A Comparison of Decay Rates and Leaf Chemistry in Native and Non-Native Plants on Cape Cod.

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Abstract

Human impacts such as global climate change and increase global shipping has caused an influx of invasive species into the United States. These invasive species have taken footholds in native ecosystems throughout the United States and are posing risks to the health and integrity of these systems. This study focuses on leaf chemistry, decomposition rates, and leached nutrients in order to investigate the impacts that invasive species may have on nutrient cycling and food web integrity.

Keywords: Invasive species, leaf chemistry, leaf decomposition, lignin, nutrients leached.
Introduction

The problem and severity of invasive species has been steadily increasing due to human impacts on ecosystems such as changing climate, global shipping, and increased disturbance to ecosystems. Global shipping unintentionally carries plants all around the world introducing them to new countries. Disturbances such as bulldozing pave the way for invasive plants to invade and become established in native ecosystems. The changing climate is creating favorable weather and temperature for many invasive plants in new countries such as the United States. Invasive plants pose both economic and ecological issues.

Nutrient recycling is an important aspect to ecosystems and until recently the impact of invasive plants on soil nutrient recycling and soil microbial communities has been neglected. Decay rates of plant tissue and the activity of microbial communities dictate the rate at which nutrients recycle through a system. It has been shown that microbial communities change with the introduction of invasive plants (Elgersma, 2010). Changing the structure and composition of microbial communities will have an impact on decay rates of litter and therefore cause changes to nutrient cycling. In addition to changing microbial communities, invasive plants may be having an effect on native insects by being unpalatable (Tallamy, 2004). Unpalatability to insects can have repercussions on insect populations and the whole food chain especially on many bird species who feed their young primarily with insects. Invasive plants may be changing food web structure and having impacts on nutrient cycling through ecosystems. It is important to examine invasive plants’ effect on changing nutrient cycling rates in order to fully understand how invasive plants are changing ecosystems. If these plants are affecting nutrient recycling there may be lasting effects to the ecosystem if the plants can be eradicated.
I will be examining relative decay rates and leaf chemistry of eleven native and eleven invasive plants growing on Cape Cod. To see how these plants may be changing nutrient cycling, with emphasis on nitrogen, I will examine concentrations of nutrients leached from plant leaves. I will examine leaf chemistry and try to relate that to decay rates. I will look at leaf chemistry to see if there are any indicators that invasive leaves are less palatable than native leaves. Since there is speculation that invasive plants are less palatable to insects, I suspect that invasive plants will have higher lignin content and C:N ratios. Because of this I would also expect invasive plants to decompose slower and therefore slow down nutrient cycling. I will perform leaf chemistry analysis of % lignin and C:N ratios and set up incubations to measure decomposition rates in both the field and the lab.

**Methods**

I chose eleven native and eleven invasive plants to examine for chemical composition and relative decomposition rates. All of the species are deciduous and found on Cape Cod. I chose three vine species, four tree species, and four shrub species to examine variation within functional groups of native and invasive plants. The eleven native plants that were chosen make up the greatest portion of biomass in order to see what plants have the greatest influence on soil nutrient composition and recycling. The eleven invasive species chosen are the most commonly found invasive plants on Cape Cod. To collect species samples I walked around Beebe Woods near Peterson Farm and other locations around Falmouth to find all of the species. The samples were brought back to the lab and left to air dry for three days. Subsamples of each species were weighed and put in litter bags. Litter bags were made in triplicate for a total of 66 litter bags. The bags were then put at a common field location so that all the litter types experienced the same
environmental factors. I tied the litter bags together and flagged them so that they would be easily found in case they were covered by falling leaves or snow. After three weeks in the field I collected the bags, air dried them in the lab, and reweighed them to find the mass loss.

