28 November 2015

David Dornbos, PhD
Professor of Biology, Calvin College
3201 Burton Ave. Grand Rapids, MI
616-526-8578
dld9@calvin.edu

Influence of autumn olive (*Elaeagnus umbellata)* on plant community composition and soil fertility

DAVID L. DORNBOS, PhD, *Calvin College*, 3201 Burton Ave, SE, Grand Rapids, MI 49546, USA

KARISSA B. SMIT, *Calvin College*, 3201 Burton Ave, SE, Grand Rapids, MI 49546, USA

PHILIP J. TUBERGEN, *Calvin College*, 3201 Burton Ave, SE, Grand Rapids, MI 49546, USA

ABSTRACT The non-native, invasive shrub *Elaeagnus umbellata* is now common in the northeastern United States and Canada. *E. umbellata* outcompetes its native neighbors by growing faster and utilizing a greater proportion of light, water, and minerals due to faster photosynthesis rates. It has been established that *E. umbellata* is a nitrogen fixer and has been shown to facilitate the growth of co-habiting plants, but it is unclear whether or not it makes biologically available nitrogen accessible to plants around it. *E. umbellata* may be allelopathic or stimulatory, and may pose a challenge to native biodiversity. Modification of soil chemistry by *E. umbellata* could represent a mechanism by which *E. umbellata* gains competitive advantage and reduces species richness. This study examined impacts of *E. umbellata* on soil chemistry and plant species composition. Our hypothesis was that *E. umbellata* changes the soil chemistry of its rhizosphere by increasing the quantities of plant-available nitrogen and reducing that of calcium and magnesium, either directly or indirectly favoring the growth of neighboring *Gramineae* species. There were ten sites for study in a meadow habitat and 10 in a forest habitat; each site had one plot with *E. umbellata* and one plot without *E. umbellata*. We examined the impact *E. umbellata* had on other species by comparing leaf chlorophyll and protein content of co-habiting plant species, and also measuring the ions in the soil. We found lower total protein and greater chlorophyll content in leaves of Virginia creeper in forest habitats and in leaves of Kentucky bluegrass in field habitats when growing with *E. umbellata*. Species richness and evenness were not affected by *E. umbellata.* Lower concentrations of plant-available minerals were found in *E. umbellata* soils, potentially because this fast-growing plant extracts large water volumes and the minerals available within those soils. The germination study revealed that there was an inhibitory effect of *E. umbellata* on other seeds when

extracts were made from frozen leaves, compared with very minor effects using extracts from fresh leaves or roots, implying allelopathic tendencies may be triggered by frost. While we did support the hypothesis that *E. umbellata* changed soil fertility, we did not see a corresponding change in biodiversity.

KEY WORDS: autumn olive, *Elaeagnus umbellata*, allelopathy, biodiversity, soil chemistry, invasive, non-native, nitrogen fixer

INTRODUCTION

Non-native organisms are organisms introduced accidentally or intentionally into an ecosystem in which they have not historically inhabited. Some non-native species are further classified as invasive. Invasive organisms are capable of rapidly colonizing available niches, particularly after a disturbance. Non-native invasive plants exact a heavy price on ecosystem health. They frequently outcompete native plants for sun, water, and nutrients, driving reductions in floristic quality, biodiversity, and species richness of an area. Invasive plants cost approximately 120 billion dollars annually in lost ecosystem goods and services (Pimental et al. 2005).

Autumn olive (*Elaeagnus umbellata* Thunb.) is a non-native, invasive shrub now commonly found throughout the northeastern United States and Canada. This shrub was introduced from Asia (Ahmad et al., 2008) to aid in the reclamation of strip-mined soils in Southern Illinois, Indiana, and Kentucky because it grew well in highly disturbed soil with acidic pH, poor fertility, and minimal organic matter. *E. umbellata* was also well suited to stabilize roadside soil and supply fruit to migrating birds. The ability of *E. umbellata* to grow in disturbed environments while also providing wildlife value prompted the Michigan Department of Natural Resources to promote the planting of this species to willing land-owners (Michigan DNR, 2012). *E. umbellata* is now common on old farm fields, along roadsides, and is occasionally found in forest understories (Edwards and Dornbos, 2007).

E. umbellata represents a significant portion of the shrub layer on the Pierce Cedar Creek Institute property, Barry County, Michigan. The Institute has actively supported efforts to remove *E. umbellata* through cutting, treating, and burning to minimize the potential negative impacts of *E. umbellata* on ecosystem goods and services. In a two year period between 2005 and 2007, *E. umbellata* cover increased 14% within the boundaries of the Institute and increased from a low density of plants to a medium or from a medium to high density of individuals on

approximately 30% of the area where this species had previously invaded (Edwards and Dornbos 2007). This suggests that once *E. umbellata* colonizes an area, it can effectively become a dominant member of the landscape within a relatively short time period.

Invasive plants can exploit landscapes by using one or more mechanisms to gain a competitive advantage. One non-native invasive plant, spotted knapweed (*Centaurea maculosa*), utilizes allelopathy by exuding catechins into soil, which inhibits the growth of neighboring plants. This competitive edge results in increased colonization and establishment by *C. maculosa* (Pollock et al., 2009). Similarly, black walnut (*Juglans nigra*) releases juglone to disrupt root plasma membrane ATPase activity and to inhibit water uptake (Hejl and Koster, 2004). Orr et al. (2005) tested the potential of *E. umbellata* to be allelopathic and found that a minced leaf tea of *E. umbellata* reduced sycamore (*Platanus occidentalis*) root mass but enhanced seedling emergence. In contrast, tall fescue (*Lolium arundinacea*), known to be allelopathic, produced multiple negative effects in sycamore and other tree species. Although it is often surmised that *E. umbellata* might interfere with the growth of native plants by allelopathic mechanisms, very little evidence supports this notion.

