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Alterations in C:N ratio to understand plant metabolic pathways in response to pressure from goat grazing in the invasive species: *Rhamnus cathartica*

by:

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Abstract

The high fecundity and high germination rates of *Rhamus cathartica* serves the species to out-compete regional plant native species. Its high abundance in deciduous forest ecosystems forces species that are unable to utilize *R. cathartica* as a food source to migrate to areas of lower density, further contributing to the decline in regional biodiversity. Bare soil conditions left as the result of the high concentration of Nitrogen in senesced leaves and leaf litter, cause further amplification of the migration of non-plant species to areas of lower *R. cathartica* density. Nitrogen is essential to primary and secondary metabolism, thus minimizing the loss of N to herbivory attack serves to maintain plant performance and fitness. This research sought to explore the relative content ratio of carbon to nitrogen in the invasive species, *R. cathartica*, before and after two sessions of goat grazing to determine if herbivory attack induces nutrient reallocation to metabolic pathways. Our results demonstrate *R. cathartica* responds to the first session of goat grazing by investing N in the photosynthetic pathway. This reinvestment in N early in the growing season, looks to deplete the plant of resources so that it is unable to effectively respond to another session of herbivory attack late in the growing season. The lower Chl levels reduces plant efficiency during a time where the plant wants to maximize uptake to prepare for the approaching cold season. With successive grazing, I would expect growth in the fall season to decline, since earlier grazing looks to deplete resources necessary for the revitalization or reproduction of *R. cathartica* to restore its phenological advantage.

Background and Significance

The non-native species, *Rhamnus cathartica,* threatens Minnesota regional biodiversity as a result of its high fecundity and high germination rates (Knight 2007). The magnitude of its effects is directly dependent upon the abundance of *R. cathartica* in a given area. As a seedling, *R.cathartica* is successful in establishing itself under disturbed conditions, common in fragmented forests that appear across the Minnesota (and greater North American continent) landscape. Early and strong seedling establishment contributes to the competition for resources between native and invasive species. Once developed, *R. cathartica* forms dense monospecific thickets that, in some forests, dominate the surface area of the understory (Knight 2007). These layers of dense shrubbery threaten the survival of juvenile plant species due to the limit in photon input. With densities reaching 34,600 sapling stems per hectare, *R. cathartica* comprises up to 50% of all understory plant stems (Knight 2007) in deciduous forest ecosystems. Native herbivores who are unable to use *R. cathartica* as a food source, migrate to areas in which the native plant species have not yet been out-competed, contributing to the decline in regional biodiversity through intense grazing pressure. Thus the forest composition has transitioned in these ecosystems, leaving buckthorn one of the most dominant species. Further, buckthorn has a unique phenology compared to native species. One of the earliest to leaf out in the spring and one of the last to sceness, buckthorn maximizes the number of available growing season days (Courtney 2019). When buckthorn finally drops its leaves, they drop while they are still green, which places a nitrogen-rich leaf upon the forest floor. The high abundance of *R. cathartica*, coupled with the high concentration of nitrogen in senesced leaves and leaf litter, causes significant changes to the soil biota and microorganism community underneath. Once dropped, the high in nitrogen leaf litter rapidly decomposes leading changes in soil-Nitrogen pools ultimately resulting in bare soil conditions (Knight 2007). Bare soil conditions cause further amplification of the migration of non-plant species to areas of lower *R. cathartica* density.

Researchers have made many attempts to derive a solution that would allow us to manage the rapid growth of invasive species without having any negative impacts on the native foliage nearby. Chemicals and manual labor are time intensive and carry significant impacts to the forest community. Fortunately, the use of grazing pressure by goats may allow us to manage invasive species and conserve biodiversity. The effects of goat grazing on ecosystems is dependent upon variables such as species productivity, soil fertility, ecosystem type and grazing intensity (Fernandez-Lugo et al. 2013). While goats are very effective at controlling the size and magnitude of plant populations (Popay, Field 1996), they are just beginning to explore the use of goats for invasive species control. Since 2018 a herd of goats has been grazing on habitat plots on Bethel University’s campus in St. Paul, MN (Lillie 2018). While data suggest the goats are effective at removing buckthorn initially from these habitats (Lillie 2018, Ronken 2020, Hargrove 2020, Stockdill 2021, Kohen 2022), regrowth of plants is occurring, leaving habitats under similar pressure from this invasive species. Until we are better able to understand the physical and biochemical changes in *R. cathartica* in response to grazing pressure, the continued, long-term investment in goats as grazing control remains in question. Further investigation of the molecular impacts of goat grazing must be conducted to understand the mechanisms and properties that allow these species to regrow after defoliation.

