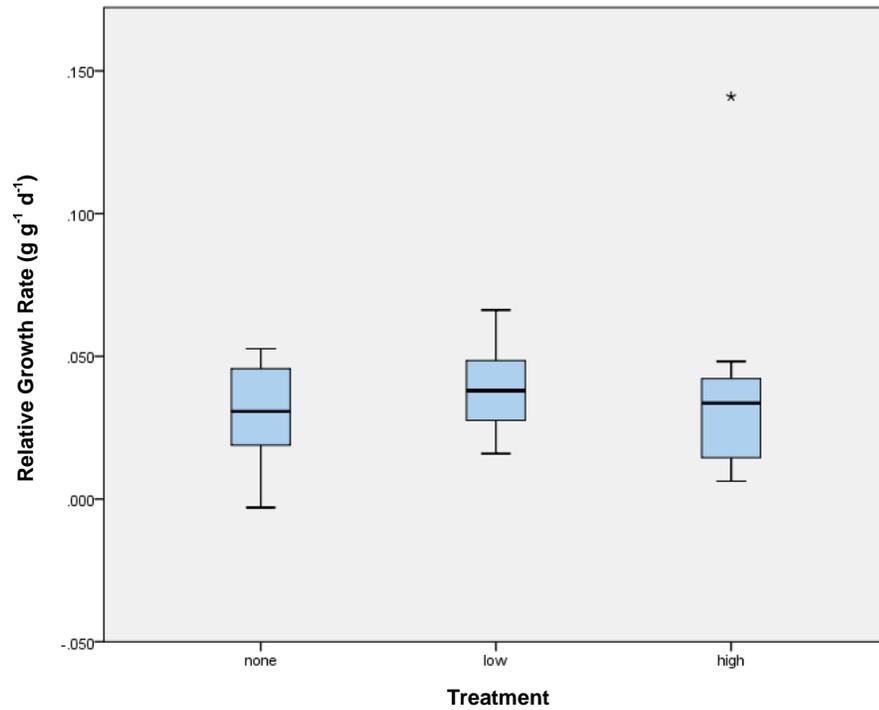
**Figure 6. Spread of data for relative growth rate of *P. munitum* by treatment. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$. Star denotes outlier that is over 3 interquartile ranges from the interquartile range.**

| Species | | none | low | high |
|---|---|---|---|---|
| *H. helix* | Median | 0.013 | 0.014 | 0.016 |
| | Q1 | 0.010 | 0.011 | 0.013 |
| | Q3 | 0.015 | 0.016 | 0.019 |
| *F. vesca* | Median | 0.025 | 0.023 | 0.028 |
| | Q1 | 0.020 | 0.020 | 0.022 |
| | Q3 | 0.031 | 0.030 | 0.029 |
| *P. munitum* | Median | 0.031 | 0.038 | 0.034 |
| | Q1 | 0.015 | 0.025 | 0.012 |
| | Q3 | 0.048 | 0.049 | 0.048 |

**Table 4. Distribution of relative growth rate (g g$^{-1}$ d$^{-1}$) of each species by treatment including median and interquartile range. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$**

**DISCUSSION**

Results indicate that increased nitrogen levels do not contribute to the dominance of *H. helix*. On the contrary, the effect of nitrogen addition was found to be dependent on species, but it was the native species that responded positively. *H. helix* on the other hand had a slightly larger increase in cover in control plots. However, *H. helix* growth was positively correlated with initial *H. helix* cover, and control plots had a higher initial *H. helix* cover. Consequently, the slightly higher growth rate observed in control plots may have been influenced by the positive correlation between initial cover and growth rate. A significant effect of species alone was also observed, as *H. helix* had a higher growth rate than native species in both treated and control plots. This suggests that regardless of treatment *H. helix* is able to outcompete native species. However, treatment made the growth rate of *H. helix* and native species more similar, which indicates that nitrogen addition may minimize the effect of species over time. If later research can confirm this pattern, this would suggest that native species may actually benefit from elevated nitrogen levels.

