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Testing the Potential Allelopathic Effects of the Non-Native Garlic Mustard (*Alliaria petiolata*) on Saprotrophic Fungi

Michelle Henson
Department of Biology
University of North Carolina at Asheville
One University Heights
Asheville, North Carolina 28804 USA

Faculty Advisor: Dr. Jonathan Horton

Abstract

Forested ecosystems around the world are becoming increasingly vulnerable to non-indigenous plant invasions. Garlic mustard (*Alliaria petiolata*), a naturalized non-native plant species, has successfully invaded woodland habitats across much of the eastern United States because of its highly competitive abilities and the production of allelopathic compounds. The species can limit the growth of native understory species and tree seedling establishment thereby altering community composition and biodiversity by disrupting relationships with various mycorrhizal symbionts. However, the potential adverse effects of garlic mustard allelopathy on saprotrophic fungi have not yet been assessed. We experimentally tested the effects of garlic mustard on growth and fruiting of a widespread saprotrophic fungus, oyster mushroom (*Pleurotus ostreatus*). First year garlic mustard was planted in plastic bins with oyster mushrooms over a 16-week period and were monitored for fruiting body production. We also examined the potential allelopathic effects of garlic mustard on decomposition rates in heavily invaded and uninvaded areas in the Pisgah National Forest of western North Carolina. Forty pairs of litterbags were placed in the field in April 2015. A random subset of these were retrieved after six months and one year in the field and decomposition rates were calculated from mass lost over time. Fruiting body mass totals had no difference between the invaded microcosms and our controls ($p = 0.8637$). Fruiting body mass was significantly ($p < 0.001$) affected by time, however, there was no difference between invaded and control plots ($p = 0.8243$). Under field conditions, we found no significant difference in decomposition between garlic mustard in invaded and uninvaded plots in our six-month treatment ($p = 0.21$) and one-year treatment ($p = 0.87$). However, there was a nearly significant ($p = 0.0596$) decrease in decomposition with increasing garlic mustard stem density. Decomposition rates may have also been confounded by the abundance of an allelopathic tree species ($p = 0.0726$), black walnut (*Juglans nigra*) at the site.

1. Introduction

Non-native invasive plants are known to threaten the integrity of highly diverse and heterogeneous environments[1] and are found in nearly every ecosystem on Earth[2]. Non-indigenous plant species that colonize natural areas have the potential to threaten the biodiversity and interactions of native species[3] by changing resource availability (e.g. carbon and nitrogen cycling), disrupting underground processes through soil community interference[4], modifying species composition, and shifting ecosystem functions (e.g. fluxes across biomes and species). Invasive species have also been shown to alter the physical properties of soil environment, which creates long-term impacts on ecosystem structure and function[5].

Garlic mustard [*Alliaria petiolata* (Bieb.) Cavara & Grande] is a European herbaceous biennial that is commonly found in shaded deciduous forests. The species forms basal rosettes in the first year that can overwinter, grows rapidly the following spring, then flowers and sets seed during mid-spring[6]. As a result of both natural dispersal and artificial introduction through anthropogenic activities[7], garlic mustard has become increasingly successful in dominating forested ecosystems across much of the eastern United States and southern Canada. The species is unique as an

invasive herbaceous plant because it is shade tolerant[6], non-mycorrhizal[4], and has the ability to invade undisturbed mature second-growth forests, habitats that are typically considered to be resistant to invasion[8].

Like most species of the Brassicaceae family, garlic mustard produces biologically active compounds, or allelochemicals, which are secondary chemicals known to deter herbivory[9], and suppress plant-to-plant interactions and fungal growth[10]. They release allelopathic compounds into the soil by root excretion or from leaves during rainfall[11]. The main group of secondary metabolites in this family is glucosinolates, flavonoid glucosinolates, cyanide, and a cyanoallyl glycoside known as alliarinoside[5, 11]. These secondary metabolites have various degrees of half-lives in the soil during different plant growth stages and attempts to quantify the levels of glucosinolates and flavonoid glycosides has proven difficult due to instability of the compounds[11]. When allelopathic chemicals are released into the soil, studies have shown they can increase in field concentrations over time, and have the ability to alter the physical properties of soil environment[12]. It is also been known to decrease abundance of various mycorrhizal fungi[12, 13] and inhibit native plant growth, which can create long-term impacts on ecosystem structure and function[5]. However, little is known about garlic mustard's effect on other components of the fungal community, such as saprotrophic fungi[4].

