Determining the effects the combined growth *Alliaria petiolata* and *Rhamnus cathartica* have on soil composition.

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Abstract

With invasive species becoming a topic of increasing concern in the scientific and economical realms, it important to understand exactly how invasive plant species are able to alter and affect an ecosystem. Invasive plants affect an ecosystem in many different ways including out-competing the native plants, reducing biodiversity, and altering soil composition. Two such plants known to alter soil composition are garlic mustard (*A. petiolata*) and buckthorn (*R. cathartica*), common species in the forests of southeastern Wisconsin. Although research has been conducted exploring just how these two plants have affected soil composition, few if any have been performed focusing on their relationship with one another and the combined effect their growth has on the soil. In order to address this issue, this study has been conducted to test the soil from areas with just buckthorn stands, just garlic mustard patches, and then areas where the two plants are growing together. Based on observations from previous research concerning the two plants separately, it was hypothesized that with the combined growth of the two plants the soil will show a significant difference when focusing on soil nutrients, moisture, bulk density, and pH, specifically that the soil with the two plants combined will be higher than the average of the qualities of the soil where the plants grow apart. Though not statistically significant the results showed a trend that supported the hypothesis that soil composition differed when the two plants were growing together. This information collected is useful to gaining a better understanding of how the two plants interact and their possible effects on the soil.

Literature Review

Introduction

Invasive species are regarded as one of the highest causes of biodiversity loss, second only to habitat loss (Soule, 1990), costing the Unites States specifically hundreds of billions of dollars annually in environmental degradation, lost agricultural productivity, and prevention and eradication efforts (Lodge, 2006). Invasions by non-native plant species are increasing, along with increasing damages to the ecosystem (Lodge, 2006). These species whose introductions are likely to cause economic, environmental, or human harm (WDNR, 2011) differ widely in trait characteristics that make them successful in a variety of environments. For example, for plants, some invasives primarily impact biodiversity because their early and prolific aboveground growth provides dense shade that prevents the later-emerging native species from establishing due to lack of sunlight (Heneghan, 2006). Other invasives are able to exude allelochemicals that deter root establishment of the surrounding plants (Wolfe, 2008). Some invasives may be superior competitors because of faster growth (Heneghan, 2006) or decreased pressure from herbivores in their new environments.

Although the study of invasive species is a heavily explored topic, scientists still face the challenge of determining what drives invasive success. This is a challenge considering that although these species are most likely interacting with their surrounding environment, most invasive studies are done in isolation, such as focusing on one plant in the environment or conducting a study only in a greenhouse. Looking at invasive species that are different forms- such as a tree, shrub, or herbaceous, is especially important because these relationships are not explored much in previous research. For example, in the Midwest two of the most common invasive plants are buckthorn (*Rhamnus cathartica*, a shrub) and garlic mustard (*Alliaria petiolata*, an herbaceous plant), and though these often co-occur, little is known about how these species interact. Individually, both of them are known to have impacts on the soil – buckthorn through aboveground leaf litter inputs (Heneghan, 2006) and garlic mustard via root exudates (Wolfe, 2008). If these are non-additive responses, it may be that we need to adjust our long-term management techniques for areas that have been influenced by both. Thus, a better understanding of the combined effects of these species on soil properties will improve our ecological understanding of these invaders as well as aid in the refinement of control methods.

Characteristics of Invasive Species

In order to determine whether a plant would be considered invasive there are certain characteristics which may be observed. Because invasive plant species are able to out-compete the natives, researchers have found it necessary to carefully examine the qualities these invasives have that the natives seem to be lacking. One theory concerning invasive success focuses on the invasives' lack of natural enemies. There is a specific hypothesis known as the 'natural enemies hypothesis' that states that invasive introduced plants spread rapidly because they are liberated from their co-evolved natural environments (Liu, 2006). This hypothesis was supported in that the researchers discovered herbivore damage levels were greater on native plants than on introduced invasive; however, the herbivore damage levels were only marginally greater for the native plants. These results are most likely due to the small numbers of each type studied (Liu, 2006). Studies have shown the herbivores in an area drawn to the native plants in said area rather than the invasives, contributing to the idea that the invasive species have escaped their natural predators and are therefore able to thrive in this new area. The fondness of the herbivores to the natives rather than the invasives may be due to the chemical makeup of both species (Parker, 2007). In the Parker study it was determined that the invasive species were preyed upon less by herbivores than the natives. One hypothesis given for this is that the plant used in the study, *T. repens,* contained an unusual cyanogenesis defense as opposed to the natives used in the study. A second hypothesis for these results is that the native herbivores will feed on plants that they are familiar and comfortable with (Parker, 2007). According to the principle of energy allocation, if less energy is used for defense, then more energy is available for growth. It stands to reason that since these plants no longer need to focus energy on protection against foes, this excess energy would be used towards promoting their success in a new environment. Although this theory has not been abundantly tested research has found the ability of plants to reallocate their energy when natural enemies have been removed is possible (Blossey, 1995). In a study conducted by Blossey (1995) concerning Purple Loosestrife, it was determined that the plants which had the lowest herbivore pressure showed an increase in vegetative growth, with the plant growing to 99.7cm in height in 1991where it was considered invasive compared to 82.6cm in height where it was considered native. In 1992 the pattern was consistent, the invasive plant growing to 177.8cm and the native only 109.7cm.

Determining Invasiveness

Factors affecting the growth of plants, including energy use, have all been observed when concerning whether a plant will be invasive in a new area. Daehler (2003) discovered that when resources were abundant in an environment the native species were easily able to out-compete the invasives, but when the situation was reversed and the resources such as water and light became scarce, the invasive species became the dominant species in the environment. Daehler (2003) involved the comparison of 79 independent native-invasive plant species. In 94% of 55 comparisons the natives were able to out-compete the invasives. These results were concluded to be due to the low availability of resources, specifically light, nutrients, and water. The invaders, however, were found to have higher leaf area, lower tissue construction costs, and greater phenotypic plasticity when resources were higher (Daehler, 2003). When an invasive species has greater energy acquisition compared to native plants, it is able to use that energy to grow faster and thus, spread farther than the native plants. The more resources available to the plant, the better it is equipped for growth and spread. This is due to the fact that many invasives are able to expend less energy when they grow cutting construction costs (Leishman, 2007). Leishman (2007) showed native plants and invasive plants to have an equal ability to capture carbon, but the invasive species' leaf traits enabled them to have a higher growing ability. This was determined by obtaining community-level data for 55 vascular plant species nutrient-enriched and undisturbed land in Australia. The leaf-trait data was obtained from literature for 75 native and 90 exotic invasive coexisting species (Leishman, 2007). One particular invasive species that has been found to be able to out-compete the native plants in areas where it is found, especially in disturbed sites with plenty of available resources, is *Alliaria petiolata*, or garlic mustard.

Effect of Invasive Plants on Soil Composition

There are many ways in which a plant is able to affect the soil composition of the area in which they live (Fig. 1). The effect exotic plants displacing the native plants has on the soil microbial communities is important to gaining a better understanding of how invasive plant species are able to affect soil composition and in turn affect an ecosystem. In a study by Kourtev (2002) it was determined that microbial communities under three different plant species differ in structure and function, supporting the hypothesis that different plant species each have their own effect on the soil composition. It is understood that these soils are affected with the growth of the plants, either through the litter inputs through the soil or through the growth and activity of the roots (Kourtev, 2002).

In terms of organic matter, nitrogen levels, moisture, and texture, soil can sometimes be easily affected by the types of plants found growing within the soil. Although these components of soil are affected when exotic plants are introduced, another aspect is the actual soil biota living within the soil, directly affected by the types of plants it interacts with which can potentially change the composition of the soil itself. It may seem as though exotic plants have a very miniscule effect on what is happening below the ground, but changes in the plant community can have a direct effect on the soil community. Each plant in an ecosystem supplies the soil with organic matter from its own leaves and from the substances it exudes from its roots, affecting the chemical make- up of the soil itself. The change of plant inputs in the soil can alter the nutrient levels, moisture content, acidity, fire resistance, and many other attributes of the soil. Although there is a large amount of research focusing on the effects invasive species have on the native plants in their invaded ecosystems, there are relatively few studies concerning the effects these invaders have on the soil itself.

It is also important to note that the effects an invasive species has on the soil composition or on an entire ecosystem itself may also vary depending on the area. The effects on the soil may also be altered if more than one invasive species is interacting with the area (Kourtev, 2002). One such study concerning interacting invasive plant species was done by Wolfe (2005) where it was concluded that the microbial composition was altered with the two invaders when compared to the native plant's microbial composition. Another study done in New Jersey with *Vaccinium palladium*, or blue ridge blueberry, showed the shrub's high levels of nitrogen in the leaf litter, giving the soil a higher pH and nitrification rates than under a native shrub in the area (Heneghan 2006). According to some of the research previous conducted these plants are able to have a positive, negative, and neutral effect on the soil and soil biota. Because of these results it becomes even more necessary for more research to be conducted, directly focusing on specific invasive species and their specific effects on the soil composition and soil biota (Wolfe, 2005). An example where two different invasive species have affected the soil composition in one area is a study of *B. thunbergii* (Japanese Barberry) and *M. vimineum* (Japanese Stiltgrass). These two plants were responsible for the increase in pH because of their high rates of nitrate uptake by their roots (Ehrenfeld, 2001). One of the main reasons for examining the soil composition in areas where exotic species were or are prevalent is due to the changes these species produce long term on the soil. In an area where restoration projects have been established it is especially important to note the changes in the soil composition considering many believe with the removal of the invasive species will come the flourish of the native species. If this were the case, the soil composition under both the native plant and invasive plant would have had to remain the same (Heneghan, 2006).