After the subsamples were taken for field incubation, the rest of the collected litter was oven dried to calculate an oven dry weight to air dry weight equivalent. Once oven dried, a subsample of each species was ground using a WIGLBUG. Multiple leaves of each species were ground in order to get a composite samples. I used 5-6g of ground sample to pack and analyze for CHN using an elemental analyzer. I used another 400 mg of ground sample for lignin analysis. Lignin analysis was only performed once per species type. However, since the ground samples were composites of many leaves this should be a good representation of lignin content. The 400 mg of sample was put into BD 20 tubes. I then pipetted 3 mL of 72% Sulfuric acid into each BD tube making sure that all of the sample was covered. Then I placed the tubes in a water bath set at 30°C and left them for one hour. I swirled the tubes frequently by hand being careful not to get material stuck to the sides of the tubes. After this initial digestion I added 42 mL of DI to the BD tubes. I then autoclaved the BD tubes for one hour at 120°C. Once the samples were cooled I filtered them onto pre-weighed ashed 47 mm GF/D Gooch filters. The filters were dried at 60°C for 36 hours and then reweighed. The remaining mass was the lignin content of the initial samples.

For the in-lab incubation approximately I placed 1-2 g of leaves into 125 mL Erlenmeyer flasks which I then filled with 125 mL of water and incubated in the dark at 25°C for two days. Each species was repeated in triplicate for a total of 66 incubations. After two days of incubation I filtered the fluid in the Erlenmeyer flasks through GF/F 47mm filters. Before filtering I collected 5 mL of samples as an inoculant to add to the BOD bottles. I collected the filtered
samples and divided them for different analyses; 10 mL for Ammonium, 10 mL for Nitrate and TDN, and 15 mL for DOC. I recorded the remaining volume of leachate and put it into 300 mL BOD bottles. Then I added the unfiltered inoculant to the BOD bottles and filled them with DI making sure there were no air bubbled when topped. I recorded initial oxygen concentrations using an oxygen probe then stored the BOD bottles in the dark at 25°C. I measured oxygen concentrations every twelve hours until the concentrations were below 1 mg/L. I stored the nitrate/TDN samples in 20 mL scintillation vials and froze them. I stored the ammonium samples in 20 mL scintillation vials, fixed them with 20 µL of 5 M HCL, and stored them in the refrigerator. I stored the DOC samples in DOC bottles, fixed them with 100 µL 43% Phosphoric Acid, and stored them in the refrigerator. I again filled the Erlenmeyer flasks with 125 mL of DI and left them to incubate a second time for six days in the dark at 25°C. I repeated the previously done steps of filtering, collecting samples for ammonium and nitrate, and putting the remaining measured leachate into 300 mL BOD bottles for oxygen concentration measurements. Like previously done, I measured the oxygen concentrations every twelve hours until concentrations were below 1 mg/L. I did not take DOC samples from the second leachate.

For nitrate analysis I pipetted 5 mL of sample into culture tubes and analyzed them on a Lachat Flow Injection Analyzer. For the ammonium analysis, I pipetted 5 mL of sample into culture tubs, added three ammonium reagents each sample, and then incubated the samples in the dark for one hour. I analyzed the samples on an ammonium Color Spectrophotometer. Samples that had an absorbance higher than 1.5 I diluted 10:1 and re-analyzed. For DOC analysis, I pipetted 300 µL of sample into a microplate and analyzed on a SpectraMax®M2/M2e Multimode Microplate Reader using SoftMax®Pro Software, Molecular Device. I used tannic acid for the standard. I first ran the samples at a 5:1 dilution and then ran undiluted samples. I ran
the undiluted samples at multiple wavelengths to find the best fit because the samples contained a lot of color.

To find total dissolved nitrogen (TDN) concentration in the leachate, I pipetted 1mL of sample and 4 mL of DI into TDN tubes for a 5:1 dilution. Then I added 125 µL of 1,000 µM Urea to each sample. I added an additional 1 mL of reagent and then autoclaved the samples at 115°C for 90 minutes. I then ran the samples on the Lachat Flow Injection Analyzer to find the concentrations of TDN. To find dissolved organic nitrogen (DON) concentrations I subtracted the ammonium and nitrate concentrations from the TDN concentrations.