A second mechanism by which invasive species gain a competitive edge is through their ability to simply grow faster than its neighbors, thereby garnering a greater proportion of light, water and soil minerals. Previous research at Pierce Cedar Creek Institute demonstrated that *E. umbellata* has similar or faster photosynthetic rates than native woody shrubs and early colonizing trees in meadow environments. Results collected in 2006 and 2008 indicate that *E. umbellata* exhibited net photosynthetic rates comparable to black cherry, hawthorn, and black walnut and a 20% greater rate than in gray dogwood (Ritsema and Dornbos, 2006; Hesselink and Dornbos, 2008). These data support the idea that *E. umbellata* competes well with native species in meadow environments, but does not fully account for the ability of *E. umbellata* to aggressively invade such areas. *E. umbellata did, however,* exhibit extraordinarily faster growth associated with higher photosynthetic rates in forest understories. *E. umbellata* produced growth rates that were twice that of native juvenile trees of similar size and exhibited net photosynthesis rates of 4.7 μmols $CO_2/m^2/s$, whereas those of black cherry, beech, black oak, and red maple produced rates between 1.9-2.5 μmols/m^2/s. Leaf chlorophyll content closely correlated with growth rate of *E. umbellata* indicating that it has more photosynthetic capacity with which to capture and to utilize sunlight. Ritsema and Dornbos (2006) and Hesselink and Dornbos (2008) also found that *E. umbellata* is no more efficient at water use efficiency, but

rather transpires greater water volumes, producing significantly higher transpiration rates of 2.4 mmols/m^2/s in comparison with the native seedlings 1.8-1.9 mmols/m^2/s.

E. umbellata is also recognized as a nitrogen fixing plant by virtue of a symbiotic mutualism with *Frankia* spp. (Mirza et al., 2009). Access to adequate biologically available nitrogen in otherwise nitrogen-limited environments like forest understories or sandy meadows could enable *E. umbellata* to produce substantively higher chlorophyll amounts contributing to faster growth rates than its competitors.

Non-native plants can impact the biodiversity of ecosystems indirectly or directly. Rudgers and Orr (2009) reported that the non-native grass tall fescue (*Lolium arundinaceum*) indirectly affects biodiversity by promoting the presence of a symbiotic fungal endophyte that inhibits the growth of some native trees, yet enhances that of others. *E. umbellata* has been implicated in the indirect alteration of aquatic ecosystems. Goldstein et al. (2009) found the percent of *E. umbellata* cover was positively correlated with mean stream nitrate concentrations, but not with ammonium ion concentrations. While other studies have demonstrated a significant relationship between native nitrogen-fixers and stream nitrate, this is the first study to document this relationship for an invasive, exotic N-fixing species. *E. umbellata* can impact species diversity directly through competition and indirectly by altering stream nitrogen levels.

A potential concern is whether *E. umbellata* could alter soil chemistry by virtue of its ability to fix nitrogen. There is some evidence that *E. umbellata* facilitates the growth of native plants growing nearby. Black walnut trees growing in plantations with interplanted *E. umbellata* shrubs grew taller and produced greater diameter at breast height than black walnut trees growing in monoculture (Funk et al., 1970). Bouma et al. (2011) found similar results at Pierce Cedar Creek Institute. Over the course of the growing season, native plants growing with an *E. umbellata* consistently produced higher leaf numbers and leaf chlorophyll content than those growing with another member of the same species. *E. umbellata* always exhibited the fastest growth rates and highest chlorophyll content, but clearly the native seedlings received some benefit during the growing season by growing with *E. umbellata*. Curiously, approximately 40% of the native plants failed to resume growth after overwintering whereas all of the *E. umbellata* plants and most of the native plants growing with another native plant of the same species successfully overwintered. These results suggest that some benefit was conferred to native plants from *E. umbellata* during the growing season. Presumably the benefit was biologically available nitrogen, because these plants would be

competing for water and light during the growing season. This benefit conferred upon native plants was insufficient, however, to overcome the capacity of *E. umbellata* to limit overwintering success.

The presence of biologically-available nitrogen in soils or soil water would provide a plausible explanation for the growth advantages of plants in juxtaposition with *E. umbellata*. Efforts to identify higher levels of biologically-available nitrogen in soils near *E. umbellata* have been marginally successful. Goldstein at al. (2009) found a positive correlation between the concentration of nitrate-N in stream water and higher percent *E. umbellata* cover, implying that *E. umbellata* makes nitrate available in the soils it inhabits. A study conducted at the Pierce Cedar Creek Institute by Aljobeh and others (2010) found a trend for higher nitrate and ammonium ion under *E. umbellata,* but these differences were not significant. It is unclear if nitrogen was undetected in these samples because *E. umbellata* did not enrich soils during the time of the growing season when the water samples were collected, or if the testing methods were not sufficiently sensitive. Both significantly higher nitrate-nitrogen and ammonium-nitrogen were extracted from soil samples collected under *E. umbellata* shrubs compared to extracts with no *E. umbellata* (unpublished data, Dornbos). No differences in sulfate, phosphate, or potassium ions were found in the *E. umbellata* rhizosphere when compared with *E. umbellata* free soil, while less calcium and magnesium were present under *E. umbellata*. This study further suggests that *E. umbellata* may be altering its soil environment.

Field observations hint that *E. umbellata* might selectively interfere with some plant types and not others. Observations suggest that grasses seem to flourish under an *E. umbellata* canopy, whereas woody plants seldom seem to be successful in surviving or penetrating an *E. umbellata* canopy. Our hypothesis is that *E. umbellata* changes the soil chemistry of its rhizosphere by increasing the quantities of plant-available nitrogen and reducing that of calcium and magnesium, either directly or indirectly favoring the growth of neighboring *Poaceae* species.

METHODS

General Methods

All field work was done at Pierce Cedar Creek Institute in Barry County, Michigan during the summer of 2015. We identified 20 matched pair replicate plots—half in a forest and half in a meadow habitat—either with or without an *E. umbellata* plant, for a total of 40 plots. All plots exhibited a loam soil type: forest plots were located

within Marlette loam soil and meadow plots in Coloma sandy loam soil with the exception of one replicate pair (Perrinton sandy loam). *E. umbellata* plots were separated by at least 20 meters from other *E. umbellata* plots. Each *E. umbellata* plot had a matched plot that was within 20 meters of itself, but more than 5 meters from another *E. umbellata* plant (control plots). We marked every plot with GPS, forester tape, and flags to ensure the same plants were used throughout the project (See Figure 1).

Soil Testing and Extraction

Five soil cores were combined from each *E. umbellata* plot to form a soil composite of about 500g of soil. The same procedure was used to produce composite soil samples for each of the control plots. To reduce variability caused by abiotic factors among plots, we sampled all the plots within field or meadow on the same day. After we took soil from both the meadow and forest replicates, we measured roughly 150g from each sample bag and dried it in a convection oven at 50°C for 24 hours. We took the total mass of the dry soil and compared it to the wet soil to get percent water in the soil. We determined soil carbon by placing approximately 20g of dried soil in a ceramic cuvette and put in a muffle oven at 350°C for 24 hours. After 24 hours, we massed the cuvette with soil, discarded the soil, and placed the cuvettes back into the muffle oven at 350°C. After 24 hours, we massed the empty cuvettes to get an accurate mass of the soil. We used the mass of the soil to calculate percent soil carbon.