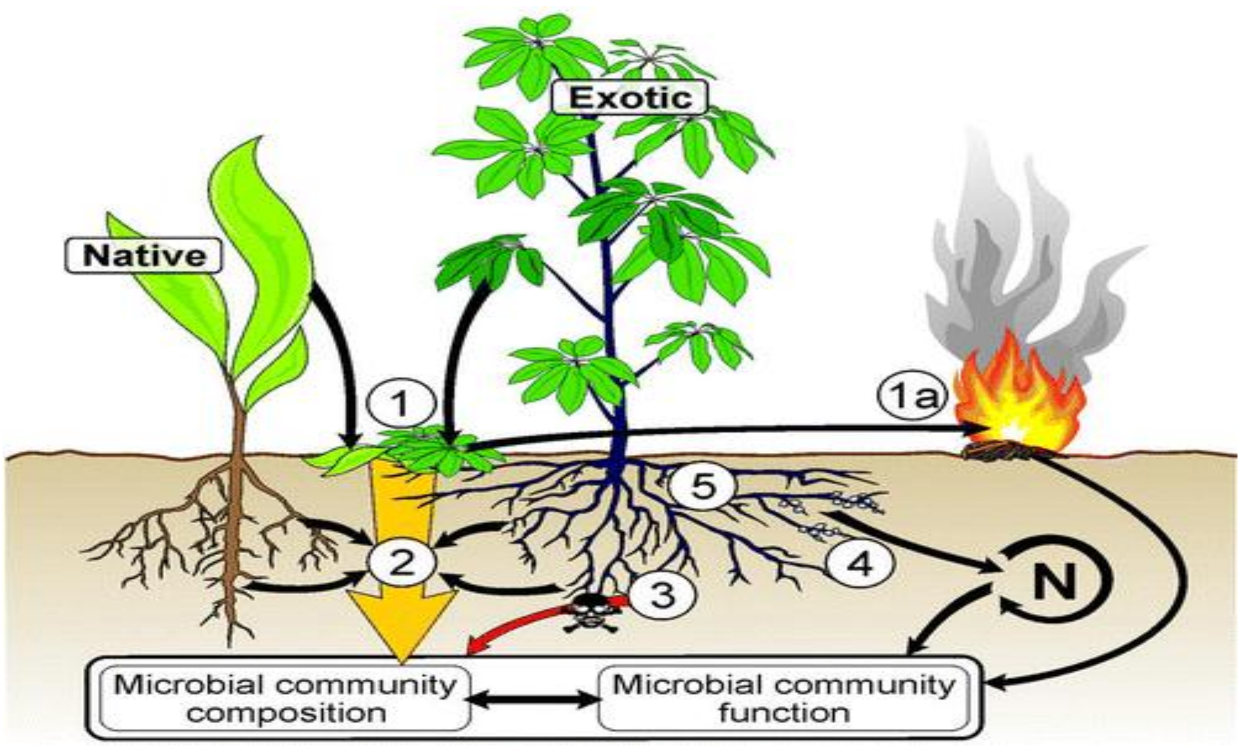


Figure 1: "Conceptual diagram illustrating potential pathways by which the displacement of native plant species (plant on left) by exotic plants (taller plant on right) can cause shifts in soil community composition and function. Five direct pathways are illustrated: differences in the quantity or quality of (1) litter production or (2) root exudates, altering resource availability for belowground communities; (3) release of novel chemicals with antimicrobial activities; (4) novel nutrient acquisition strategies such as nitrogen fixation that can alter biogeochemical processes; and (5) differences in the local soil environment induced by changes in root architecture or function. These direct mechanisms may lead to indirect effects, such as changes in disturbance regimes, including fire (1a). The effects of one exotic plant species on soil biota may be manifested by one of these mechanisms or by several mechanisms acting simultaneously" (Wolfe, 2005).

Garlic Mustard

Garlic mustard, or *Alliaria petiolata*, is a well- known invasive species in the Midwest and particularly Southwest Wisconsin. It is one of two (the other being *Microstegium vimineum*) of the most common invasive herb layer species in the Eastern United States (Morrison, 2007). It originated in Europe and was introduced to the United States in the 19th Century. Figure 1 shows the distribution of garlic mustard across the United States from the National Agriculture Library. The plant was most likely brought by early settlers for its use as a medicine, an herb in cooking, and as a way to prevent erosion. Garlic mustard is believed to be spread by White Tailed Deer, which, instead of eating the plant because they prefer the native species in the area, will simply spread the seeds wherever they travel. They do this by transporting the seeds they obtain when they walk among the plants, picking up seeds on their legs and fur. When the seeds finally do fall off the deer have usual moved at least some distance away from the original plant, thus spreading its seeds to new territory. Garlic mustard begins its growth in April when the seeds germinate. These plants overwinter as rosettes and if they survive they disperse their seeds the following spring. From the beginning of garlic mustard's first occurrence in the United States it has taken only about 20 years for the plant to spread to almost 30 states (invasiveplantsnet). Because this plant is self-pollinated it takes only one plant to pollinate a whole area (SE-EPPC). These monospecific stands dominate the forest understory, hurting the native plants that grow in those areas (Dhillion, 1999). These plants also have a prolific reproduction, which means they produce a large quantity of seeds in order to ensure the survival of the species. Instead of producing a few seeds which are protected enough to ensure they will last to reproduce themselves, garlic mustard's producing of many seeds that are not as protected can lead to a high yield of new plants, even if there is a large amount of seeds that do not survive long enough to grow. Garlic mustard also has a fast growth rate, only adding to the plant's ability to spread and displace the native plant species (Anderson, 1996). Garlic mustard prefers partially shaded forests and does not do well with an increased amount of direct sunlight; however, it also does well with a variety of soils and moisture levels, making it able to thrive in many different environments.

Even though this plant does do well in many different conditions, it does not tolerant highly acidic soils well and has been found to be less competitive in soils with a low pH. An experiment done by Anderson (1995) hypothesized that the reason garlic mustard is unable to colonize large areas in southern Illinois is due to the increased acidity found in the soil in that location. This experiment demonstrated a significant positive correlation (r=0.98; <0.001) between plant dry weight and soil pH (Munger, 2001). Garlic mustard is fond of colonized disturbed areas of forests and riparian zones. Areas such as floodplains are particularly vulnerable due to the increase in transport ease for the garlic mustard seeds (SE-EPPC). Garlic mustard has also been found to contain a cyanide concentration as high as 100p.p.m, which is toxic to many vertebrates. This high concentration of cyanide has been known to reduce herbivory and suppress the growth of nearby plants, increasing the success of garlic mustard (Rodgers, 2008).

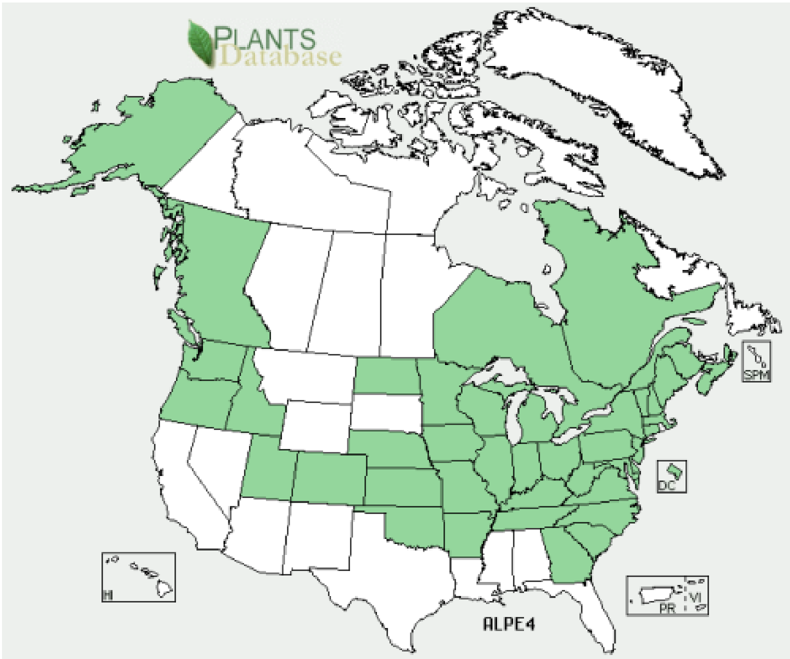
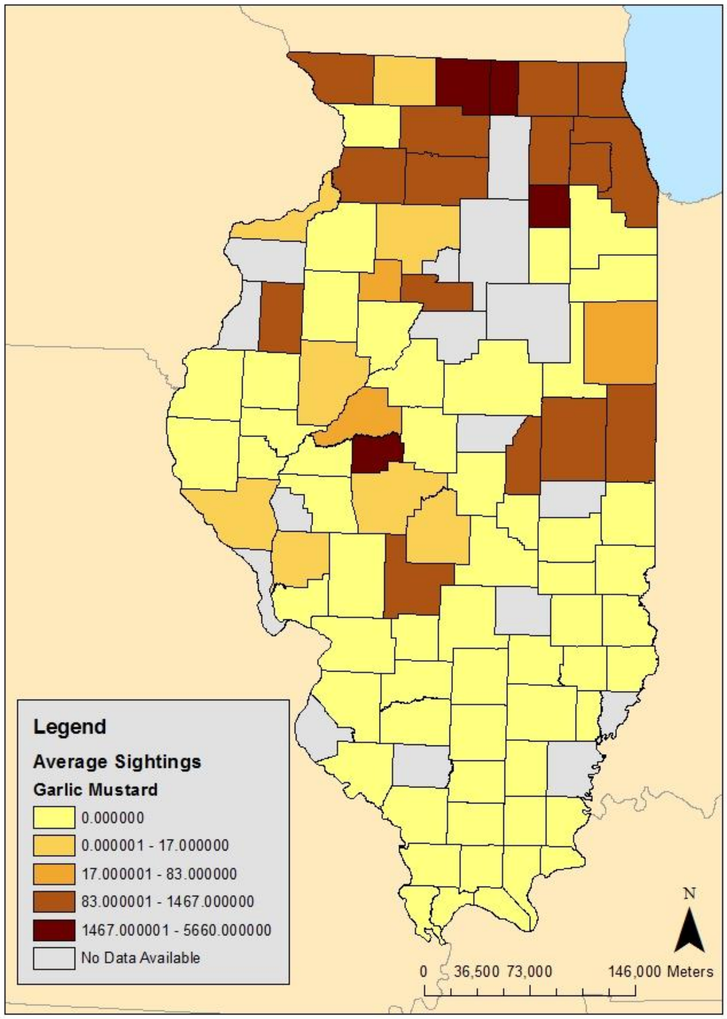