For statistical analysis, I used standard error of the mean.

**Results**

In the field incubation the invasive shrubs and trees lost a greater percent of mass than their native counterparts (Figure 1). Native vines though lost more mass than invasive vines. However, all functional groups had a fairly wide range a percent mass loss. Then looking at the mass loss of the samples that were leached in Erlenmeyer flasks all native functional groups lost more mass than the invasive functional groups (Figure 2). In general a greater percent of mass was lost during leaching with approximately 50-80% lost while in the field approximately 20-60% of mass was lost. In the field, it seems that natives get broken down slower but they decompose faster in the Erlenmeyer flasks.

Looking at % Lignin the native trees and shrubs have a greater lignin content than invasives (Figure 3). The invasive functional groups have a much greater range of lignin content than the native plants. When looking back at the % mass loss in the field incubation the observed lignin contents support what was seen in the field. The functional groups with greater lignin
content lost less mass in the field. Native trees and shrubs which had the greatest % lignin lost
the smallest % mass loss in the field.

Carbon and nitrogen ratios are also consistent with field results and percent lignin. Native
plants have higher C:N ratios than invasive plants (Figure 4). Higher C:N ratios means less
decomposable because microbes will preferentially brake down plants with higher nitrogen since
nitrogen is usually the limiting nutrient in terrestrial ecosystems. Looking at the C:N ratios of the
individual species and functional groups of invasive species all the species have 40-50% carbon
content (Figure 5). There is no clear functional grouping in regards to % nitrogen. Native species
have % carbon ranging from 45-55% carbon content and have functional groups with clear
separation in regards to % nitrogen (Figure 6). Vines have the highest nitrogen content of 1.17-
1.63%. Native shrubs have 1.17-1.63% nitrogen and native trees have 0.90-1.24%.

Analysis of the initial leachate showed that there were no trends in DOC concentrations
after the first leaching (Figure 7). All functional groups have a wide range DOC concentrations.
However, there were trends in the ammonium concentrations of the leachate from the first and
second leaching. After the first leaching invasive trees and vines had leached more ammonium
than their native counterparts (Figure 8). They leached approximately 25-30 µg N/ g sample
while the native trees and vines leached approximately 20-25 µg N/ g sample. The native shrubs
leached more ammonium than invasive shrubs with natives leaching 20-30 µg N/ g sample while
the invasive shrubs leached approximately 17 µg N/ g sample. After the second leaching all of
the invasive functional groups had leached more than their native counterparts (Figure 9). They
also leached greater amounts of ammonium than they had after the first leaching. Invasive vines
leached the most ammonium with approximately 200 µg N/ g sample when it had leached about
30 µg N/ g sample after the first leaching. Invasive trees and shrubs leached about twice as much
ammonium in the second leaching as they had in the first. The native species leached less ammonium after the second leaching than they had after the first.

From both the first and second leaching nitrate concentrations were undetectable. So to find DON only ammonium was subtracted from the TDN concentrations. Like the first ammonium leached, invasive trees and vines leached more DON than natives while native shrubs leached more than invasive shrubs after the first leaching (Figure 10). However, by the second leaching all invasive functional groups had leached more DON than natives and invasive vines were leaching the most DON (Figure 11). Invasive shrubs and vines leached about the same amount of DON in the second leaching as they did in the first leaching while the invasive trees leached less.