Saprotrophic fungi play an important role as primary regulators in the decomposition of plant matter and nutrient cycling. They produce lignocellulolytic enzymes to help aid in the decomposition of complex organic compounds, making them a key component in terrestrial nutrient cycling. Their ability to translocate carbon, nitrogen, and phosphorous through cords (aggregations of hyphae) also demonstrates their importance in soil nutrient redistribution[14]. This cycling of nutrients maintains ecosystem stability ensuring soil nutrient retention in late-succession soils[15].

Because of the secondary compounds present in garlic mustard and the direct and indirect negative effects on various mycorrhizal fungi, we predict that garlic mustard will suppress growth and fruiting in saprotrophic fungi, and will result in slower litter decomposition rates due to altered nutrient cycling in invaded areas. To test this, we performed two experiments to analyze the impact of garlic mustard on fungal body mass, litter decomposition rates and soil nutrient availability in the Pisgah National Forest in western North Carolina.

2. Methods

2.1 Microcosm Experiment

A microcosm experiment was performed to test effects of garlic mustard allelopathy on saprotrophic fungi. We obtained 2.3 kg commercial fruiting kits of oyster mushrooms (n=10) that were placed in 51 x 18 cm (diameter x height) plastic pots and covered in potting soil. First year garlic mustard plants were planted in five of the bins in densities to match those observed in heavily invaded naturalized areas[16], 17-170 plants m^{-2}, while the other five bins served as controls. Microcosms were arranged under a shaded forest canopy in a randomized block design, rotated weekly, and were monitored throughout the growing season for fruiting body production. Plants were watered daily and no additional nutrients were added. Once fully expanded, fruiting bodies were collected, dried and weighed.

2.2 Litterbag Decomposition Experiment

A litter decomposition experiment was performed to compare decomposition rates in invaded and uninvaded plots. Forty pairs of litterbags were constructed with 9 x 9cm filter paper (Whatman number 1. GE Healthcare Life Sciences, Pittsburg, PA), so that the substrate will be standardized and our results can be compared with other studies on garlic mustard that are part of the Global Invader Impact Network[2]. In April 2016, these bags were placed in invaded (n=20) and paired uninvaded (n=20) sites in a heavily invaded region along Hurricane Ridge in the Harmon Den area of the Pisgah National Forest (35.745217 N 82.983067 W, 1120 m elevation). At each litterbag location, we collected soil samples that were analyzed by the North Carolina Department of Agriculture and Consumer Services laboratory and NC State University laboratory for plant available nutrients. We characterized the understory vegetation around each litterbag in 1.0 m^2 quadrats and identified woody vegetation and measured distance and diameter at breast height (dbh) of woody vegetation within 5 m of plot center and larger than 15 cm (dbh). We retrieved random samples of half the litterbags (10 invaded and 10 uninvaded) at the end of the growing season on October 6, 2016 and retrieved the remaining bags in Spring 2017. Remaining filter paper was removed from each bag, dried and weighed. Decomposition rates were calculated from mass lost over the time from the bags that were incubated in the field.

Because of the high abundance of black walnut (*Juglans nigra* L.) near the plot sites and the allelopathic compounds associated with the species[17], we calculated the Individual Tree Influence Index (ITII) for each tree[18], which calculates the index as a function of the size of the tree (dbh) and its distance from the plot.

$$ITII = [10^4/(2 * \frac{d}{D})^2 \quad (1)$$

where d = distance to plot center (m), D = diameter at breast height (m). These were summed to calculate the Tree Species Influence Index (TSII) for each plot.

$$TSII = \sum_{i=1}^{n} In[(ITII)_i + 1] \quad (2)$$

where n = number of individual trees around each plot.