Figure 2**-** Distribution map showing the spread of garlic mustard throughout the United States (National Agricultural Library, 2011).

Average Sightings of Garlic Mustard By County in 2003



Data: Illinois Department of Natural Resources

Figure 3- A distribution map for the Illinois counties of garlic mustard. This is just an example of what the distribution of the invasive plant looks like in a Midwest state. There is a clear increase in sightings towards the northern part of Illinois, which will most likely continue into the southern parts of Wisconsin showing a large abundance of the plant in those areas.

Garlic Mustard's Effect on Soil Composition

Garlic mustard has been found to emit glucosinolates from its root systems which are transferred to the soil, affecting the microbial makeup of that soil (Kourtev, 2002). Garlic mustard is a dominant invasive species in many forests which allows it to generate a larger influence on soil biota and composition. Dominant species are able to influence the EM fungi in a soil specifically. Garlic mustard has been known to eliminate the arbuscular mycorrhizal fungi from the roots of tree seedlings in forests where it has been found as a dominant understory species (Wolfe, 2008). This shows that the plant has the ability to degrade the local fungi communities in the soil, allowing the plant to further its own growth. The results in Wolfe's study showed *A. petiolata* to have the ability to cause significant declines in the mycorrhizal colonization in mature tree roots which in turn affects the functionality of said trees to uptake nutrients. Since so many native plants form symbiotic relationships with mycorrhizal fungi, the disruption of these relationships by garlic mustard can hurt the native plants by decreasing their growth and abundance (Rodgers, 2008). Specifically in a study by Stinson (2006) it was found that when garlic mustard was added to the soil the percent mycorrhizal colonization and abundance of competing native plants was decreased (Rodgers, 2008). Garlic mustard has been known to have been used as a biofumigation in agricultural systems, which basically means that it is used to hurt unwanted organisms in the soil, by tilling the soil so volatile chemicals released by the plant are exposed (Rodgers, 2008).

In a study performed by Rodgers (2008) where plots invaded by garlic mustard were studied, it was determined that the plots heavily invaded by garlic mustard were significantly higher in nitrogen, phosphorous, calcium, magnesium availability, and soil pH. It is believed that garlic mustard is able to raise the pH of the soil as a consequence of root exudation (Rodgers, 2008). Garlic Mustard was also found to have significantly increased the rate of decomposition of leaf litter of the native tree species. These results showed that garlic mustard may be able to create a positive feedback between site occupancy and continued proliferation. This study showed that garlic mustard raised the nutrient availability in each soil it was found in, regardless of whether the plots already contained high or low nutrient availability (Rodgers, 2008).

Buckthorn

Along with garlic mustard, another main focus of this research due to its success in the native forests of Southeastern Wisconsin is common buckthorn, or *Rhamnus cathartica*. Buckthorn is a small tree or large shrub native to Europe and Eastern Asia that was brought to the United States from as early as 1849 to be used as ornamentals. Figure 4 shows the distribution of this plant throughout the United States. These plants obtain their common name from the distinctive look of their terminal bud, having the shape of a buck's hoof. Buckthorn is a problem in a variety of areas including oak forests, riparian zones, savannahs, abandoned fields, roadsides, and even rocky sites, being able to grow in a variety of environments and conditions. Although they are able to thrive in many types of habitats, this plant does best with full sun with relatively alkaline soil. One of the reasons buckthorn is so successful is because of its ability to leaf out very early and stay leafed for a longer period of time than most natives in the area. Because of this ability the plants are able to take advantage of the canopy above not yet having leafed out which will allow the buckthorn to capture carbon for a longer period of time than most native plants (Knight, 2007). Their length and speed of growth is directly related to the amount of light the plants receive with the more light they obtain increasing their ability to grow faster and longer.

Another reason for success is their long distance distribution of their seeds, usually done by birds that eat the seeds, although rodents have also been known to disperse the seeds by storing them in different areas. These are sometimes forgotten by the rodents and the seeds will germinate after a period of time (Knight, 2007). The growing period for buckthorn begins in May through June when the plants flower and continues through September when the fruit ripens. With buckthorn being able to produce seeds only a few years after establishment they are certainly able to produce a large amount of seeds in their lifetime. Due to this plant's ability to grow in a variety of conditions, resprout after having been cut, spread far and quickly, and take over an entire area in a relatively small amount of time they are having a large effect on the native plants with which they grow.

Buckthorn's success outside of its native habitat is also due to its chemical makeup. Emodin has been found in the plant which is thought to deter insects and other herbivores from the plant. Emodin is a resin found in some plants that can act as a laxative if consumed. The emodin is also believed to protect the plant from pathogens and protect the plant's seeds from early consumption. Emodin covered fruit is avoided by birds and other predators, protecting the seeds from predation and allowing the seeds to grow until their full ripeness. Emodin in the pulp of the fruit kept invertebrates and microbes away but allowed the birds to continue to eat the plant. It is also possible that with buckthorn the seeds dropped (close to the parent plant) could be harming the native plants nearby, due to the emodin (Knight, 2007). In summary, the emodin this plant contains is thought to affect the soil microorganisms, have allelopathic effects on nearby plants, and keep predators away (Knight, 2007).

There are many factors concerning buckthorn that have been previously studied to try and understand what exactly makes them so successful (WDNR). The main characteristics approached are the plant's shade tolerance, rapid growth, high photosynthetic rates, tolerance of moisture, phenology, high germination rates, seedling success in disturbed areas, and bird-dispersed fruit. Because buckthorn has been found to have a high percent of nitrogen in its leaves throughout the growing season it was able to have higher rates of photosynthesis and carbon gain, with carbon gain being the primary source of strong growth for the plant. One reason for buckthorn's advantageous growing period length is due to the fact that in its native forests of Europe this plant has to contend with frost much sooner than in the United States. In Europe this plant has been found to not have a superior leaf out period, but rather to use much of its energy to protect itself from colder conditions. Because the plant does not have to contend with such harsh environments it has allocated its energy to be used for longer growth periods (Knight, 2007). Since buckthorn tend to grow and form dense thickets they tend to block the sun from reaching the forest floor, creating more shade and harming the native understory plants which would usually receive more light. These plants are also difficult to maintain due to their ability to grow swiftly and in long distances (WNDR).

Figure 4- Distribution map showing the spread of buckthorn throughout the Unites States and Canada (Kurylo, 2007).