Oxygen concentrations from the first leachate all decreased very rapidly for both native and invasive functional groups (Figure 12, Figure 13). By 20 hours of incubation all oxygen had been drawn down. Oxygen in the BOD bottles containing the second leachate took much longer to be drawn down. All concentrations in the invasive species bottles had leveled off by 208 hours of incubation (Figure 14). Native trees and vine oxygen concentrations had been reduced by 136 hours of incubation (Figure 15). However, the native shrub oxygen concentrations were still decreasing at 300 hours of incubation when the measurements were stopped. The highest rates of oxygen consumption for invasive species was in the first 50 hours of incubation and invasive shrubs had the highest rate at -0.027 mg O₂/g sample/hr (Figure 16). Native trees had the highest oxygen consumption rate at -0.021 mg O₂/g sample/hr (Figure 17). Both native and invasive functional groups had the highest rate of oxygen consumption between the third and fourth oxygen measurements. The native shrubs do not follow the same consumption patterns as
everything else. At some points oxygen concentrations increased instead of decreasing which is shown by positive rates (Figure 17).

**Discussion**

The field incubation would indicate that invasive trees and shrubs have faster decomposition rates than natives (Figure 1). Comparing this to the in-lab incubation where all native plants lost more mass than invasive plants there is a different trend. The in-lab incubation had much smaller margin of error than the field incubation and had much higher amounts of mass lost. The in-lab incubation samples may be getting to the point where all substances that are easily decomposable have decomposed. The field incubation may better represent decomposability of these species since the samples were exposed to accurate environmental factors where the in-lab incubation samples experience very different factors than the field. Some differences that samples would experience in the field and not in the lab is changing temperatures, consumption by insects, and rain. Rain may contain substances that could affect decomposition rates where the water added in the lab was DI so it had no substances.

Both the % lignin and C:N ratios are consistent with the observed mass loss from the field experiment. Functional groups with higher % lignin and C:N ratios lost less mass meaning that they decomposed slower. This may indicate that the in-lab incubation is not a good representation of decomposition rates and the field incubation is a more accurate representation. These would suggest that native trees and shrubs are less easily decomposable than invasive trees and shrubs because of their high % lignin and C:N ratios.

The observed leaching of high levels of ammonium and DON from the invasive plants however would indicate that they are decomposing faster than the natives. Since the invasive
plants have lower C:N ratios they contain more nitrogen. This may explain why high levels of ammonium and DON were observed in the leachates since the invasive species contain such high levels of nitrogen to begin with. Invasive vines leached the most amount of ammonium by far compared to both other invasives and native plants. Invasive vines also have the lowest C:N ratios so they have more nitrogen in them than the other functional groups. The observed leaching of ammonium and DON indicates that the invasive species leach more nitrogen than natives, at least in the initial phases of leaching. This increase nitrogen leaching has implications on nitrogen cycling.

The C:N distribution of native plants show that these functional groups have distinct C:N ratios. The invasive plants however, do not have these distinct functional group C:N ratios. The invasive plants are from different countries and even continents which may cause them to have a wider range of C:N ratios and not have specific functional group separation. This may also be observed in the % lignin of invasive functional groups. All invasive functional groups have a large variation in % lignin while the native functional groups have much smaller variation. Invasive species may be disrupting nutrient cycling. The invasive plants having lower % lignin and C:N ratios would indicate that they may be more easily digestible and therefore palatable to insects. However, there may be other secondary chemical compounds that cause the unpalatability to insects that has been observed.

The oxygen drawdown in the BOD bottles is attributed to microbial community activity. The drawdown in both native and invasive samples from the first leaching shows that the microbial communities were very active in braking down organic material (Figure 12, Figure 13). With the samples from the second leachate the native shrubs have the least oxygen drawdown (Figure 15). By the end of the experiment all of the oxygen had not been drawn down
while for all of the other native species it had. The invasive species from the second leachate all had similar rates of drawdown (Figure 16). The oxygen measurements show that microbial communities were very active in the first leachate and less active in the second. The native shrub functional group showed the only functional group specific differentiation in that most of the native shrubs had much slower rated of oxygen drawdown than any other samples (Figure 17). Although there were some invasive samples that did show less oxygen draw down (Figure 14) there was no functional group specific trends. In terms of microbial activity, there was no observed difference in the invasive species samples than the native species samples other than the native shrubs.