2.3 Statistical Analyses

Fruiting body production was compared between invaded and uninvaded plots using repeated measures analysis of variance (ANOVA) with each date of sampling and an ANOVA on final cumulative biomass (SAS Institute, Cary, NC). Decomposition was compared between invaded and uninvaded plots with a one-way ANOVA t-test at each collection date (October 5, 2016 and April 17, 2017). The influence of garlic mustard on decomposition was further evaluated by comparing decomposition to garlic mustard stem density and walnut TSII with separate linear regressions.

3. Results

3.1 Fruiting Body Mass

All first-year garlic mustard transplants survived the duration of the microcosm experiment. Total mean biomass for oyster mushrooms in invaded soil plots was slightly less than the control plots mean over the 16-week growing period, but this difference was not significant ($p = 0.8637$, $F = 0.03$ $df = 9$). After two weeks, the substrate was fully colonized. The repeated measures ANOVA showed a significant time effect ($p < 0.001$, $F = 12.47$, $df = 14$) with the majority of fruiting body mass occurring within the first two weeks of the growing season for both treatments, but there was no difference between invaded and control plots (Figure 1; $p = 0.8243$, $F = 0.05$, $df = 1$).

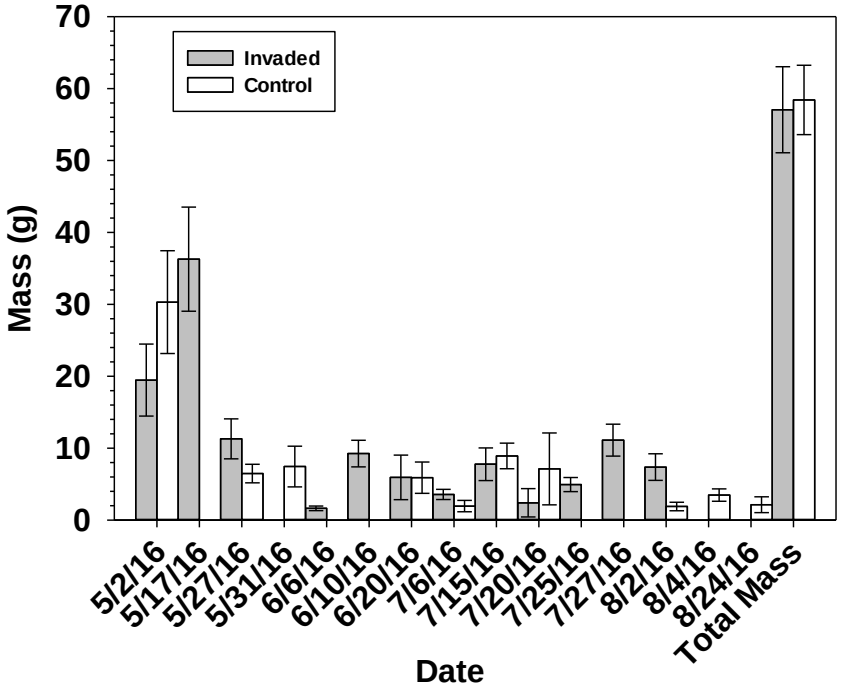

Figure 1. Mean fruiting body mass (± 1 S.E.) of invaded and uninvaded (n=10) across the 16-week treatment course. Means obtained from data collected between May 2016 and August 2016. Fruiting body production (total mass) was compared between invaded and uninvaded plots with analysis of variance (ANOVA). Repeated measures ANOVA showed a significant time effect ($p < 0.001$) with the majority of fruiting body mass occurring within the first two weeks of the growing season but with no difference between invaded and control microcosms.

3.2 Soil Characteristics

Soil characteristics did not show a significant difference to changes in soil nutrient availability between garlic mustard densities and our controls (Table 1) for any soil parameter.

Table 1. Mean soil nutrient characteristics (± 1 S.E.) in invaded and uninvaded plots along Hurricane Ridge in the Harmon Den area of the Pisgah National Forest. Soil report by NCDA&CS on September 8, 2016. Soil characteristics showed no significant difference between garlic mustard densities.