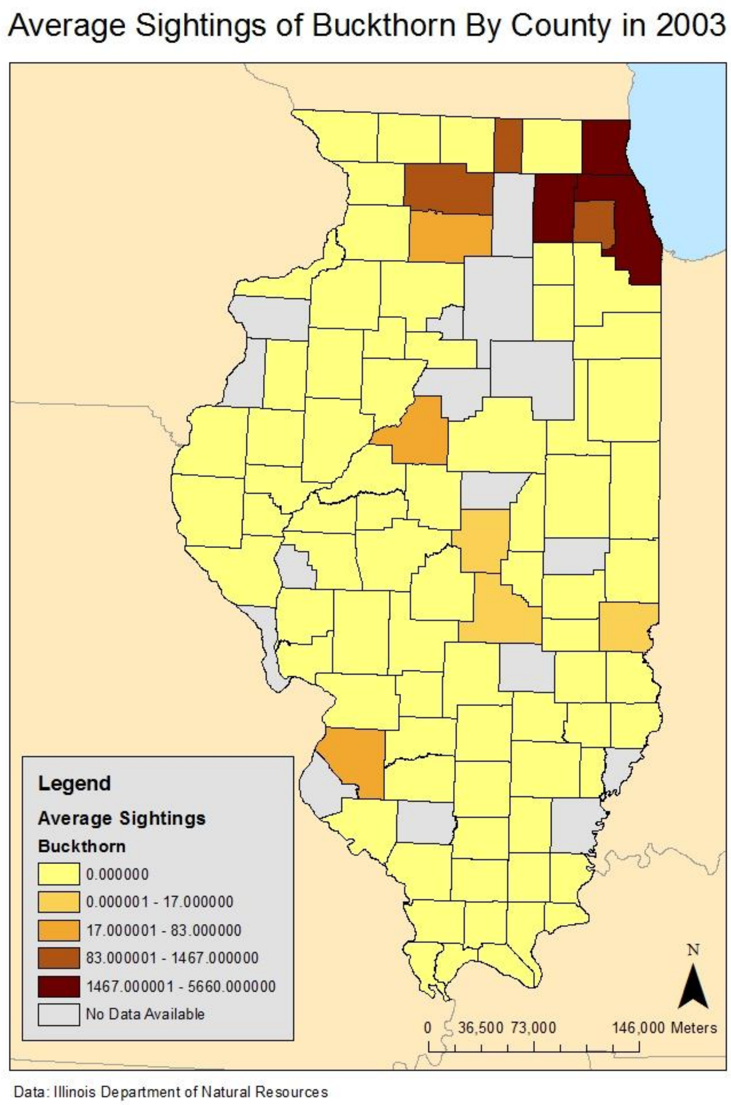
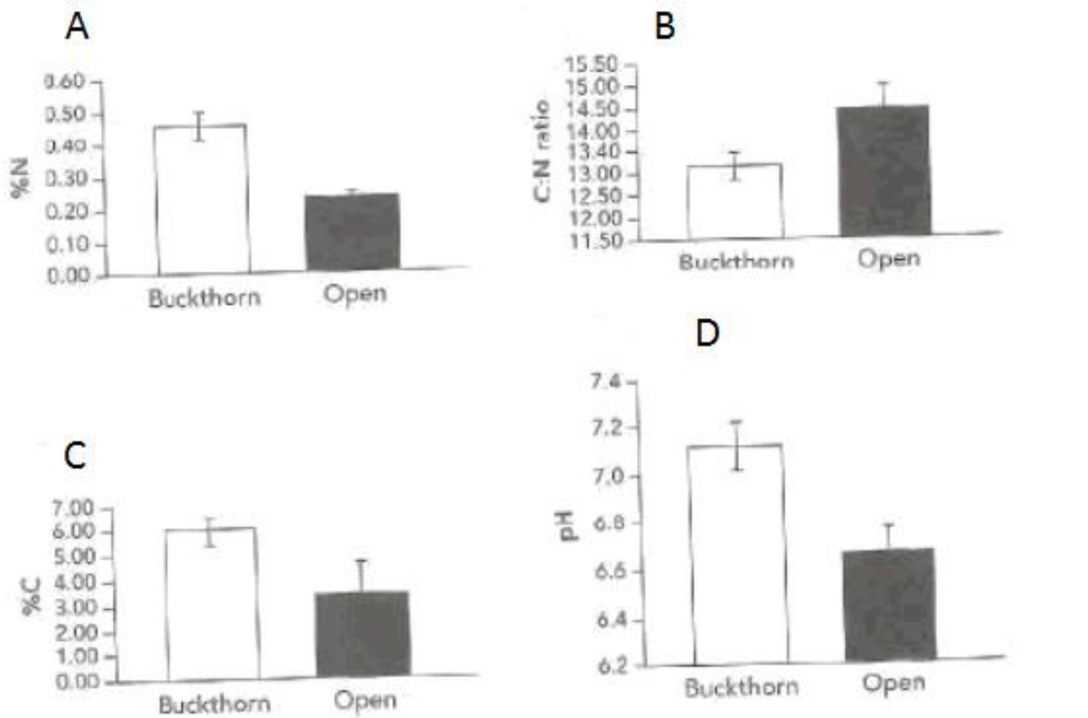


Figure 5- A distribution map for the Illinois counties for buckthorn. This is just an example of what the distribution of the invasive plant looks like in a Midwest state. The higher abundance of sightings is found to the north, which will most likely continue into southern Wisconsin, where this research is based.

Buckthorn's Effect on Soil Composition

The effects of a plant on soil composition is more difficult to determine than the effects such a plant would have on above ground resources, such as light, and may be more difficult to deal with. Buckthorn also has a direct effect on the soil composition where it is found. Because buckthorn’s leaf litter is high in Nitrogen and causes fast decomposition rates, the nitrogen levels in the soil are directly affected. The higher decomposition rates of buckthorn also caused higher decomposition rates in the leaf litter of the native plants nearby (Knight, 2007). With buckthorn specifically the soils beneath the large thickets support large populations of the Eurasian earthworm, especially when compared to areas of a forest lacking buckthorn, thus causing the higher decomposition rates. The worms and high nitrification rates create a rapid rate of incorporation of litter material into the soil (Heneghan, 2006).

Heneghan also concluded that the soil under buckthorn thickets had a higher percentage of N and C content, elevated pH, and higher water content than areas where these plants were not present. In this particular study (Heneghan, 2006) the results indicated an accumulation of nitrogen and carbon and alterations to N mineralization rates, most likely due to the high productivity of buckthorn and increased abundance of earth worms in the soil under the plants. These changes in the makeup of the soil are believed to remain even after the plant has been removed, furthering the need for new control methods, rather than the simply removal of the plant. In another study performed by Heneghan (2004) it was determined that plots where buckthorn has invaded consisted of a higher pH level, higher percent of carbon, and a higher percent of nitrogen than plots without (Figure 6). This same study showed that areas containing buckthorn had gravimetric water content 59 percent higher than in open areas, along with twice the amount of nitrogen found in the buckthorn invaded areas (Heneghan, 2004).



. Soil properties (mean ± S.E.) under buckthorn and in buckthorn-free ("open") areas of McDonald Woods. a) percent Nitrogen , b) percent Carbon, c) C:N ratio, d) soil pH.

Figure 6- The results from the Heneghan 2004 study.

Alliaria petiolata* and *Rhamnus cathartica

Although many studies have been conducted focusing on the effects both *A. petiolata* and *R. cathartica* have on the natural environment, there have been none conducted concerning their combined relationship. This lack of research is most likely due to the structure of both plants, one being an herb (garlic mustard) and the other a larger shrub (buckthorn). These two plants often occur in the same areas, most likely due to a shared response to environmental factors (Morrison, 2007). Both prefer shaded, temperate environments and disturbed sites in such environments. They are often found along the edges of trails and farther away from any rivers or streams, preferring soils a little less moist. One study compared the relationship of *A. petiolata* and another invasive species, *M. vimineum,* both part of the herb layer species in Eastern North America and both containing similar characteristics (Morrison, 2007). Although the purpose of this particular study was to determine which of these invasive plants would be considered more of a threat, it is still important to note the comparison of these two plants, due to their close relationship and similarities. This study looked at the plants growth and competitive abilities, seedling growth, survivorship, susceptibility to natural enemies, photosynthesis rates, and their changing abundance. Although none of these characteristics allows the researchers to look directly at the soil composition, the chosen characteristics do have the ability to affect the soil composition. A plant's competitive ability determines whether the plant will be able to spread and grow in a given area. If the plant has a high competitive ability and is able to grow and spread, the soil under the plant will be most likely be altered due to the plant's characteristics in relation to the native plants that once grew in the invasive's place. Survivorship, susceptibility to natural enemies, photosynthesis rates, and changing abundance are all related to the plant's ability to compete in their new environments, which again relates to the amount of disturbance or changes the soil undergoes. These previous studies can be seen as steps towards determining how invasive species affect the soil in their new, non-native environments. The relationship between buckthorn and garlic mustard will vary considering their differences, yet their shared effect on the soil remains just as important to note as it would be if they were of the same plant type (herbaceous or shrub).

All of these factors are what influence an invasive's ability to outcompete the native plants, spreading farther more quickly and directly affecting the biodiversity of the ecosystem. Because the biodiversity of the ecosystem is greatly reduced when invasive species invade a native forest it is important to understand the effects these particular species have directly on particular factors that make up the environment, such as soil composition, thereby understanding the magnitude of influence these plants may have

Purpose of Study

There is increasing evidence that invasive plant species have the ability to alter soil composition by changing nutrient availability, affecting the activity of soil microorganisms, changing decomposition rates, and affecting the pH of the soil. Because of this, invasive species that have the ability to change soil properties can have a far-reaching and long term impact on an ecosystem (Rodgers, 2008). In order to gain a better understanding of such effects, more research is needed concerning invasive species in general, and more specifically their effects on soil composition. The objectives of this research are 1) to determine what differences there are in soil composition when the two different invasive plants are growing together, 2) to obtain a clearer understanding of how invasive plant species are able to affect soil composition, and 3) to obtain a clearer understanding of how invasive plant species interact with one another. It is hypothesized that with the combined growth of buckthorn and garlic mustard the soil composition will show a significant difference concerning the averages of the soil qualities when compared to soil composition of the two plants growing separately. More specifically, it is hypothesized that the amounts of nutrients in the soil, pH, water holding capacity, and bulk density of the soil where both plants grow together will be higher than the average of the soil where the plants grow apart, also known as nonadditive.