To measure soil ions, we sieved the remaining dried soil until the soil was a fine powder, then separated the powder into two 10g samples. We put one 10g sample into a beaker with a stir bar and added 100mL distilled water to produce both anion and cation extracts. We repeated the process with the other 10g sample but used 100mL of 40mM HNO_3 in place of distilled water (Jackson, 17) to produce another cation extract. We then used a magnetic plate to stir the samples at about 300 rpm for both solvent extractions. After stirring five minutes, we poured roughly 20mL of each sample into a centrifuge tube. These samples were spun at 6000 rpm for five minutes to remove sediments from the solvent. We divided the supernatant from each sample into three Dionex™ ion chromatography tubes. We sent these samples to Calvin College for testing of both cations and anions in a Dionex™ ion chromatograph with an AS40-1 autosampler, LC25 column oven, ED40-1 detector and a GP50 gradient pump.

Protein and Chlorophyll Analysis

Protein and chlorophyll analysis was performed on two species co-habiting with *E. umbellata*, each of which was common to all plots in the forest or meadow plots. From the meadow, we tested *Poa pratensis* (Kentucky bluegrass); from the forest, we tested *Parthenocissus quinquefolia* (Virginia creeper). Chlorophyll content was measured with a Minolta SPAD crop chlorophyll meter. Ten readings were taken from each plot to produce an average chlorophyll content reading for each target plant.

We measured protein content in leaves of both the *P. quiquefolia* and *P. pratensis* plants used for chlorophyll measurement. Leaves from each plant were collected when chlorophyll measurements were made then frozen within 6 hours. Upon arrival at Calvin College, we flash froze 1 gram of each of the leaf tissue with liquid nitrogen and ground them with a mortar and pestle. A pinch of sand and 1.5 mL of KPO_4 were added to the mortar to ensure complete cell rupture. Once the plant material was thoroughly ground, we added the liquid and the plant mat to a microcentrifuge tube and centrifuged at 10,000 rpm for 15 minutes. The supernatant was then saved for processing. A standard curve was made using bovine serum albumin to determine the relationship between protein concentration well plate absorption. *P. pratensis* was diluted to half the original concentration, and *P. quiquefolia* was not diluted at all. Samples were read in a 96-well plate.

Allelopathic Testing

To test for allelopathic interference or stimulation effects of *E. umbellata,* we made teas from the leaves and roots of *E. umbellata.* We did not use leaves and roots from our designated replicate plots so as not to disturb the ongoing testing. We tested forest and meadow leaves independently in case there is a difference in the populations of shrubs. After collecting the leaves or roots, we chopped, weighed, and placed them in a large container. For every 250g of fresh leaves, we added 200mL of distilled water and placed the containers in the refrigerator for 48 hours (Vaugnh and Berhow, 1999). Another tea was prepared using previously frozen *E. umbellata* leaves. This tea was prepared in the same manner as the fresh leaf tea. We prepared another type of eluent using soil from under *E. umbellata* plots and soil from control plots in the forest. We made an elution using a 1g to 1mL soil to water ratio. After 48 hours, we centrifuged and collected the liquid for application.

We assembled replicates of petri dishes by placing 3 pieces of filter paper in the bottom of each dish followed by 25 seeds of each species. The four species included white clover (*Trifolium repens),* radish (*Raphanus*

sativus), tall fescue (*Schedonorus arundinaceus*), and Canadian rye (*Elymus canadensis*). We prepared twelve dishes of each species. After the tea had steeped for 48 hours, we applied this liquid to the petri dishes. On each species of seed, we applied 5 mL of forest tea to four dishes, 5mL of meadow tea to four dishes, and 5 mL of distilled water to four dishes. We stored the dishes in the Pierce Cedar Creek Institute field lab, which was air conditioned to about 21°C with low humidity. After 8 days, we checked the dishes for germination and normalcy of the seedlings. We placed each seed within one of three categories: normal, abnormal, or non-germinate. We defined normalcy as having a developed root, a developed shoot, and no discoloration of the roots. We harvested each normal seedling in a petri dish, placed all of them into a weigh-boat, and dried them at 50°C for 24 hours. We massed each weigh-boat after drying with the seeds, then without the seeds, to get the mass of the total number of normal seeds. We then took an average weight per seedling as a measure of seedling vigor.

Biodiversity Assessment

Using the same plots as for soil testing, we assessed biodiversity by throwing a 0.5 m^2 quadrat randomly about five feet from the center of each plot. We first would sight identify the plants within the quadrat. We then counted the number of individual plants within each species. We counted grasses and sedges per tiller. After counting the species, we took samples of any unknown plants for later identification, either using a Michigan Flora (Voss and Reznicek, 2012) book and website (University of Michigan) or with the help of other botanists. We then used these data to calculate a Simpson's index and a Shannon-Weiner diversity and evenness index for within each individual plot.

Statistical Evaluation

Statistical significance of differences between means was evaluated using analysis of variance in each case. The field plot data were evaluated as a completely randomized design in which independent treatments were the presence or absence of E. umbellata in ten replications either in forest or field habitats. Dependent variables included traits like the concentration of each ion, biodiversity indices, and co-habiting plant chlorophyll and leaf protein. Germination and seedling growth test results were evaluated as a split-plot completely randomized design where extract type was used as the main plot and test species (e.g., white clover or Canadian ryegrass) were

subplots. In each case, treatment means were compared using Tukey HSD all-pairwise comparisons and standard error. All statistics were preformed using *Statistix 9* software.

RESULTS

Ion Content in Soil

Most essential nutrients were present in similar or lower concentrations in the *E. umbellata* soil rhizosphere compared to soil with no autumn olive impact. *E. umbellata* had no effect on four macronutrient cations. Magnesium and calcium seem skewed, when in reality, because of the nitric acid digest, more of the cations were pushed off the soil particles due to the complementarity between the conjugate base of nitric acid and magnesium and calcium (Figure 2), although there was a trend for lower ammonium ions in aqueous extracts (Figure 5). In contrast, anions sulfate and nitrate concentrations were significantly lower under *E. umbellata* plants (Figure 3). There were no significant findings in regards to soil cations at the 95% confidence level. However, at the 90% confidence interval, there is a trend of less free ammonia under *E. umbellata* ($p=0.091$; Figure 1, Figure 2).