Soil Characteristic	Invaded	S.E.	Control	S.E.	p-value
%C	5.3	0.3	5.3	0.6	0.938
%N	0.4	0.02	0.41	0.04	0.866
HM%	1.4	0.2	1.4	0.1	0.928
W/V (g/cm^3)	0.8	0.01	0.86	0.01	0.487
pH	6.1	0.09	6.15	0.09	0.920
BS%	88.1	1.5	88.4	1.53	0.901
Ac ($meq/100cm^3$)	1.9	0.2	1.8	0.2	0.797
CEC (mg/dm^3)	17.4	0.8	17.3	1.0	0.947
P (mg/dm^3)	25.1	2.6	24.3	3.1	0.855
K (mg/dm^3)	308.7	23.2	283.9	28.0	0.501
Ca (mg/dm^3)	2539.1	181.5	2538.5	190.9	0.998

Mg (mg/dm^3)	240.2	18.0	246.5	19.6	0.815
S (mg/dm^3)	13.7	0.4	14.1	0.5	0.551
Na (mg/dm^3)	0.1	0.009	0.11	0.006	0.558
Mn (mg/dm^3)	123.1	6.5	119.6	5.8	0.689
Cu (mg/dm^3)	1.21	0.08	1.1	0.1	0.840
Zn (mg/dm^3)	3.3	0.2	4.6	1.6	0.403

3.3 Litterbag Decomposition

A one-way analysis of variance comparing fall decomposition rates between the two treatments (Figure 2) found no difference between invaded and uninvaded plots after the six-month treatment ($p = 0.21$, $F = 1.31$, $df = 18$) and one-year treatment ($p = 0.87$, $F = 0.167$, $df = 16$). A linear regression model found that decomposition rates decreased with increasing garlic mustard density (Figure 3), but this relationship was not strongly significant ($p = 0.0596$, $F = 4.04$, $df = 18$). Diameter and distance of black walnut trees from each plot was used to calculate the ITII and TSII was calculated when there was more than one tree influencing a plot[18]. A linear regression scatterplot (Figure 4) shows a marginally significant ($p = 0.0726$, $F = 3.64$, $df = 18$) negative relationship between decomposition and increasing walnut TSII.

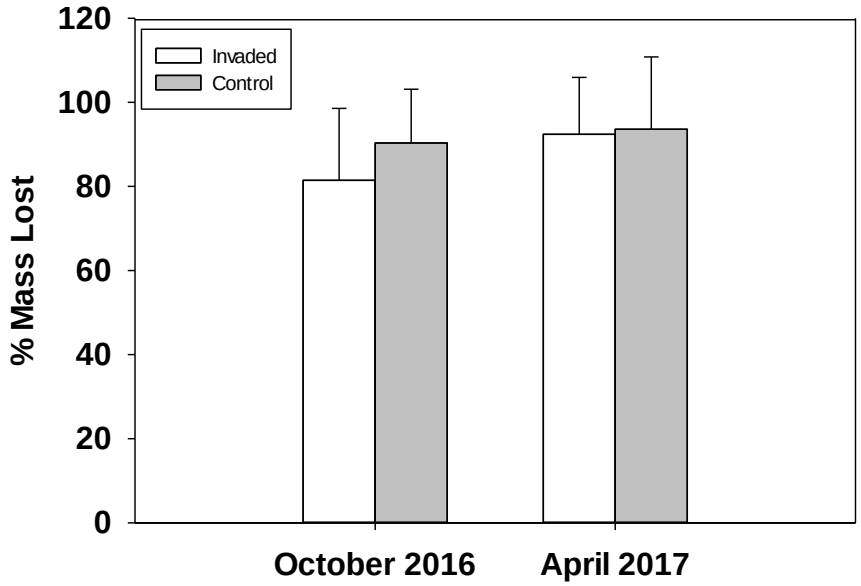

Figure 2. Total mean biomass (± 1 S.E.) of % mass lost of invaded and uninvaded across the 6-month treatment (October 2016) and one-year treatment course (April 2017). Means were obtained by calculating percent mass lost from time in the field and were compared between invaded and uninvaded plots with a one-way ANOVA.