Due to the ability of garlic mustard to affect nutrient intake into the soil and its ability to influence the pH of the soil, it should be able to create a faster rate of decomposition and raise the pH of the soil when it is growing equally with buckthorn. Considering buckthorn's ability to also increase the rates of decomposition, its relative higher moisture content than nearby soils, its ability to also raise pH, and its high nitrogen content, buckthorn should affect the soil when growing with garlic mustard. These two plant species prefer such similar types of soil and soil composition that when they grow together their effects should be beneficial to both plants. In order for this to be true, the soil needs to have a higher moisture content when the plants are growing together, have a relatively high pH, and should show an increase in nutrients in the soil.

The soil collected from plots where garlic mustard is growing in stands by itself should show a relatively high pH, with a range between 7 and 7.5, but growing with buckthorn this should increase to be around 7.5 to 8. Buckthorn should also be around a 7 through 7.5. These predictions are based on the knowledge of buckthorn and garlic mustard preferring basic soils and both plants being able to increase soil pH.

The nutrient content of garlic mustard growing in stands by itself should be relatively even, meaning the soil will not contain a high amount of nutrients but it also will not be nutrient deficient. This is due to the knowledge that garlic mustard can have a fast rate of decomposition and incorporates nutrients into the soil easily, but is not as efficient as buckthorn, which will have a high amount of nutrients. The combination of these two plants will show a rise in the soil of nutrient content.

The moisture levels of the soil when both plants are growing together will be greater than or equal to that of when buckthorn is growing by itself. Since buckthorn raises moisture content in the soil these effects should be consistent even when growing together with garlic mustard. The bulk density should have the same effects as moisture considering the two are related.

Methods:

Field Sample Collection

Buckthorn and garlic patches were identified in Carthage College's *Phil Sanders* Audubon Cooperative Wildlife Sanctuary, where both species were abundant. This site, along with the buckthorn and garlic mustard, contains a wide variety of plants, such as Box Elder, Black Walnut, and Red Maples which make up the dominant canopy species. The dominant shrub and herb species, apart from buckthorn and garlic mustard, consists of dogwoods, honeysuckle, an abundance of may apples, and geraniums. The majority of the soil in the area is silty clay. Figure 7 shows where the sample site is in relation to campus and also the different soil types found throughout campus. The soil taken for this study is all in the Dh area, meaning it is high in silt and can also have large quantities of clay. The figure also shows the sample site's relative close proximity to the main street and frequented tennis courts, displaying an area favored by both of the invasive species concerned in this study.

There have been no known previous studies conducted here concerning the effects buckthorn and garlic mustard have on soil composition when growing next to one another. The area is a trailed forest on the outskirts of Carthage's Campus, not far from a road and tennis courts. The buckthorn plants chosen to take samples from were used because of their close proximity to the trails, yet rather far distance from any garlic mustard plants. Figure 8 shows the sampled area and displays where each of the samples were taken from. There is a large cluster of buckthorn plants within 5 feet of one another (the distance each sample was taken from another), four of which samples were taken directly next to their trunks. The samples were taken by using a soil core with a maximum depth of 15inches and collecting soil from about 5 inches into the ground. Soil was taken from one buckthorn plant until a quart sized bag was filled half way. The same method was repeated for all four buckthorn plants. The garlic mustard samples were found further down the trail, yet still relatively close to it, considering they too prefer disturbed areas to grow. The distance from the first buckthorn sample site was about 30 feet. The same methods for soil collection were used with four specimens of garlic mustard. The samples were taken from the middle of the patch of garlic mustard. There is a patch of about 7 or 8 garlic mustard growing within a stand of at least three buckthorn further along the trail. Samples were taken where the two different plants were growing closest together in four different spots. Sampling techniques were again the same as with buckthorn. All 12 samples were collected within the same day so as to make conditions as equal as possible. The day was overcast yet warm and the time was between 3 and 6 in the evening. The weather had been fairly dry, the area not experiencing any rain for about a week at the time of collection.

Figure 7: This map shows the sample area and the different types of soil found here. The outlined section is the general area where the samples were collected.

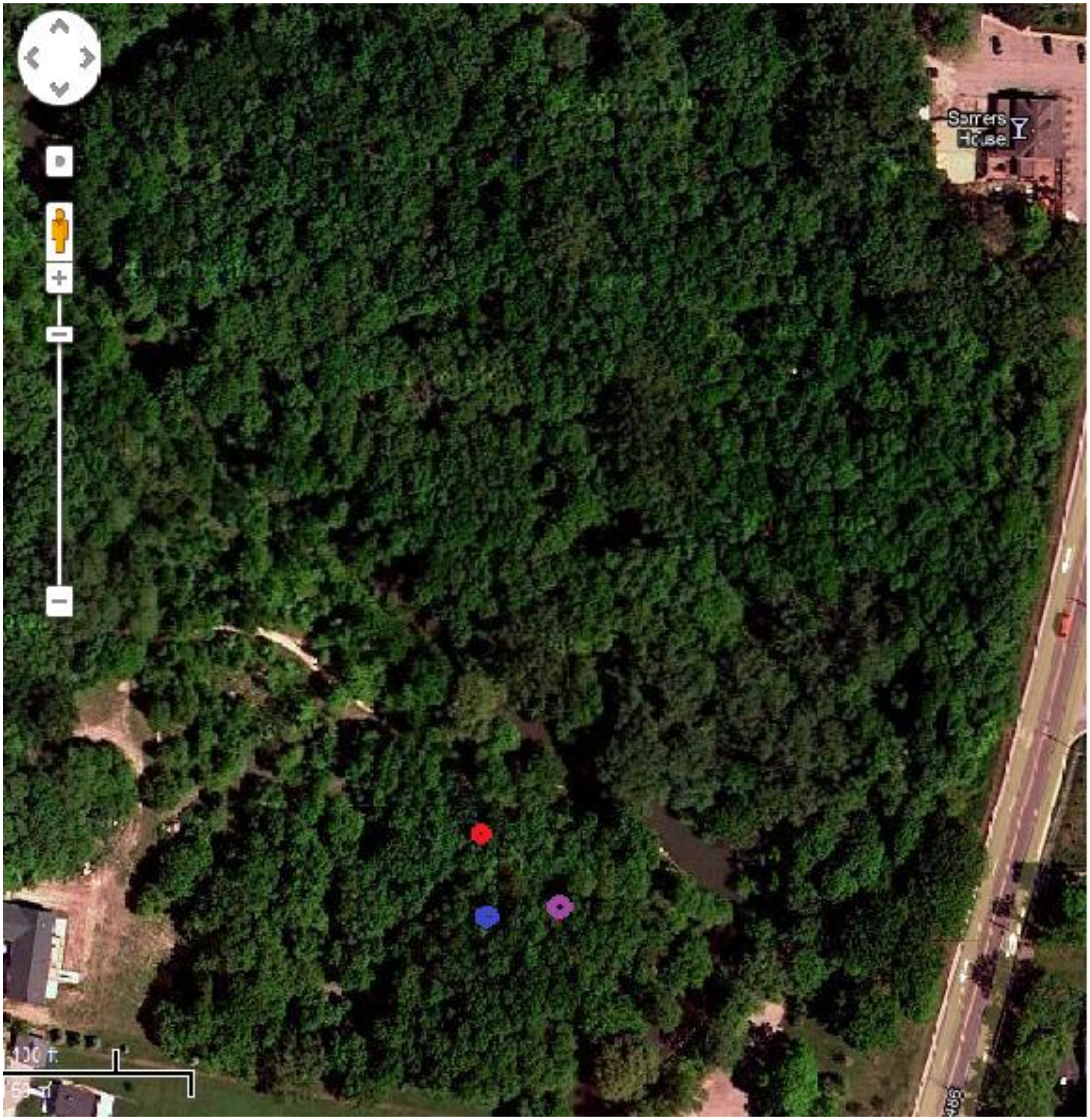


Figure 8: This map shows the area where the samples were taken. The blue area represents where both plants together were found and samples were taken, the red represents garlic mustard, and the purple represents buckthorn.

Lab Analysis

Moisture

Once the 12 samples were collected they were taken to the lab to be analyzed. The first procedure done was to test the moisture or water holding capacity of each soil sample. To do so, an empty soil tin's weight was recorded, then recorded again when a portion of the first soil sample was added to the tin and wetted until saturation (or when the soil has become sticky). The empty tin's weight was subtracted from the total weight in order to distinguish the saturated soil's exact weight. After both weights have been recorded the samples were put into an oven at 105 degrees Celsius for 24 hours. Once the 24 hours was up the soil and tin were weighed once more. This procedure was repeated for all 12 soil samples. To calculate the exact water holding capacity the weight of the water is divided by the weight of the dry soil and then multiplied by 100 to find the percentage. This total shows how much water each soil type is able to hold.

Bulk Density

The Bulk Density of a soil is simply the weight of the solid particles divided by the volume of soil. The bulk density of soil is inversely related to its porosity, meaning the lower the bulk density value the more pore space the soil contains. Bulk density is basically the mass of the combined particles divided by the volume they occupy. This is important because it tells the researcher how much available space the soil contains to be able to hold moisture. In order to obtain the bulk density soil was again added to the previously weighed tins and then weighed. The sample was air dried for at least a week and then reweighed. Once the weights were recorded the samples were oven dried at 105 degrees Celsius for 24 hours and reweighed.