The concentration of ions varied among seasons. While ion concentrations in soils with or without *E. umbellata* were similar in spring, late fall concentrations of both nitrate and phosphate (Figure 4) and ammonium ion (Figure 5) were significantly higher. While magnesium and calcium levels remained lower under E. umbellata, the difference was less than that during the summer months. Generally, ion concentrations tended to be similar or lower under *E. umbellata* in the summer and similar or relatively higher during the fall as compared with soils less affected by *E. umbellata*.

Protein and Chlorophyll Analysis:

Protein analysis revealed significant differences in both native plant species tested when growing under the influence of *E. umbellata*. Total protein of leaves from both Virginia creeper (forest habitat) and Kentucky bluegrass (field habitat) was significantly lower for plants under *E. umbellata* than for plants outside of the shrubs rhizosphere (p-value 0.049). Chlorophyll content of both native plants growing under *E. umbellata*, however, was significantly higher compared to plants outside of the rhizosphere of *E. umbellata* (p-value 0.0088; Table 1).

Allelopathy

Germination results suggest that *E. umbellata* may have allelopathic effects in some cases and not others. While tea extractions prepared from fresh *E. umbellata* did not show significant inhibition of growth, tea prepared from leaves that were first frozen then used for extraction showed significant inhibition of growth in three of the four species of seeds tested: *Trifolium repens*, *Festuca arundinacea*, and *Elymus canadensis*. *Raphanus sativus* was not significantly affected (Figure 6). When analyzed across all species tested, germination was significantly reduced by a tea prepared with frozen *E. umbellata* leaves (p-value of 0.0031). Unlike germination rates, however, seedling vigor was not significantly reduced ($p= 0.47$ for frozen leaf tea or $p=0.85$ for fresh tea). However, upon visual inspection, it was clear that seedlings were negatively affected by frozen tea applications. While seedlings exposed to water were crisp and normally colored, seedlings exposed to frozen tea were highly discolored and extremely flaccid among all four species of seeds.

Biodiversity Assessments

Both Shannon-Wiener and Simpson's biodiversity indices indicate no significant differences exist between plant communities with *E. umbellata* and those plant communities lacking the shrub (Figure 7). Species richness was not affected, whereas evenness in the plots was different between meadow and forest plots ($p<0.100$).

DISCUSSION

Our research revealed new nuances and reaffirmed previous work on the impact of *Elaeagnus umbellata* on its surrounding soil and plant environment, mostly exhibiting negative effects within the rhizosphere. Surprisingly, and inconsistent with the Third 90 Network results, soil nutrient concentrations under *E. umbellata* were similar or lower during the summer months (Figures 4 and 5). It is unclear if *E. umbellata* is not releasing nitrogen to the local soil environment or if the rapid growth of plants in these areas are simply utilizing all the available nitrogen.

The lack of successful overwintering is supported by our germination studies which support the potential for allelopathic interference by *E. umbellata*. When exposed to *E. umbellata* tea made from frozen leaves, seeds had significantly less success germinating. When the seeds did germinate in “frozen” tea, they were exceedingly

unhealthy looking, flaccid and discolored. It might be possible, therefore, that leaf fall during autumn (leaves are typically green or slightly yellow) produces compounds after freezing that are inhibitory to neighboring plants. Freezing and subsequent thawing would rupture membranes, potentially releasing an enzyme capable of rapidly modifying an innocuous biochemical (removing a glucose moiety, for example) and thereby releasing an allelochemical(s).

In regards to previous findings, we assumed that changes in soil ion content and the potential allelopathy would lead to a change in the plant community surrounding *E. umbellata*. Our results from both the Shannon Wiener index and the Simpson's index suggest that no such difference exists. It cannot be assumed that there is no difference in the plant communities, however, because the composition of the communities can be radically different. It is possible for the indices to reflect similarities in the species amount and population totals, but not reflect the overall floristic quality of the communities. Therefore, it is possible that *E. umbellata* changes not the numbers in the community, rather the quality of the surrounding plant community.

In conclusion, these results support mechanisms by which *Elaeagnus umbellata* interacts with abiotic and co-habiting plants that have been demonstrated or implicated in earlier reports. Soil testing has revealed a lower abundance of nitrogen containing ions within the rhizosphere of *E. umbellata* during the peak growing period, as well as depreciated numbers of other essential plant ions. Surrounding plant health is affected by *E. umbellata* through the increase of chlorophyll in leaves but a decrease in the total leaf protein. Germination studies support the *E. umbellata* may have an adverse effect on seeds and seedlings within its rhizosphere.

LITERATURE CITED

Ahmad, S.D. et al. 2008. Evolutionary Relationship and Divergence Based on SDS-PAGE of Elaeagnus umbellata (Thunb.) Populations, a Multipurpose Plant from the Himalayas. Turk. J. Biol. 32:31-35.

Aljobeh, Y., K. Engerer, and Z. Aljobeh. 2010. Impacts of Elaeagnus umbellata on the Soil and Water Quality at Pierce Cedar Creek Institute. PCCI Report. 28 pgs.

Bouma, C., S. Rentschler, and D.L. Dornbos. 2010. Autumn Olive, Root Nodules, and Nitrogen Fixation. PCCI Report. 14 pgs.

Edwards, K. and D.L. Dornbos. 2007. Characterization of the Competitiveness of Autumn Olive in a Mature Forest. PCCI Final Report. 17 pgs.

Funk, D.T., R.C. Schlesinger, and F. Ponder Jr. 1970. Autumn-olive as a Nurse Plant for Black Walnut. Bot. Gaz. 140:S110-S114.

Goldstein, C.L., K.W.J. Williard, and J.E. Schoonover. 2009. Impact of an Invasive Species on Stream Nitrogen Levels in Southern Illinois. J. Amer. Water Res. Assoc. 45(3): 664-672.

Hejl, A.M. and K. L. Koster. 2004. Juglone Disrupts Root Plasma Membrane H+-ATPase Activity and Impairs Water Uptake, Root Respiration, and Growth in Soybean (Glycine max) and Corn (Zea mays). J. Chem. Ecol. 30(2):453-471.

Hesselink, R. and D.L. Dornbos. 2008. Characterization of the Physiological Competitiveness of Autumn Olive in Meadow and Forest Environments. PCCI Report. 14 pgs.

Michigan Department of Natural Resources. 2012. Autumn olive: Invasive Species – Best Control Practices. 7 pgs.