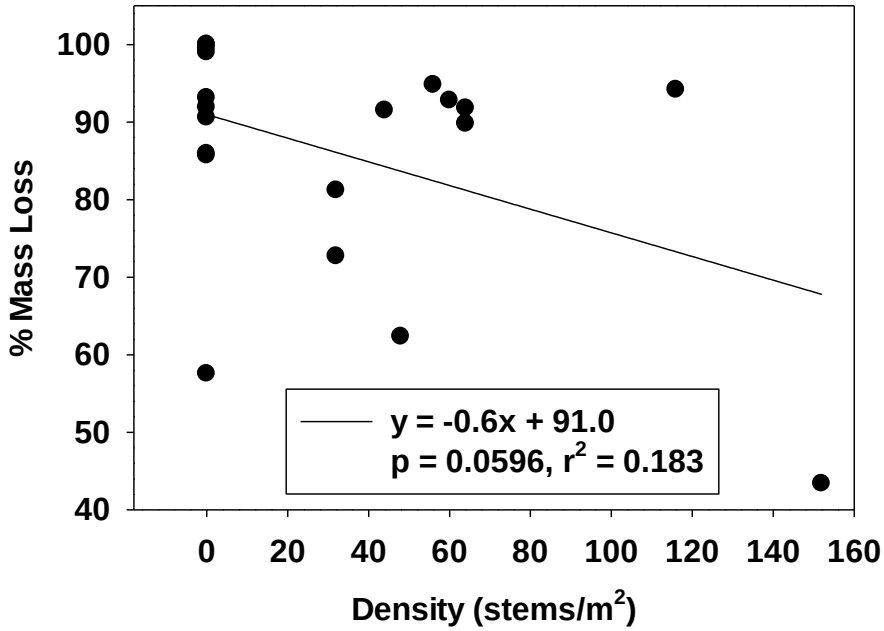

Figure 3. A linear regression model displaying the relationship between rates of decomposition (% mass lost) with respect to garlic mustard densities (stems/m^2) in the field.

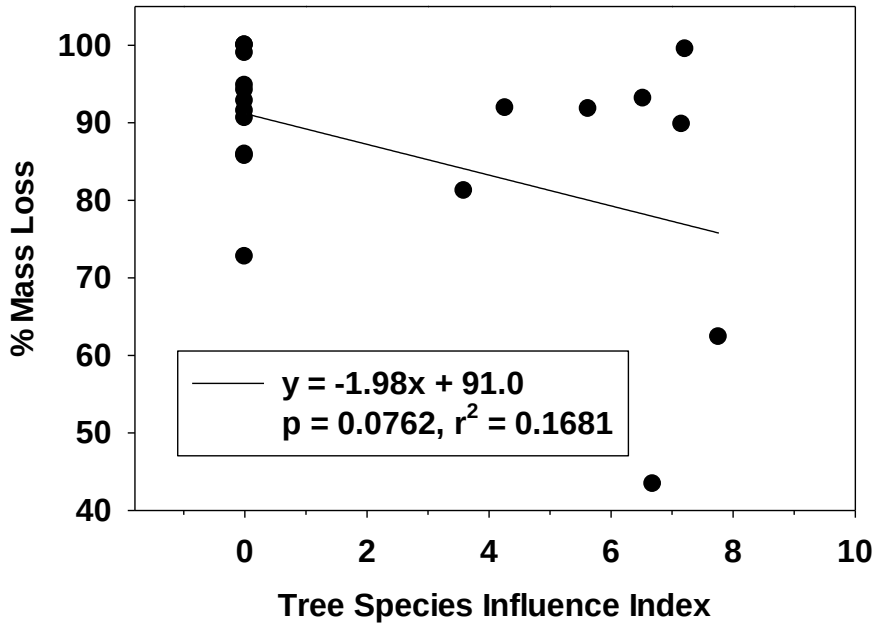

Figure 4. A linear regression model displays the relationship between rates of decomposition (% mass lost) with respect to walnut TSII in the field.