Carbon(C), Nitrogen(N) and Chlorophyll(Chl)

C and N nutrients are essential for various cellular functions, and an adequate supply of these two nutrients is crucial for plant growth, development, and response to a wide array of stressors (Nunes-Nesi, Fernie, & Stitt 2015). The carbon content of a plant is composed of various carbohydrates, more specifically sucrose and glucose. These photosynthetic products provide the energy and the C-skeletons necessary for ammonium assimilation during amino acid biosynthesis. N nutrients include nitrates, amino acids, and proteins. Insoluble N is also found in cell walls, membranes, and other structures. An insufficient supply of N stunts plant growth and development, reduces photosynthesis and leaf area, and ultimately decreases plant productivity (Klem et al, 2022). Furthermore, minimizing the loss of N to herbivory attack serves to maintain plant performance and fitness since N is essential to primary and secondary metabolism (Gomez, 2010). The enzyme Rubisco (Ribulose-1,5-bisphosphate carboxylase-oxygenase), responsible for catalyzing the carboxylation of RuBP (Ribulose-1,5-bisphosphate) in the first step of the Calvin Cycle, makes up roughly 30-50% of soluble leaf protein (Gruber & Feiz 2018). Expression of Rubisco is regulated by light and temperature (Weis 1981), thus levels of Rubisco are expected to be highest at the front end of the growing season. Synthesis of Rubisco requires N and often diverts N from other biosynthetic processes, including Chl synthesis (Matt, Krapp, Haake, Mock & Stitt 2002). This means that N supply and direction of allocation has a significant impact on biomass production (Zheng 2009). Due to plant phenology and seasonal changes, plants like *R. cathartica* reallocate N throughout the growing season. Stored N is released on a competitive basis and dependent upon seasonal signals in order to maximize plant growth and development given the environmental conditions of that time of year (Liu, Ren, White, Cong, Lu 2018). Plants typically invest more N during the spring to support new leaf growth to prepare for the upcoming season of increased temperature and photon input (Weis 1981). At leaf maturity, Rubisco levels plateau as the plant prioritizes shuttling sugars. Thus any stressor that induces increased metabolite transport, may require Rubisco to serve a source of N to uphold adequate generation of energy (Liu et al. 2019).

Chl is distributed into the pigment-protein complexes of photosynthesis I (PSI), photosynthesis II (PSII), and light-harvesting complexes (LHCs). Chl is made up of C, hydrogen, oxygen, N, and magnesium. This means, since N is a component of Chl, Chl content has a positive relationship with N supply (Mu, Cheng, 2020). In other words, if Chl content were to decline this would indicate total N also declined. Chl is a necessary biochemical component of photosynthesis as it is responsible for light energy absorption, transport, and conversion. The light energy absorbed by Chl can follow one of three fates: (1) Driving photosynthesis (photochemistry), (2) Dissipating as heat or (3) Re-emitted as Chl fluorescence. Since it can drive photosynthesis, it's not surprising that as Chl content increases, photosynthesis rates also increase (Mu, Cheng, 2020).

Grazing and Photosynthesis

Herbivory is a significant disruptor of photosynthesis in plants. Specifically grazing alters the absorption and utilization of solar energy by plants, thus influencing the biosynthetic pathways and photosynthetic pathways in plants. Plants can respond to such stressors through the reallocation of N or the releasing of stored N which allows plants to cope with environmental stressors (Field, Mooney 1986; Liu et al. 2019). In general plants respond to the loss of photosynthetically active leaves as the result of herbivore attack by increasing chlorophyll content and upregulating photosynthetic enzymes to improve the efficiency of the photosynthetic apparatus. This allows the plant to increase the yield of light dependent reactions in C fixation, providing the plant with an influx of energy to repair damaged tissues or organs (Huang 2013; Lui et al. 2019). Additionally, grazing removes dead or decaying plant matter, altering the mass allocation of the plant from which it was removed. The redirected flow of nutrients can stimulate new leaf production to ensure the plant has sufficient energy for daily function and reproductive purposes (Lui et al. 2019).

After grazing, the reduction in leaf area may limit the ability of the plant to acquire sufficient light, decreasing photosynthetic efficiency in return. On the other hand, the remaining leaves have more access to light due to the decrease in plant matter in the sun-obstructing canopy. If this increased input of photons exceeds the plant's photosynthetic capacity, photosynthetic efficiency may decrease as a result (Liu et al, 2019). This occurs when the remaining leaves accumulate reactive oxygen species (ROS) in consequence of excess energy that cannot be safely dissipated. The accumulation of ROS results in lipid peroxidation, suppression of the synthesis of PSII proteins, and inflicts damage to the photosynthetic apparatus (Liu et al, 2019). Therefore, herbivory pressure, such as that exerted by a grazer, has the potential to negatively impact photosynthetic rates. In the case of invasive species, this could be a positive outcome for native habitats if this reduction happens at critical growth windows *and* causes a fitness differential.

Research Aims

This research sought to investigate the carbon and nitrogen ratio in *Rhamnus cathartica* on Bethel University campus in Minnesota, United States. I explored how C:N ratios respond to grazing by sampling before and after two summer grazing cycles. One grazing cycle happened early in the growing season, and the second occurred in late summer, after a period of rest between then June and September grazing timepoints. Specifically, I sought to determine if there is a significant difference in the C:N content ratio in grazed vs ungrazed plants at each time of sample collection, and if there is a significant difference in the trends of C:N ratio throughout the season between the two plots. I anticipate there will be a significant difference after both grazing periods because plants are known to alter macromolecule production and modify nutrient allocation under the stress of other environmental factors such as salinity, drought and high temperatures (Liu et al. 2019).