Mirza, B.S. et al. 2009. Variation in Frankia Populations of the Elaeagnus Host Infection Group in Nodules of Six Host Plant Species after Inoculation with Soil. Microb. Ecol. 58:384-393.

Orr, S.P., J.A. Rudgers, and K. Clay. 2005. Invasive Plants Can Inhibit Native Tree Seedlings: Testing Potential Allelopathic Mechanisms. Plant Ecol. 181(2):153-165.

Pimental, D., R. Zuniga, and D. Morrison. 2005. Update on the Environmental and Economic Costs Associated with Alien-Invasive Species in the United States. Ecol. Economics 52:273-288.

Pollock, J.L. et al. 2009. Catechin-Metal Interactions as a Mechanism for Conditional Allelopathy by the Invasive Plant *Centaurea maculosa*. J. Ecol. 97(6):1234-1242.

Ritsema, M.R. and D.L. Dornbos. 2006. Characterization of the Photosynthetic Competitiveness of Autumn Olive (Elaeagnus umbellata). PCCI Report. 13 pgs.

Rudgers, J.A. and S. Orr. 2009. Non-Native Grass Alters Growth of Native Tree Species via Leaf and Soil Microbes. J. Ecol. 97(2):247-255.

ACKNOWLEDGEMENTS

Pierce Cedar Creek Institute

Willard G. Pierce and Jessie M. Pierce Foundation

Calvin College Biology Department

Calvin College Chemistry Department

APPENDICIES

Tables

Table 1: Mean protein and Chlorophyll content of the combined Virginia Creeper (Forest plots) and Kentucky Blue Grass (Meadow Plots) under and away from *E. umbellata*.

	Under Autumn Olive	No Autumn Olive	P-value
Protein Content (mg/g dry wt.)	1.45	1.56	0.0487
Chlorophyll Content (SPAD units)	22.5	18.5	0.0088

Figures

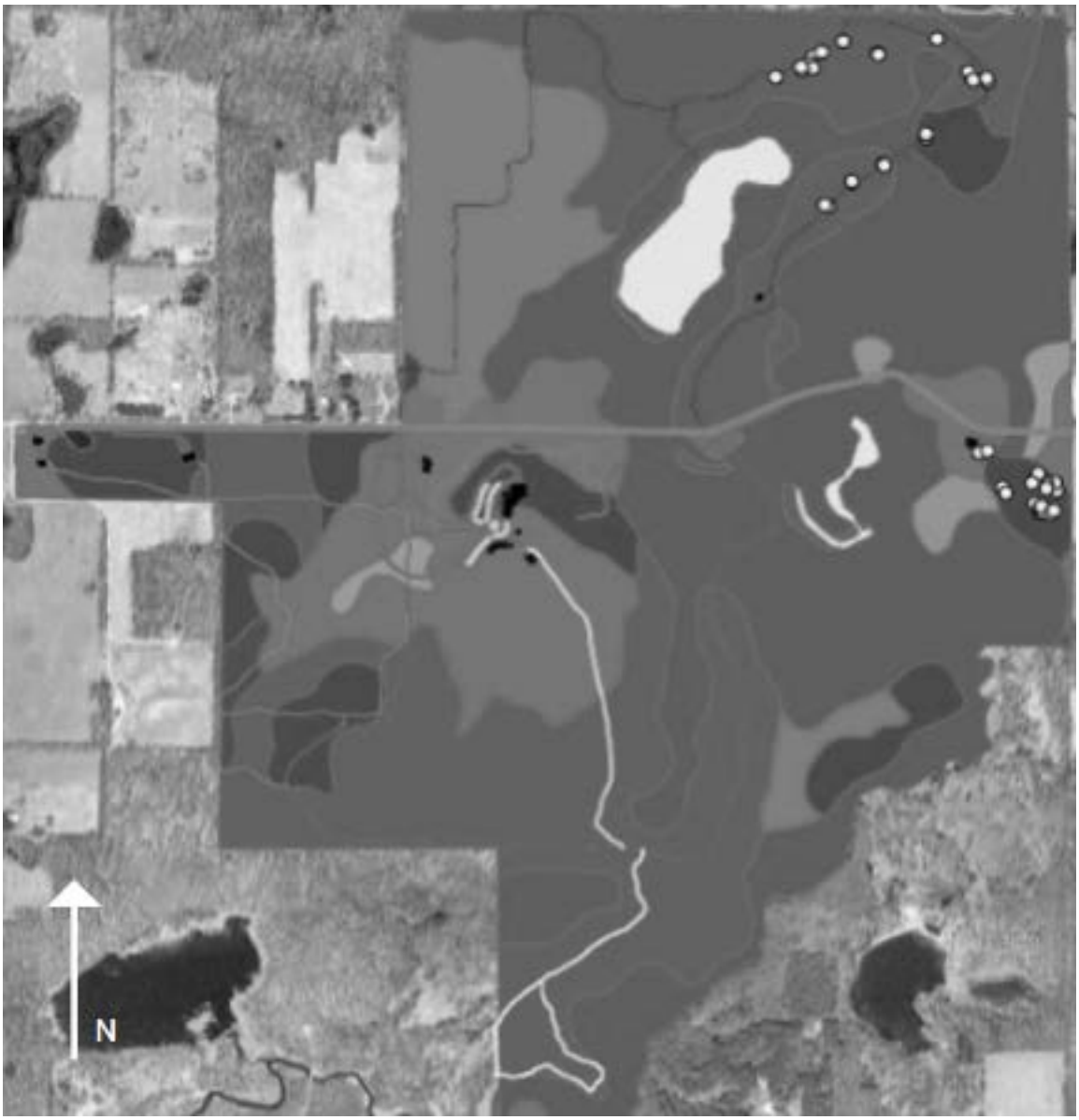

Figure 1: Points of the study plots on Pierce Cedar Creek Institutes' property in Barry County, MI. The 20 points in the upper right corner make up the matched pair forest plots, and the points near the middle of the right edge are the meadow area. The differently shaded blocks on the map show different soil types.