The observed mass loss indicates that at least the invasive trees and shrubs decompose faster than native species. With the faster decomposition rates and the increase nitrogen leaching by invasive plants these invasive plants are having an impact on the ecosystem. The faster decomposition rates will lead to less organic matter build up in future years. This may cause the organic layer of soils to become smaller and smaller. Invasive plants have low C:N ratios so they will need more nitrogen than native plants. However, with the increased leaching of nitrogen by these invasive plants they are starting a feedback loop that will make these ecosystems more suitable over time. The high leaching will lead to increased nitrogen in the soil. In the next growing season the invasive plants will then have an advantage because of the high leaching from the previous year. The invasive plants that were examined do have the potential to alter nitrogen cycling due to their differences in leachate and effect the food web by having different chemical structures which may be unpalatable to fauna.

By examining secondary chemical compounds in invasive leaves we will better understand if they are less palatable to native insects. In addition, by examining soil chemistry
and nutrient concentrations in the soil we will be able to see if the increased leaching from the
native plants does have impacts on nitrogen cycling. This would have to be performed of
multiple years and growing seasons to be able to observe changes in concentrations in the soils.
Lastly, longer field decomposition experiments will need to be performed to better understand
the difference in decomposition rates of invasive and native plants.

Acknowledgements

My greatest gratitude goes too Chris Neill for help designing this project and interpreting the
results. Additional thanks to Rick McHorney, and the TAs Leena Vilonen, Madeline Gorchels,
and Helena McMonagle for advice and help with execution of this experiment.
References


Table 1. Species on native and invasive functional groups that were examined.

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Native</th>
<th>Invasive</th>
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<tbody>
<tr>
<td>Vine</td>
<td>Catbrier</td>
<td>Bittersweet</td>
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<td></td>
<td>Fox Grape</td>
<td>Black Swallowwort</td>
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<td>Blackberry</td>
<td>Porcelain Berry</td>
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<td>Barberry</td>
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<td>Bush Honeysuckle</td>
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<td>Sweet Pepperbush</td>
<td>Japanese Knotweed</td>
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<td></td>
<td>Arrowwood</td>
<td>Multiflora Rose</td>
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<td></td>
<td>White Oak</td>
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<tr>
<td></td>
<td>Red Maple</td>
<td>Norway Maple</td>
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<tr>
<td></td>
<td>Sassafras</td>
<td>European Spindle-tree</td>
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Figure 1. Percent mass loss in field of native and invasive functional groups.
Figure 2. Percent mass loss from leaching of native and invasive functional groups.
Figure 3. Percent lignin of native and invasive functional groups.
Figure 4. Carbon to Nitrogen ratios on native and invasive functional groups.
Figure 5. Percent carbon and nitrogen of invasive species functional groups.
Figure 6. Percent carbon and nitrogen of native species functional groups.
Figure 7. DOC from first leachate of native and invasive functional groups.
Figure 8. Ammonium concentrations after the first leaching of native and invasive functional groups.
Figure 9. Ammonium concentrations after the second leaching of native and invasive functional groups.
Figure 10. Dissolved organic nitrogen concentrations after the first leaching in native and invasive functional groups.
Figure 11. Dissolved organic nitrogen concentrations after the second leaching in native and invasive functional groups.

Figure 12. Oxygen consumption in invasive functional groups from the first leachate.
Figure 13. Oxygen consumption in native functional groups from the first leachate.
Figure 14. Oxygen consumption in invasive functional groups from the second leachate.
Figure 15. Oxygen concentration in native functional groups from the second leachate.
Figure 16. Rates of oxygen consumption in invasive functional groups from the second leachate between time point measurements.
Figure 17. Rates of oxygen consumption in native functional groups from the second leachate between time point measurements.