PH

Acidity was tested using LaMotte Soil Test Kit Guide. For each soil tested a 1-8mL test tube was used. The test tubes were filled with 4mL of pH indicator solution. Then 1.5g of soil was added to the test tube with the indicator. This was capped and mixed gently for a minute and let stand for ten minutes until the soil settled. Once the ten minutes were up the color of the solution was matched to a pH color chart to determine the results.

Nutrients

In order to test for nutrients the LaMotte Soil testing Kit was used. All measurements of test tubes and the exact solutions used came from the standards of this kit.

To test phosphorus levels a standard sized test tube was filled to the 6th line with Phosphorous Extracting Solution. 1.5g of soil from each sample were added to the test tube. The tube was capped and then mixed for one minute. After the minute the test tube was set to stand and settle until there was a clear liquid above the soil. The clear liquid was transferred using a pipet to a clean test tube to line 3. Six drops of Phosphorus Indicator Reagent were added to the liquid in the second test tube which was then mixed. After mixing, a Phosphorous test tablet was added and mixed until a color change occurred. A phosphorous color chart was used to determine results.

Potassium was tested in about the same way. A test tube was filled to line 7 with Potassium Extracting Solution and 2g of soil were added to the test tube. This was shaken for a minute and then allowed to sit for the soil to settle. The clear liquid at the top was extracted using a pipet and transferred to a new test tube to line 5. One Potassium Indicator Tablet was added to the liquid in the second test tube and was mixed until the tablet dissolved. Potassium Test Solution was added two drops at a time until the color changed from clear to blue. A Potassium End Point Color Chart was used to determine results.

To test Nitrogen content a test tube was filled to the line 7 with Nitrogen Extracting Solution. 1g of soil from the samples were added to the test tube and then mixed for one minute. The soil was allowed to settle and then a pipet was used to extract the clear liquid at the top and placed into a second, clean test tube to line 3. Two measurements of Nitrogen Indicator were added using a .25g spoon. This was mixed and then let sit for 5 minutes until a color change occurred. The results were determined using a Nitrogen Color Chart.

Data Analysis

The results were analyzed by performing statistical analysis which assessed whether the means of two groups are statistically different from one another. For each test performed in the methods a t-test was used to test if there was a significant difference between garlic mustard with buckthorn, buckthorn with both, and garlic mustard with both. Statistical significance was accepted if p-value was < or = to 0.05.

Results

When calculating the results many factors were taken into consideration. First of all the locations for all 3 of the sampled areas were relatively the same. Each sampled area was within the *Phil Sanders* Audubon Cooperative Wildlife Sanctuary. This means the soils would have received about the same about of precipitation and other weather effects. The 3 locations were also almost equidistant from the river, so that should not have been much of a factor that would affect the results. With the calculated results, much of the data is showing the combined soil to be almost an average of the other two separate soils. This is different when looking at pH where the combined soil is higher than the two separate soils, and when looking at nitrogen and potassium.

Water Holding Capacity

The results of the water holding capacity testing showed garlic mustard as having the highest percent of holding capacity with an average of 69.02%, followed by the soil with both plants at 56.53%, and then buckthorn at 46.52% holding capacity. Although there is a visible difference between the 3 soil types in figure 9, statistically they are not different. The p-values for each of the three comparisons were well above .05, making the data insignificant.

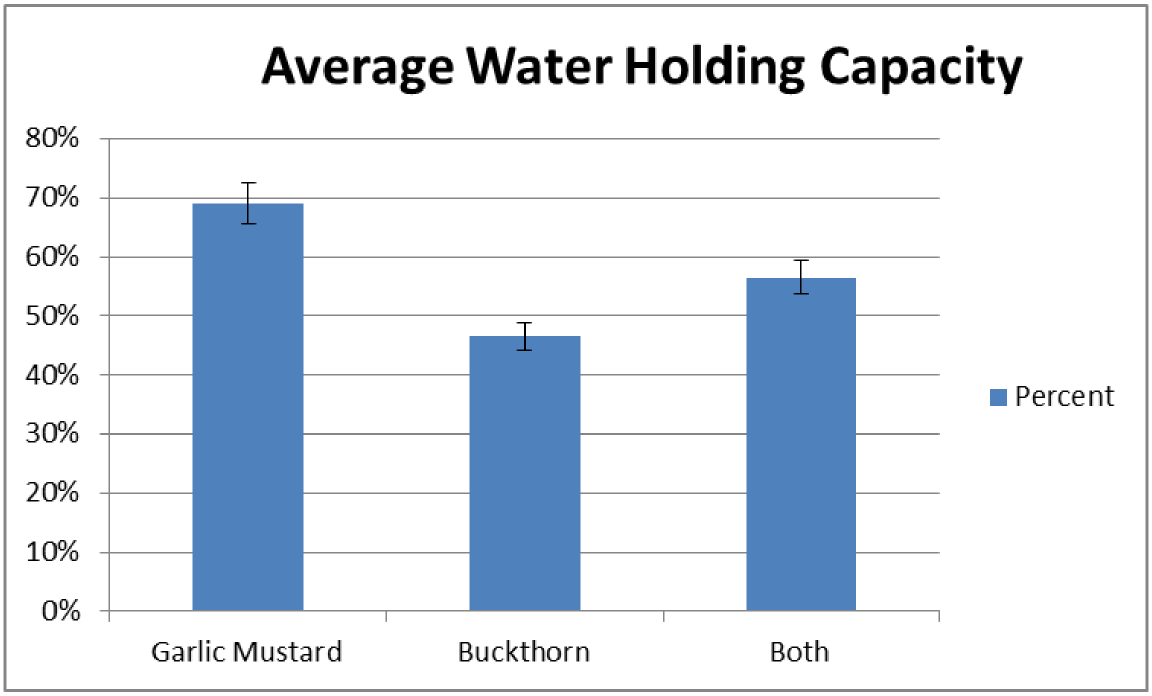


Figure 9: The average percent water holding capacity for the 3 different soils.

Bulk Density

The average bulk density was highest with buckthorn at $6cm^3$, followed by both soil with $.57cm^3$, and garlic mustard with $.54cm^3$. There is not a clear difference between the samples even in figure 10. Statistically, there was also no significant difference. The p-values were greater than 0.2 for each of the 3 samples, much higher than the .05 they would need to be under in order to be significant.

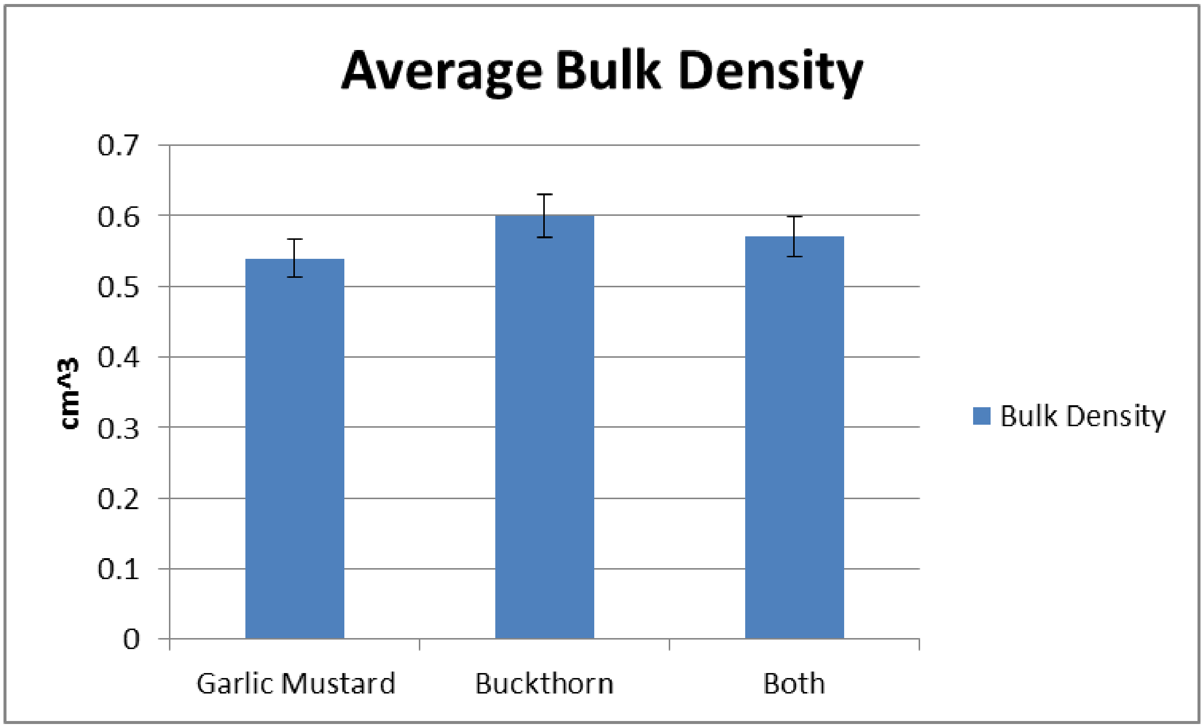


Figure 10: The average bulk density of the 3 soil types.

PH

There is a clear difference shown in figure 11 between the 3 soil types with buckthorn being the lowest at an average pH of 7.1, garlic mustard next with an average pH of 7.3, and the soil from both plants reaching the highest with an average pH of 7.6. Statistically, there is a significant difference when comparing the buckthorn samples with the samples of the combined plants. This p-value was 0.0567. Garlic mustard when compare to buckthorn showed a p-value of 0.195 and garlic mustard compared to the combined soil showed a p-value of 0.244.