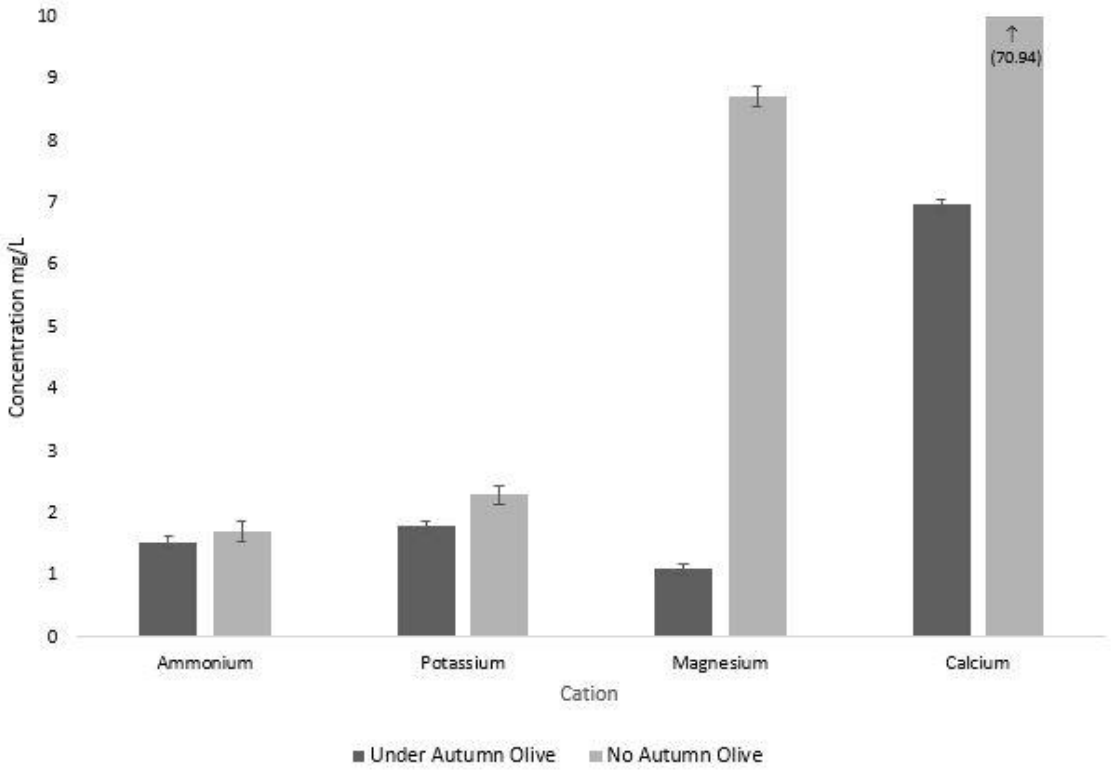

Figure 2: Impact of *E. umbellata* on cations in aqueous soil extracts. An overall comparison combining both forest and meadow and water soil extractions and nitric acid soil extractions. Ammonium p-value: 0.091. Potassium p-value: 0.0097. Magnesium p-value: 0. Calcium p-value: 0.

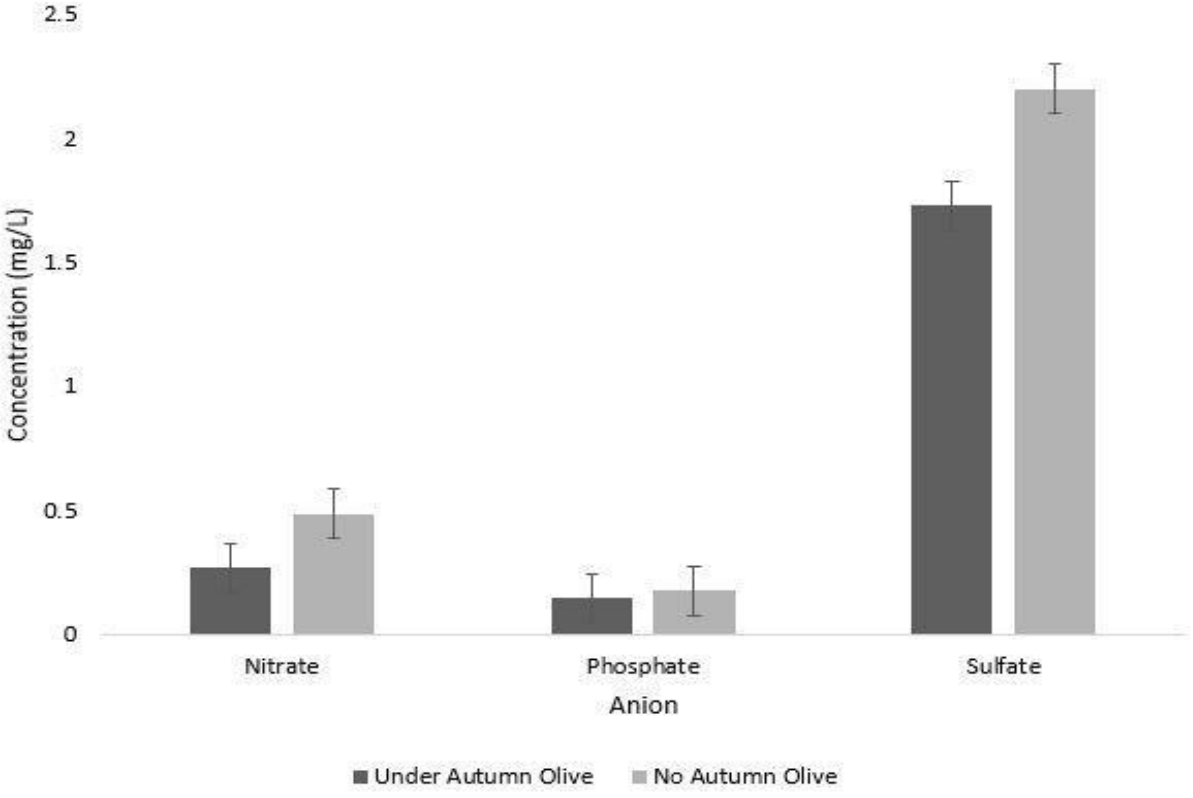

Figure 3: Impact of *E. umbellata* on anions in aqueous soil extracts. An overall comparison combining both forest and meadow plots. Nitrate p-value: 0.007. Phosphate p-value 0.75. Sulfate p-value 0.04.