In the greenhouse the median RGR of *H. helix* increased as nitrogen levels increased, though increases were slight and not significant. Differences in the RGR of *F. vesca* and *P. munitum* were also not significant, and there was no discernible pattern as the median RGR of *F. vesca* decreased with the addition of 3 g N m$^{-2}$, but increased with the addition of 9 g m$^{-2}$, whereas the median RGR of *P. munitum* increased with the addition of 3 g N m$^{-2}$, but decreased with the addition of 9 g m$^{-2}$. For *P. munitum*, this improbable pattern was likely due the large variance in the data, as this species had the

largest spread of data and an extreme outlier. For *F. vesca*, this pattern was likely due to the fungus that caused several leaves in all treatments to begin to decay and die over a week before harvest. Conclusions based on these results would therefore be unreliable. Unfortunately, what was intended to be an experiment comparing the growth of *H. helix* to 3 other native species was reduced to an experiment comparing only the growth of *H. helix* to the growth of *P. munitum*. General conclusions cannot be drawn about the response of *H. helix* versus native species based on comparison to a single species. Additionally, it appears the growth rate of these species, particularly *P. munitum*, is simply too variable to show significant differences with a small sample size.

Results from both experiments are contradictory to many other studies that have found nitrogen addition to increase the dominance of invasive species (Brooks 2003, Lowe et al. 2003, Littschwager et al. 2010, Rickey and Anderson 2004). Interestingly, though Lowe et al. (2003) found that increasing nitrogen levels resulted in a much greater biomass gain in an invasive grass than a native grass, an experiment with the same design testing multiple species including the same two grasses showed no overall pattern differentiating native and invasive species response to added nitrogen (Lowe et al. 2002). Out of all native and invasive species tested, some invasive species had the strongest positive response to increasing nitrogen levels, but others had the weakest response. These results show that while some invasive species may gain a competitive advantage due to increased nitrogen levels, a generalization cannot be made to explain the success of all invasive species under such conditions. Padgett and Allen (1999) also obtained

results similar to the current study, with three native shrub species showing a greater relative yield response than three non-native annuals to increased nitrogen.

Rapid growth has been associated with nitrophilous species (Funk and Vitousek 2007), so the rapid growth rate of *H. helix* indicates that it would incur an advantage in nitrogen-rich environments. Perhaps *H. helix* is so successful not because it acquires nitrogen at a faster rate but because it is better at efficiently converting it into a gain. This could be achieved through apportioning more nitrogen to photosynthetic tissues or by having a higher photosynthetic efficiency through higher carbon gain per unit leaf nitrogen (Laungani and Knops 2009, Poorter et al. 1990). It could therefore thrive in a low or high nitrogen environment, but added nitrogen would not necessarily increase its competitive ability.

Invasive species may also thrive in nitrogen-limited environments if they can reduce the soil nitrogen pool through higher plant nitrogen residence time, which can be achieved through tissue longevity or redistribution of nitrogen from senescing tissue to living tissue (Laungani and Knops 2009). A longer residence time would lead to a larger leaf nitrogen pool and a depletion of the soil nitrogen pool (Laungani and Knops 2009), which over the long-term would benefit the invasive species to the detriment of native species. Padgett and Allen (1999) found that increased nitrogen deposition favored native shrubs over non-native annuals, but they also found that tissue nitrogen concentrations increased in the non-native annuals while there was little difference in native shrubs. They speculated that this difference could indicate poor regulation of growth in native species under high nitrogen conditions, which could result in shortened life spans due to

the inability to respond to environmental cues to prepare for dormancy or stressful conditions. Laungani and Knops (2009) found that the longer residence time of nitrogen in invasive *Pinus strobus* allowed it to accumulate twice as much nitrogen in its tissues and four times as much nitrogen in photosynthetic tissue as compared to native species. If *H. helix* possesses a longer nitrogen residence time than native species, this could lead to the dominance of *H. helix* in nitrogen-limited environments.

It is also possible that *H. helix* may dominate in a high nitrogen environment, but the deposition rate in both experiments was not high enough to elicit a significant response. Over exaggeration of deposition is common to many studies, probably because this is more likely to lead to significant results. A meta-analysis of 23 nitrogen addition studies evaluated species loss following nitrogen addition, but deposition rates in these studies ranged from 60-120 kg N ha$^{-1}$yr$^{-1}$ (Clark et al. 2007). Phoenix et al. (2006) predicted that by 2050 global deposition rates will have increased to an average of 11.8 kg N ha$^{-1}$yr$^{-1}$ with the highest deposition rate across India and Sri Lanka at 33 kg N ha$^{-1}$yr$^{-1}$, while Galloway et al. (2008) predicted that by 2050 rates in some regions could be as high as 50 kg N ha$^{-1}$yr$^{-1}$. Even if deposition rates reach 50 kg N ha$^{-1}$yr$^{-1}$, the deposition rates employed in many studies clearly surpass any realistic future rates. Not only have most studies added nitrogen at an unrealistic rate, treatments often only occur seasonally when plants are photosynthetic (Brooks 2003, Rickey and Anderson 2004), or they are staggered for short-term experiments to provide the greatest nitrogen input during the most rapid growth period (Lowe et al. 2002, Lowe et al. 2003). This would further make simulation more unrealistic as this would allow plants to maximize nitrogen use for

photosynthesis and growth, when in natural ecosystems nitrogen is continuously

deposited throughout the year and plants would not have a year's supply of nitrogen

deposited during the photosynthetic period.