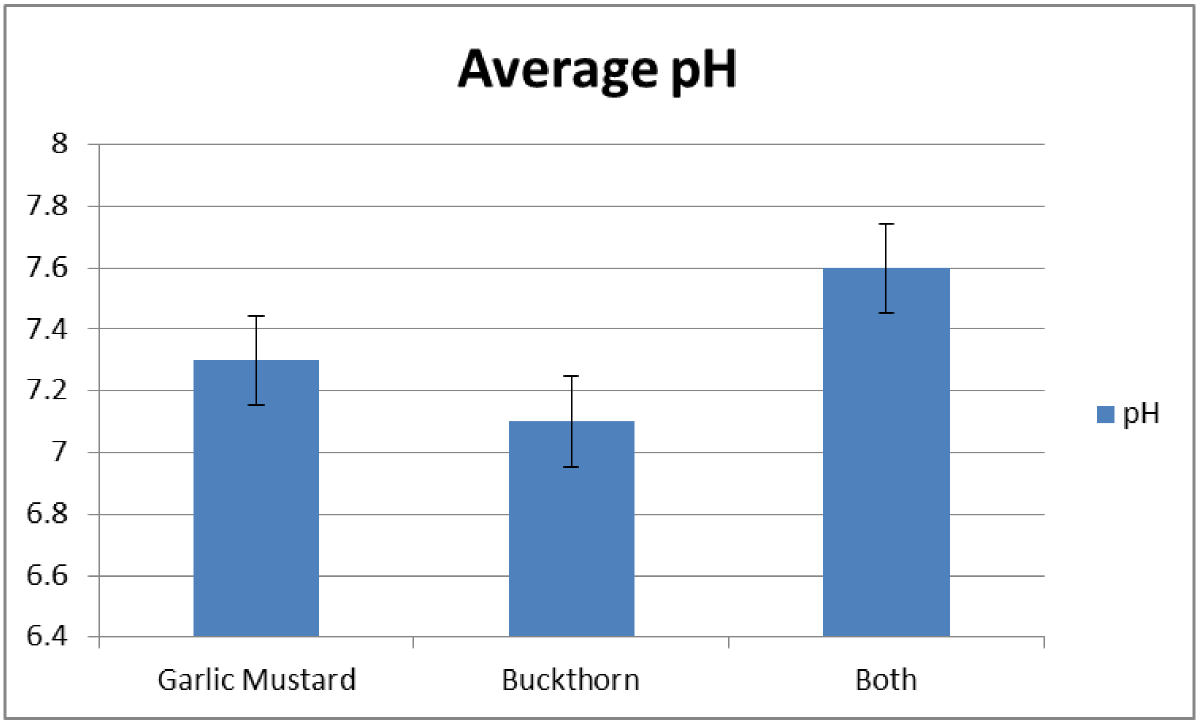


Figure 11: The average predicted pH values for the 3 soil types.

Nutrients

The nutrient levels show the most significant difference between the different soil types than any of the other tests. Figure 12 shows the buckthorn soil and combined plant soil to have an almost equal average phosphorous level at 95 and 97 pounds per acre respectively and garlic mustard to be much lower at 82 pounds per acre. Statistically garlic mustard vs. buckthorn showed a significant difference with a p-value of 0.006. Garlic mustard vs. the combined plant soil also showed a significant difference with a p-value of 0.039. Buckthorn vs. the combined plant soil was not significantly different with a p-value of 0.358. Nitrogen levels were very close between the soil types, but only garlic mustard vs. both showed a statistically significant difference with a p-value of 0.045. Garlic mustard vs. buckthorn was well above 0.4 and buckthorn vs. both had a value of 0.273. With the use of figure 12 one can see how closely related the three types are. Potassium levels follow the pattern on the other two nutrient counts, as evidenced in figure 12. Only one of the three combinations showed a statistically significant difference when a t-test was performed. Buckthorn vs. the combined plant soil contained a p-value of 0.090. Garlic Mustard vs. the combined plant soil contained a p-value of 0.318. Garlic Mustard vs. buckthorn contained a p-value of 0.028, showing this comparison to have a significant difference.

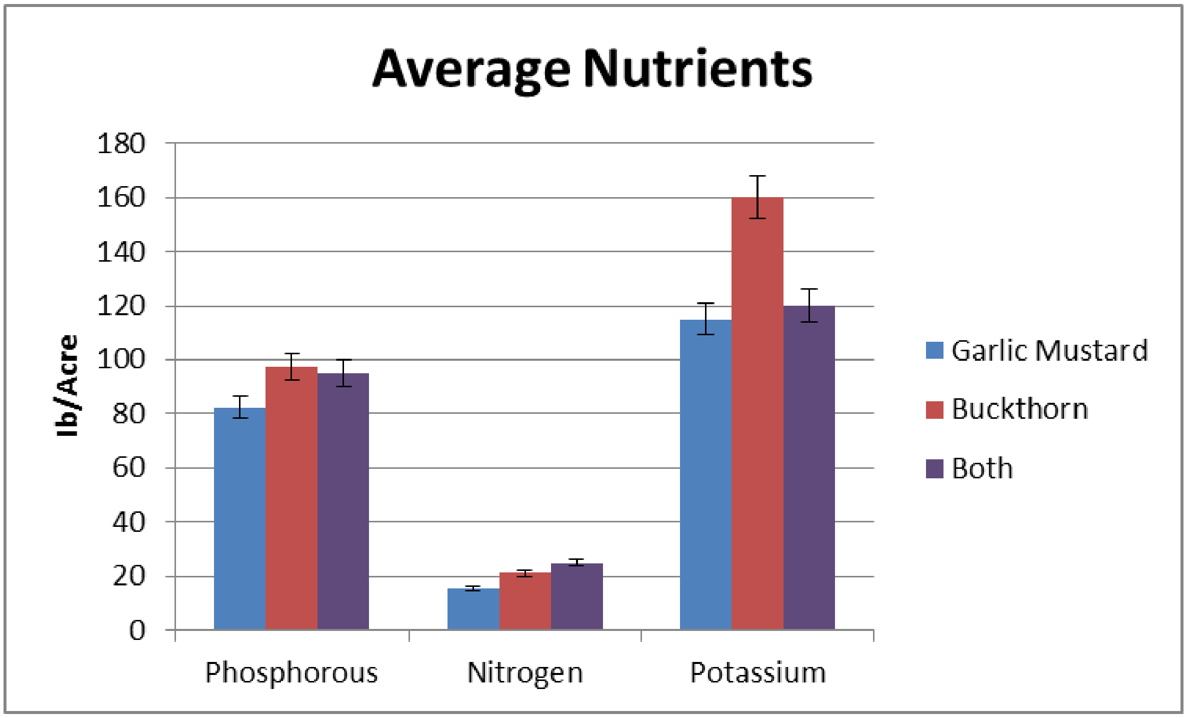


Figure 12: The predicted results in pounds per acre of average nutrients for the 3 soil types.

Discussion

The purpose of this study was to determine if there would be a significant difference in soil composition between three types of soil: soil where only stands of buckthorn are growing, soil where only patches of garlic mustard are growing, and soil where the two invasive plants are growing together. Though not many results proved to be statistically significant, there were clear differences in the soil composition when concerning bulk density, moisture, pH, and nutrient levels. The hypothesis that with the combined growth of buckthorn and garlic mustard the soil composition will show a significant difference concerning the averages of the soil qualities when compared to soil composition of the two plants growing separately was supported with the results, however the idea that the amount of nutrients in the soil, pH, water holding capacity, and bulk density of the soil where both plants grow together will be higher than the average of the soil where the plants grow apart was not fully supported. This second part was supported with the nitrogen results and the pH. With the other results it appears the soil containing both plants was closer to an average of the properties of the two soils where the plants are growing apart.

Although not statistically significant according to the t-test results, the water holding capacity for the soil where the two plants were growing together was between the other two soil types at 56.53% on average (fig. 9). This is most likely due to the idea that garlic mustard has the ability to raise moisture content, allowing it to hold more water. Buckthorn has also been known to do this (Heneghan, 2006), but research on just how much is scarce. If the two plants were growing together it makes sense that the garlic mustard would raise the soil's ability to hold water, but not enough to its usual abilities because of the buckthorn growing in the area. Three of the four samples from the garlic mustard sites showed a significantly higher water holding capability than those from the buckthorn sites (not shown).

The bulk density results are similar to the results of the moisture testing in that they show the area where the two plants were growing together to be between the soils of the two plants growing separately (fig. 10). This is most likely again due to the idea that garlic mustard is able to hold more water than buckthorn due to its higher amount of pore space, so it raises the soils ability to hold more water also. Since bulk density is lower for garlic mustard, it means that this plant has a higher porosity and that water is able to penetrate the soil and trickle into it, allowing it to contain more water for a longer period of time. There is no statistically significant difference between the soils, which is understandable considering their closely related traits. Although garlic mustard may be able to hold more water than buckthorn, this does not mean buckthorn is unable to hold any water. On the contrary, these two plants are almost equal in their abilities, but according to the data received in this study the bulk density of garlic mustard of all four samples collected were lower than the four samples collected from buckthorn.