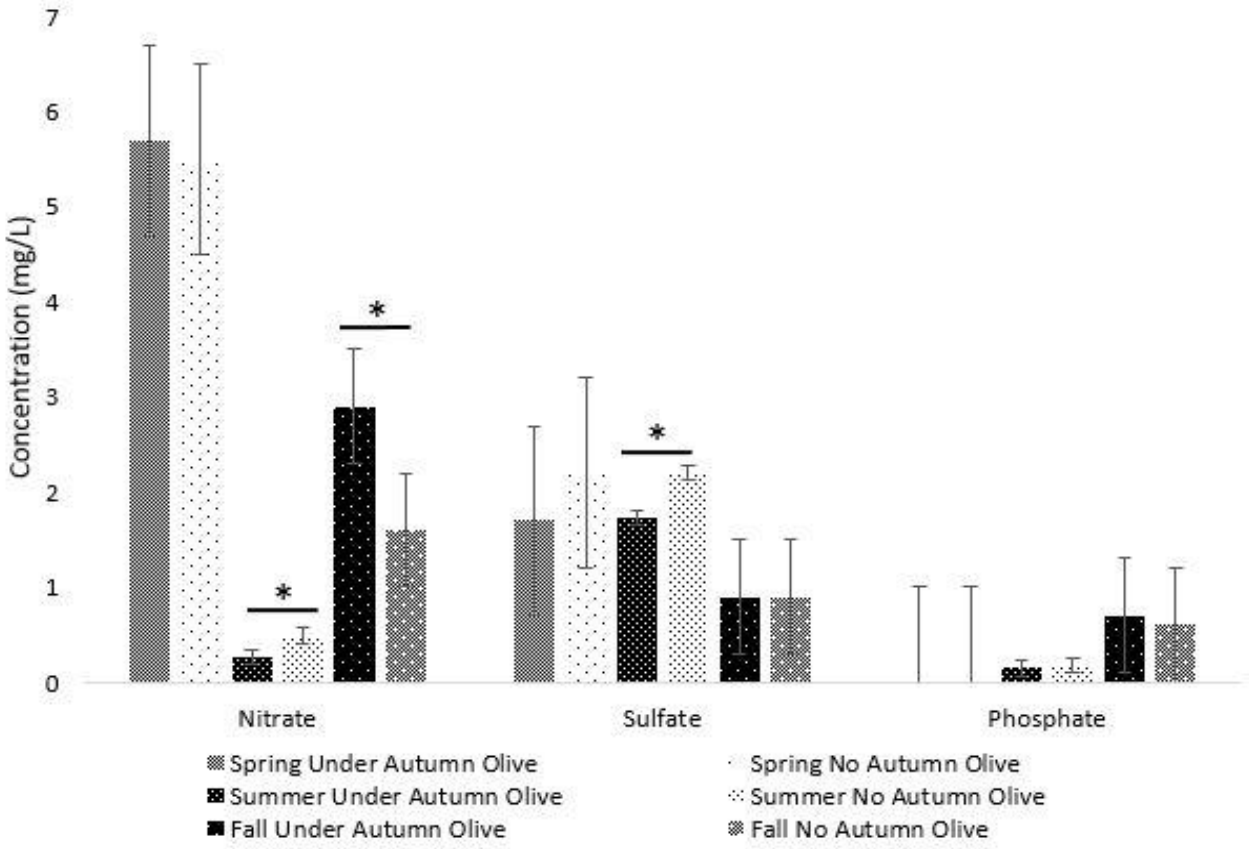

Figure 4: Seasonal comparison of the impact of autumn olive on anions in aqueous soil extracts. Summer data taken at Pierce Cedar Creek Institute. Spring and Fall data taken by the Third 90 pre-collegiate research group at the Blandford Nature Center in Grand Rapids, MI. Significant differences between anions under and away from *E. umbellata* delineated by *.

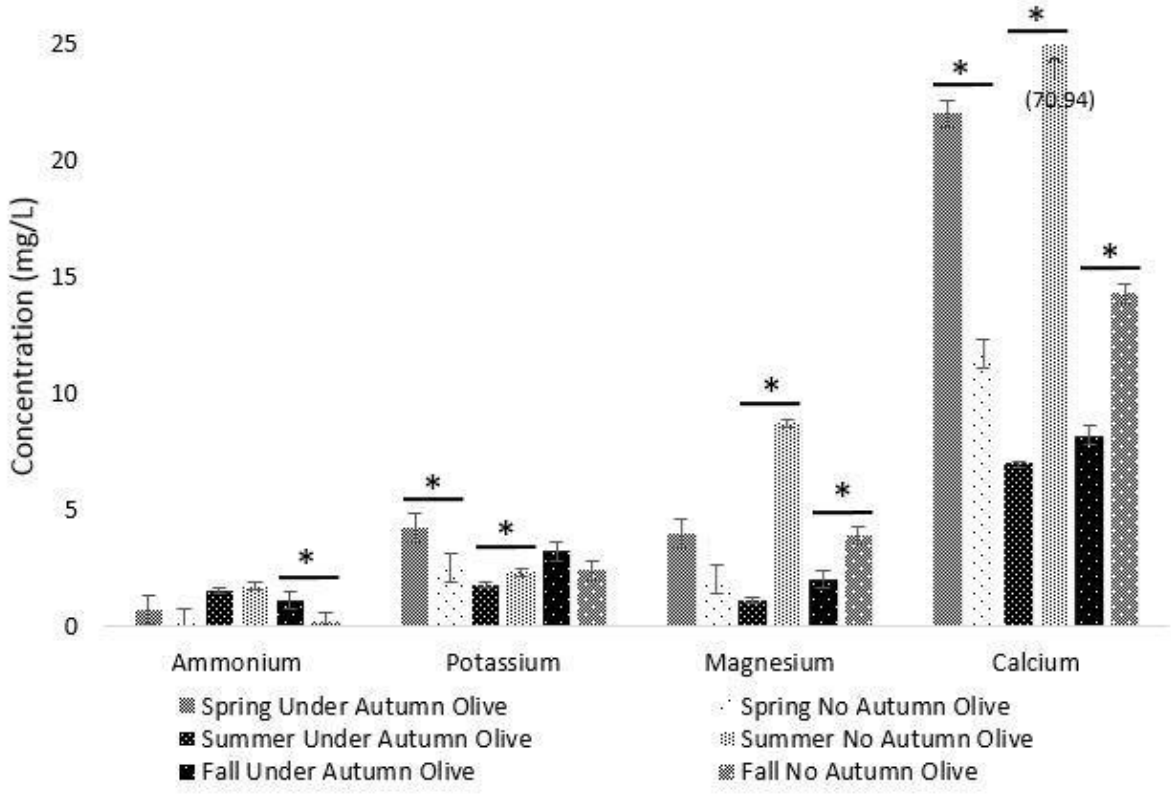

Figure 5: Seasonal comparison of the impact of *E. umbellata* on cations in aqueous soil extracts. Significance between the ions under and away from E. umbellata indicated by *. Spring and fall data from the Third 90 pre-collegiate research group at Blandford Nature Center in Grand Rapids, MI.