Additionally, the duration of the current study may not have allowed for accurate

simulation of long-term nitrogen addition. Chronic low-level nitrogen addition may take

several years to significantly impact plant communities. One long-term nitrogen addition

experiment found that even at the lowest rate of addition (10 kg N ha$^{-1}$yr$^{-1}$) species

richness significantly declined (Clark and Tilman 2008). However, it was not until after

the fourth year that species loss began to occur. Given that the field experiment was one

year and the greenhouse experiment only twelve weeks; this may not have allowed

enough time to accurately predict community response after long-term nitrogen addition.

Furthermore, an inverse relationship has been observed between background nitrogen

deposition levels and response time (Gilliam 2006). Areas that receive high levels of

ambient nitrogen addition will take longer to show a response to nitrogen addition,

whereas areas that receive low levels of ambient nitrogen addition will show a quicker

response time. Portland, being a populous urban center, has likely received relatively

high levels of nitrogen deposition for quite some time, which would lead to a longer

response time to nitrogen addition.

There also may not have been a positive response from *H. helix* in the field

because of the application method. Many plots had dense groundcover which resulted in

mostly foliar application of the ammonium nitrate solution. Leaves of *H. helix* have a

thick waxy cuticle which often inhibits herbicide absorption (Soll 2005). This waxy

cuticle could also inhibit fertilizer absorption, making foliar application less effective for

*H. helix* than other species without this extra barrier. Additionally, there may have been a

positive growth response in *H. helix* but it went undetected due to its growth pattern. *H.

helix* is a long growing vine, so it is possible vines within the plots that were fertilized

grew outside of the plot and did not contribute to an increase in percent cover. However,

this effect should have been somewhat negated by only sampling the center square meter.

Despite these limitations, it appears that nitrogen availability does not contribute

to the competitive success of *H. helix*. Instead, results suggest that it reduces the

competitive ability of *H. helix*, as nitrogen addition bridged the gap between growth rates

of *H. helix* and native species. Further testing with a larger sample size could verify these

results and further test the response of *H. helix*. Field studies should employ deposition to

soil only to ensure that the waxy cuticle of *H. helix* leaves does not impede absorption.

Additionally, growth of *H. helix* should be measured not only by change in percent cover

but also by change in length of vines. It would also be beneficial to have a larger sample

size so that different levels of nitrogen addition could be tested, and to have plots where

plants could be harvested so underground biomass could be included in measurements as

well.

While another greenhouse experiment could be employed to try to obtain

significant results, it would be more informative if natural conditions were simulated.

Soil nitrogen concentrations should be measured and the soil mixture used in

experimental pots should maintain this background nitrogen level aside from treatments.

A larger sample size would more clearly show patterns if any exist, and would allow for

final biomass to be utilized as the parameter of growth. This would not only eliminate the need to make assumptions about water content to infer an initial dry mass, it would also eliminate the need to thoroughly wash the roots at the start of the experiment to attain fresh weight measurements. This would prevent plants like *G. shallon* from suffering transplant shock and dying. Finally, as it could take up to four years to observe changes in plant communities as a result of nitrogen addition (Clark and Tilman 2008), the duration of both the field experiment and greenhouse experiment should be extended.

**CONCLUSION**

Results from this study indicate that increased nitrogen deposition does not affect the growth of *H. helix* or its ability to compete with native species. On the contrary, native species experienced an increase in cover with nitrogen addition, while *H. helix* cover slightly decreased. However, the positive correlation between an increase in *H. helix* cover and the abundance of already present *H. helix* may have influenced the slight decrease in treated plots, as treated plots had a lower initial abundance. Nonetheless, results indicate that the effect of treatment is dependent on species and native species clearly had a positive response to nitrogen addition while *H. helix* had a slightly negative response if any at all. Though an interaction between the effects of species and treatment was expected, the direction of this interaction was not. Therefore, further testing should be done to investigate this unexpected pattern.