The pH values show a significant difference graphically for all three soil types (fig. 11) and statistically for two of the three comparisons. The only non-statistically different values were when the buckthorn soil pH was compared to the garlic mustard soil pH. This is most likely due to the idea that both garlic mustard and buckthorn prefer the same type of soil (higher in pH) so they will have the same basic pH originally. Since the combined soil pH is higher than the two individuals, it can be concluded that the two plants working together to raise the pH of the soil have succeeded in doing just that. The two plants have the ability to raise soil pH to a higher pH that suits their growing abilities (Rodgers, 2008; Heneghan, 2004). In this case, the two being in the same location has not hindered this ability, but increased it. This explains why there is a significant difference when looking at the comparison between buckthorn and the combined plant soil and garlic mustard with the combined plant soil.

Buckthorn has been known to be able to increase the amount of phosphorous in the soil once the plant has successfully invaded an area (Heneghan, 2004). Because of this ability, the combined growth of buckthorn and garlic mustard in an area should show an almost equal amount of phosphorous in the soil than if buckthorn were growing alone. This is evident in figure 12 where buckthorn and the area with both plants have an almost equal amount of phosphorous. The buckthorn in this area may have a slightly higher amount of phosphorous due to higher productivity of the specific plants in the sample area. Garlic mustard does not hinder the intake of phosphorous in the soil, so its presence in the area does not have any kind of effect. Garlic mustard does however have the ability to raise phosphorous levels as well, which could explain why the combined soil has a high level of phosphorous (Rodgers, 2008). Buckthorn also has been known to incorporate more nitrogen into the soil, an ability garlic mustard has but to a smaller scale (Heneghan, 2004; Rodgers, 2008). This is evident in figure 12 as well. Since both plants have the ability to incorporate nitrogen into the soil, the results showing the area with the compared plants follow this theory by having a higher amount of nitrogen than the two separate soils. Potassium showed a statistically and graphically (fg. 12) significant difference between the two soils growing separately and the combined soil. This is most likely due to both garlic mustard and buckthorn being able to incorporate different amounts of potassium into the soil (Heneghan, 2004; Rodgers, 2008). With both plants incorporating the nutrient into the soil at different rates the amount in the soil at one time varies between sites. With the two plants growing together in the same area you can either obtain a higher amount in the soil due to both plants producing a large amount of potassium, or an average amount in the soil, due to one plant producing a smaller amount than the other. Buckthorn has a stronger ability for fast incorporation of this nutrient into the soil, which is supported by the results.

Conclusion:

Overall, there are clear differences in soil composition depending on if garlic mustard and buckthorn are growing together or separately, even if they are minimal. More research is needed to see how these two plants affect soil in different environments, over different periods of time, or in other aspects aside from moisture, bulk density, pH, and these specific nutrients. Another method of improving this research would be to use more efficient testing methods on the soils that will give more accurate results. Using a much larger amount of samples will also help to give a clearer picture of the results. However, this research can be used to supplement future studies concerning the relationships between different invasive species, or how invasive species are able to affect soil composition. Understanding the abilities these invasive species possess may possibly help to improve measures of removal of the plants in that in order to prevent long-term effects on an ecosystem the plants would need to be removed before they are able to germinate. Existing plants should be completely removed rather than simply cut back. Future studies could use more samples from different forests in order to obtain a broader picture of what is occurring and to see if the results in this study show a pattern. Also, performing studies to show how each individual plant affects the soil composition rather than just doing a comparison study of how the two plants work together will show more of why the differences between the soils occurred rather than just knowing that they did occur.

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References

Anderson, Roger C, Shivcharn S Dhillion, and Timothy M Kelley. "Aspects of the Ecology of an Invasive Plant, Garlic Mustard (Alliaria petiolata), in Central Illinois." *Restoration Ecology* 4.2 (1996): 181-191. Print.

Anderson, Roger C, and Timothy M Kelley. "Growth of garlic mustard (Alliaria petiolata) in native soils of different acidity." *Transactions, Illinois State Academy of Science* 88.3-4 (1995): 91-96. Print.

Blossey, Bernd, and Rolf Notzold. "Evolution of Increased Competitive Ability in Invasive Nonindigenous Plants: A Hypothesis." *Journal of Ecology* 83.5 (1995): 887-889. Print.

Daehler, Curtis C. "Performance Comparisons of Co-Occurring Native and Alien Invasive Plants: Implications for Conservation and Restoration." *Annual Review of Ecology, Evolution, and Systematics* 34 (2003): 183-211. Print.

Davis, Adam S, et al. "Demographic Models Inform Selection of Biocontrol Agents for Garlic Mustard (Alliaria petiolata)." *Ecological Applications* 16.6 (2006): 2399-2410. Print.

Dhillion, Shivcharn S, and Roger C Anderson. "Growth and photosynthetic response of first-year garlic mustard (alliaria petiolata) to varied irradiance." *Torrey Botanical Society* 126.1 (1999): 9-14. Print.

Ehrenfeld, Joan G, Peter Kourtev, and Weize Huang. "Changes in Soil Functions Following Invasions of Exotic Understory Plants in Deciduous Forests." *Ecological Applications* 11.5 (2001): 1287-1300. Print.

Gordan, Doria R. "Effects of Invasive, Non-Indigenous Plant Species on Ecosystem Processes: Lessons from Florida." *Ecological Applications* 8.4 (1998): 975-989. Print.

Heneghan, Liam, et al. "European Buckthorn (Rhamnus cathartica) and its Effects on Some Ecosystem Properties in an Urban Woodland." *Ecological Restoration* 22.4 (2004): 275-280. Print.

"Invasive Species." *Wisconsin Department of Natural Resources*. Wisconsin Department of Natural Resources, 23 June 2011. Web. 11 Oct. 2011. <http://dnr.wi.gov/invasives/>.

Knight, Kathleen S, et al. "Ecology and ecosystem impacts of common buckthorn (Rhamnus cathartica): a review." *Biological Invasions* 9 (Feb. 2007): 925-937. Print.

Kourtev, Peter S, Joan G Ehrenfeld, and Max Haggblom. "Exotic plant species alter microbial community structure and function in the soil." *Ecology* 83.11 (2002): 3152-3166. Print.

Kurylo, J S, et al. "Rhamnus cathartica: Native and Naturalized Distribution and Habitat Preferences." *Journal of the Torrey Botanical Society* 134.3 (2007): 420-430. Print.

Leicht-Young, Stacey A, et al. “Effects of an Invasive Plant Species, Celastrus orbiculatus, on Soil Composition and Processes.” *American Midland Naturalist* 161.2 (2009): 219-231. Print.

Leishman, Michelle R, et al. “Leaf Trait Relationships of Native and Invasive Plants: Community- and Global-Scale Comparisons.” *New Phytologist* 176.3 (2007): 635-643. Print.

Liu, Hong, and Peter Stilling. “Testing the enemy release hypothesis: a review and meta-analysis.” *Biological Invasions* 8.7 (2006): 1535-1545. Print.

Lodge, David M, Susan Williams, and Hugh J MacIssac. “Biological Invasions: Recommendations for U.S. Policy and Management.” *Ecological Application* 16.6 (2006): 2035-2054. Print.

Morrison, Janet A, et al. “Ecological comparisons of two co-invasive species in eastern deciduous forests: alliara petiolata and microstegium vimineum.” *Torrey Botanical Society* 134.1 (2007): 1-17. Print.

Munger, Gregory T. “Alliaria petiolata.” *Fire Effects Information System*. U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory, 2001. Web. 12 Nov. 2011. <http://www.fs.fed.us/database/feis/>.

Parker, Ingrid M, and Gregory S Gilbert. “When there is no escape: the effects of natural enemies on native, invasive, and noninvasive plants.” *Ecology* 88.5 (2007): 1210-1224. Print.

Read, D B, et al. “Plant Roots Release Phospholipid Surfactants That Modify the Physical and Chemical Properties of Soil.” *New Phytologist* 157.2 (2003): 315-326. Print.

Roberts, Karl J, and Roger C Anderson. “Effect of Garlic Mustard [Alliaria Petiolata (Beib. Cavara & Grande)] Extracts on Plants and Arbuscular Mycorrhizal (AM) Fungi.” *American Midland Naturalist* 146.1 (2001): 146-152. Print.

Rodgers, Vikki L, et al. “The invasive species alliaria petiolata (garlic mustard) increases soil nutrient availability in northern hardwood-conifer forests.” *Oecologia* 157.3 (2008): 459-471. Print.

Soule, Michael E. “The Onslaught of Alien Species, and Other Challenges in the Coming Decades.” *Conservation Biology* 4.3 (1990): 233-239. Print.

Warren, J, C J Topping, and P James. “An Evolutionary Modeling Approach to understanding factors behind plant invasiveness and community susceptibility to invasion.” *Journal of Evolutionary Biology* 24.10 (2011): n. pag. Print.

Wolfe, Benjamin E, and John N Klironomos. “Breaking New Ground: Soil Communities and Exotic Plant Invasion.” *BioScience* 55.6 (2005): 477-487. Print.

Wolfe, Benjamin E, et al. “The invasive plant Alliaria petiolata (garlic mustard) inhibit sectomychorrhizal fungi in its introduced range.” *Journal of Ecology* 96.4 (2008): 777-783. Print.