4. Discussion

4.1 Fruiting Body Mass

Contrary to our expectations, we found little influence of garlic mustard on fruiting body production. This could be due to using potting soil instead of using native soils. Past studies have shown abundance of glusinolates, sinigrin and glucotropaeolin in garlic mustard field soils in belowground tissues and have varying degrees of half-lives and can accumulate in the soil over time[11]. It is possible that by using potting soil we did not see the inhibitory effects because of low levels of allelochemicals in the soil during our short duration study. Additionally, we used first-year plants, which were generally small in biomass and likely had a smaller influence than larger second year plants. It is possible our design may not have captured the full inhibitory effect of garlic mustard allelochemicals. There is ample evidence that garlic mustard allelopathy negatively effects mycorrzhizal fungi, however, more work is needed to elucidate these effects on the saprotrophic community. Future research on fruiting body mass should include second-year plants found in naturalized areas or possibly grow garlic mustard from seed in the microcosm, and allow the study to proceed in a controlled environment using invaded field soils.

4.2 Soil Characteristics

Past research has shown that non-native plants can alter soil activity and soil nutrient availability, which can have a negative effect on ecosystem structure and function[5]. Carbon and nitrogen present in invaded soils has shown reduced C:N ratios relative to uninvaded soils as a result of lower organic carbon concentrations in invaded soils[4]. We speculate a possible reason for the lack of difference in soil properties could be the fact that the entire area had once been more extensively invaded by garlic mustard, but had been treated by the U.S. Forest Service three times between 2012 and 2016. There may be legacy effects from past higher densities of garlic mustard in the plots that we designated as "uninvaded".

4.3 Litter Decomposition

With a high concentration of secondary metabolites present in the soil, results in vegetation type having a dominant effect on soil microbial community can have a large affect on substrate quality[19] since soil microflora is regulated by litter quality that determines decomposition processes. Previous research has shown that garlic mustard allelopathy can induce changes in ecosystem properties by altering microbial nutrient uptake, resource availability[4], and by modifying native plant species composition[11] and its mycorrhizal symbionts[9].

Mycorrhizal associations resemble a compatible and specialized relationship with native plant species and altering such relationship can cause a reduction of mycorrhizal colonization of plant roots. It is been shown that reduction of arbuscular mycorrhizae could be due to the release of glucosinolate products from the roots of garlic mustard and inhibit germination of AM fungal spores in the soil[20, 12]. This inhibitory effect of allelopathy creates a microenvironment that only garlic mustard plants can tolerate[20]. It is also known that garlic mustard allelopathy inhibits the growth of ectomycorrhizal fungi both experimentally and in forests in its introduced range and can influence native plant communities, tree seedling establishment, and biogeochemical cycling[13].

In our study, decomposition did not differ between invaded an uninvaded plots. However, decomposition decreased marginally with increasing garlic mustard density in the field. It is possible that the slower rate of litter decomposition is a result of the abundance of allelopathic compounds present in the soil from black walnut trees. Black walnut is a notoriously allelopathic tree[17] and has been documented to have toxic effects to a variety of plants and organisms[21]. The principal chemical responsible for walnut allelopathy is called juglone and has been noted in soil culture to have inhibitory effects on growth of herbaceous and woody plants[17]. The abundance of walnut trees at our site led us to examine the possible interference it could have had on litter decomposition rates.

4.4 Future Implications

With the accumulating evidence that garlic mustard allelopathy negatively affects mycorrhizal communities, future attempts to quantify the level of metabolites present in field soils is recommended to help us better understand garlic mustards potential allelopathic effects on various organisms and ecosystem processes. Since fungi play an essential role in the decomposition of organic plant matter, further investigation on allelopathy on litter decomposition rates are

also recommended in order to determine how the various levels of metabolites can affect soil nutrient availability and various fungal species.

5. Acknowledgments

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