Another outcome of this study was a significant effect of species, and a greater increase in *H. helix* cover than native species in both treated and untreated plots. It is clear that regardless of the environmental conditions, *H. helix* is fast gaining on native species. If increased nitrogen levels do not aid in the invasion of *H. helix*, further research should focus on what mechanisms contribute to its success and why it is most invasive here in the Pacific Northwest.

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**APPENDIX A: Conversion Factors**

   Table A1 shows the average conversion factors for each treatment with standard

errors. There was no clear pattern across all species in the direction of increasing or

decreasing water content and consequently conversion factor (Figure A1); and three

separate ANOVA tests showed no significant difference between conversion factors of

different treatments ($p > 0.05$) (Table A2). Therefore, the average conversion factor for

each species across all treatments was retained for calculations of inferred initial dry

mass.

| Treatment | | *H. helix* | *F. vesca* | *P. munitum* |
|---|---|---|---|---|
| 0 g N m$^{-2}$ | Mean | 0.320 | 0.384 | 0.288 |
| | N | 8 | 8 | 8 |
| | Std. Error | 0.010 | 0.017 | 0.016 |
| 3 g N m$^{-2}$ | Mean | 0.297 | 0.382 | 0.295 |
| | N | 8 | 8 | 8 |
| | Std. Error | 0.006 | 0.015 | 0.009 |
| 9 g N m$^{-2}$ | Mean | .309 | 0.401 | 0.335 |
| | N | 7 | 7 | 7 |
| | Std. Error | 0.006 | 0.006 | 0.015 |

**Table A1. Mean conversion factor of each species by treatment with standard errors.**

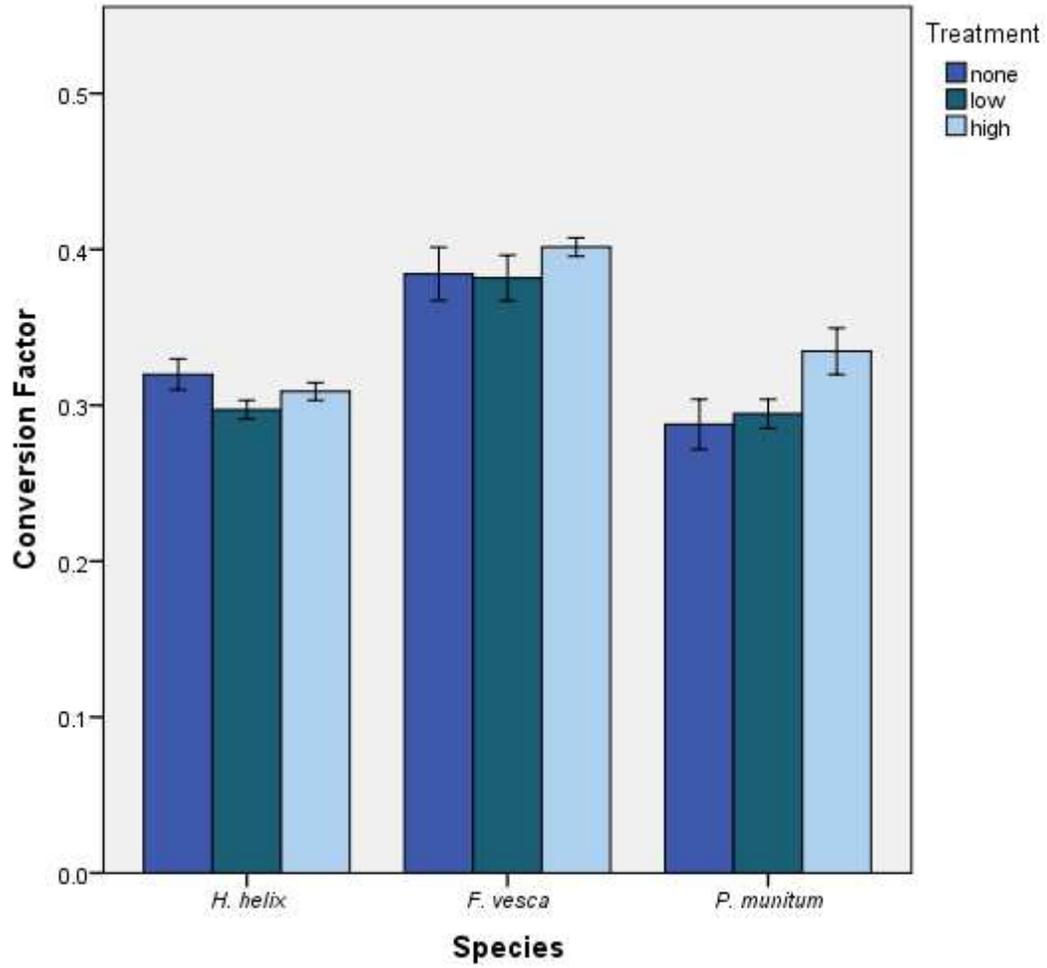**Figure A1. Means ± 1 S.E. for conversion factor of each species by treatment.**