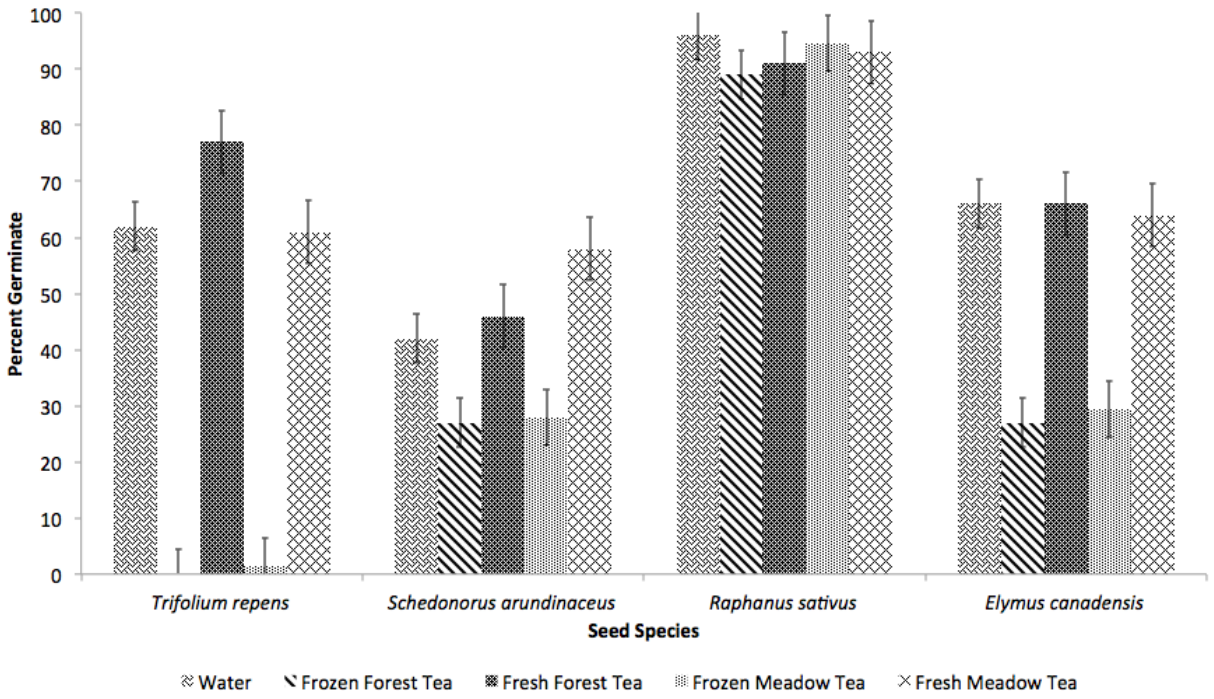

Figure 6. Comparison of water, frozen tea extracts, and fresh tea extracts inhibition on germinating seeds. The *Raphanus sativus* seedlings were clearly very vigorous, so no significance was found. For the other species of seed, frozen teas had a clear inhibitory effect.

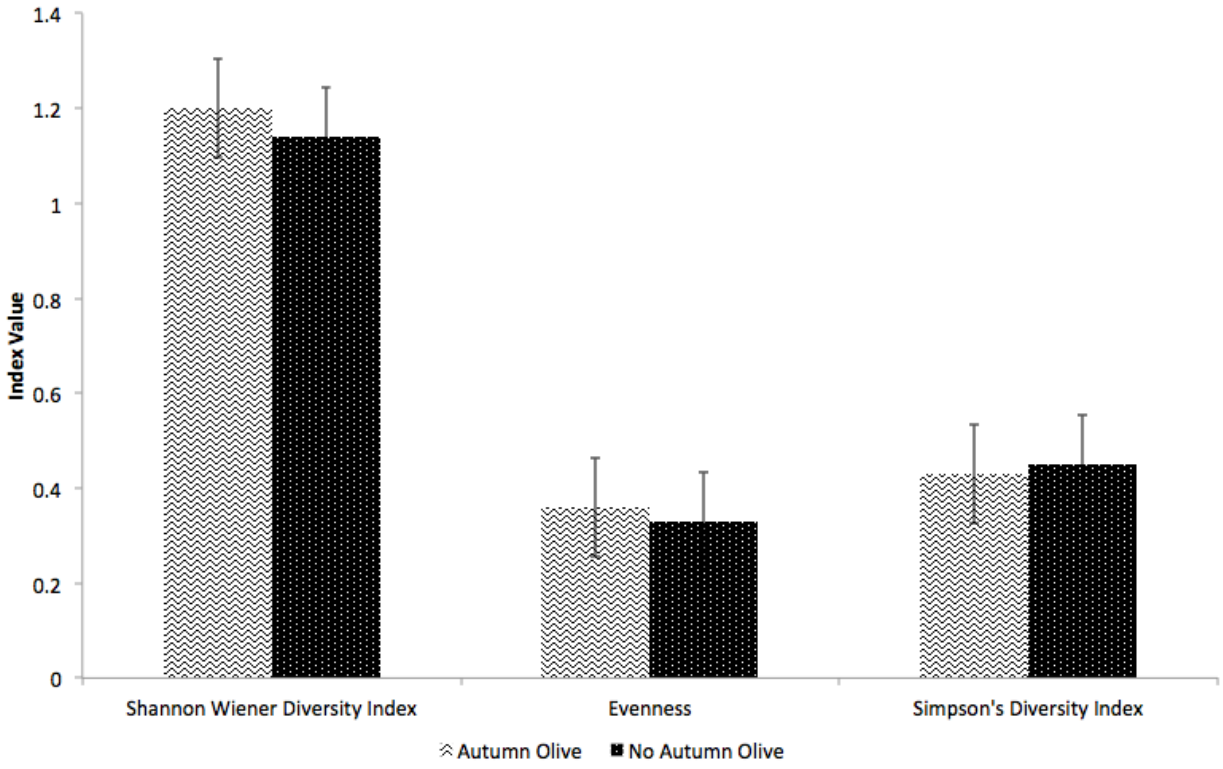

Figure 7. Effect of *E. umbellata* on neighboring plant communities illustrated through Shannon-Weiner diversity index and Simpson's diversity index.