|            | df | F     | p     |
|------------|----|-------|-------|
| *H. helix* | 2  | 2.320 | 0.124 |
| *F. vesca* | 2  | 0.567 | 0.576 |
| *P. munitum* | 2 | 3.286 | 0.058 |

**Table A2. ANOVAS for conversion factors of each species between treatments.**

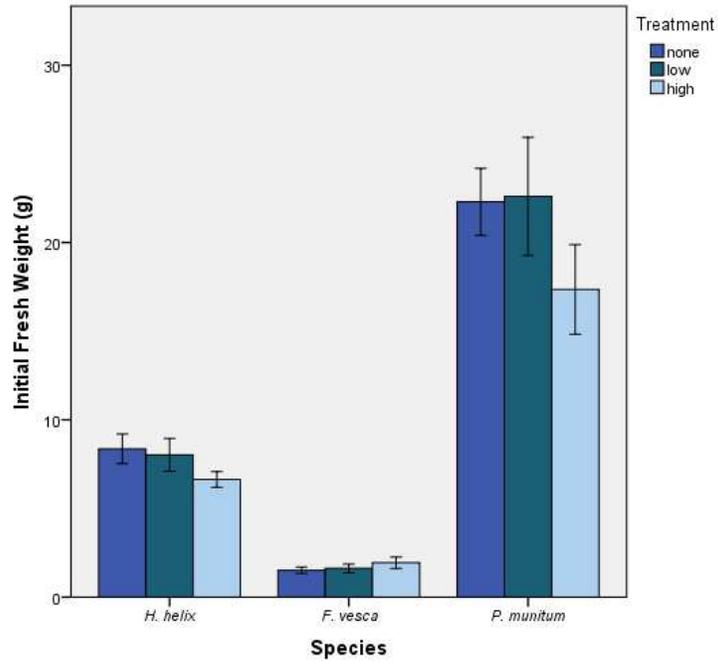**APPENDIX B: Initial Fresh Weight and Final Dry Mass**



**Figure B1. Means ± 1 S.E. for initial fresh weight (g) of each species by treatment. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$**
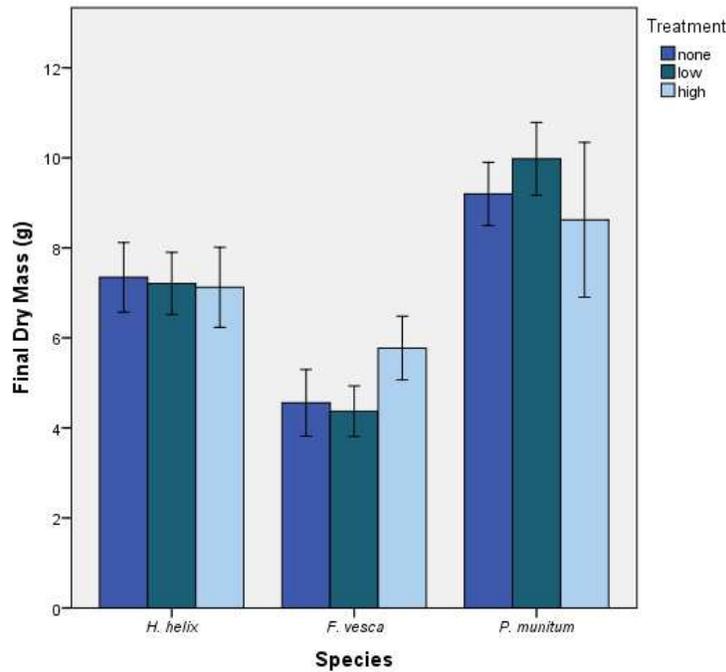


**Figure B2. Means ± 1 S.E. for final dry mass (g) of each species by treatment. Nitrogen treatments: none = 0 g N m$^{-2}$; low = 3 g N m$^{-2}$ high = 9 g N m$^{-2}$**