Methods

Study Site & Description

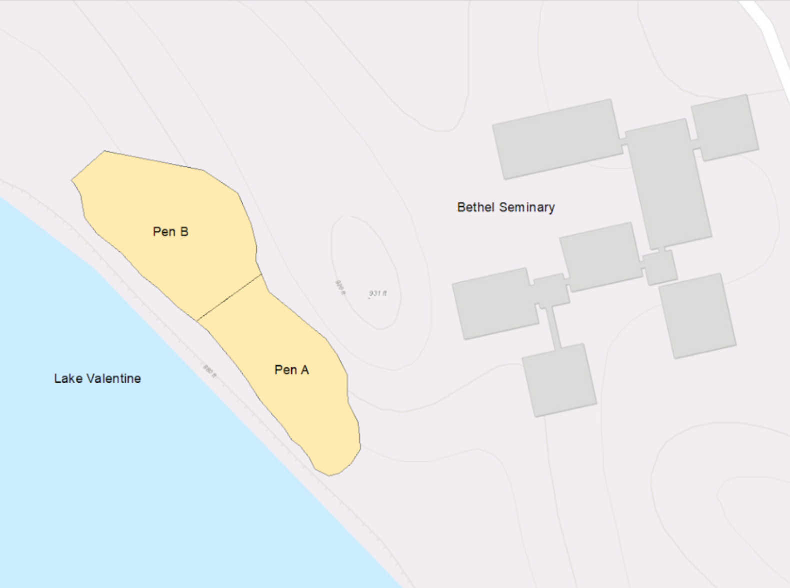

Figure 1. A map showing pens A and B on Bethel University's campus in St. Paul, MN. Both pens are located near Lake Valentine and Bethel Seminary. Pen B represents the plot of deciduous forest grazed by the goats from July 24 through July 31, 2022, and from September 27 through October 4, 2022 for the second graze. Photo made by Daniel Drechsel in 2021 with ArcMap.

Since this research aims to investigate two different periods of grazing, it is important that a control plot which is not grazed be established in order to compare changes that resulted from grazing and not natural seasonal changes. The pens (Figure 1) are located adjacent to Lake Valentine on Bethel University campus in St. Paul, Minnesota and enclose an area of temperate deciduous forest. These pens were previously established and were selected based on their vegetative profile as an area vulnerable to the growth of invasive species, *R. cathartica* (Dreschel 2021, Figure 2). Pen B represents the plot of land grazed by the goats from July 24th through July 31st, 2022 and from September 27th through October 4th, 2022. This area of temperate deciduous forest was previously grazed in 2020, however only pen B was previously grazed (Dreschel 2021). With a high gradient, the topography of pen A makes this pen unfavorable for goat grazing (Kohen 2022). Prior to the arrival of the goats, *R. cathartica* densities were visibly lower in pen B since this plot has been previously grazed. 8 adult goats were donated by Munch Bunch for each of the grazing sessions. The use of electric collars? and invisible fencing enabled the grazing patterns of the goats to be limited to pen B, so that no grazing occurred in pen A.

Figure 2. Photo of sample collection site in the ungrazed plot. A 1x1 meter PVC pipe square was placed so that the marker for that collection site was in the Southeast corner. The *Rhamnus cathartica* tree with viable leaves with the 0-1.5 meter height range closest to the marker was flagged with a red tie so that it may be easily identified in subsequent sample collections.

Field Data Collection

Within each of these plots, 20 markers were randomly placed as locations for data collection to study the effects of goat grazing on stem growth (Lillie 2018, Ronken 2020, Hargrove 2020, Stockdill 2021, Kohen 2022). These markers remained in place throughout the duration of the project, and were replaced if disturbed by the goats. From each plot, 10 markers with sufficient *R. cathartica* biomaterial were randomly selected in order to assess C and N content. This assessment was done upon visual inspection of the area enclosed by a 1x1 meter PVC pipe square, placed at each site of data collection with the stake located in the Southeast corner of the outline (Figure 2). If the enclosed area at each marker did not contain at least one *R. cathartica* plant with height of at least 1.5 meters, it was not selected as a site for sample collection. At each of the 10 selected markers from each plot, the *R. cathartica* tree with sufficient leaves in the 0-1.5 meter range that is closest to the marker were flagged and served as the plant from which biomass and chlorophyll fluorescence were quantified. To collect samples for in lab analysis, 3 random leaves, with no visible damage or decay, from the 0-1.5 meter range, and with a length and width greater than 2 centimeters were selected from the flagged tree. Leaves were collected on July 21, 2022 (pre-1), September 6th, 2022 (post-1), September 28th, 2022 (pre-2), and November 8th, 2022 (post-2). To monitor the vitality of leaf samples throughout storage, SPAD501 values were collected weekly on 3 leaves collected from the ungrazed plot, but not a flagged tree on September 6th, 2022. These 3 leaves were stored alongside the leaves from each sample collection in the dark, at 5°C. SPAD values were collected weekly throughout the duration samples were stored. Live SPAD values were recorded for quality control, and averaged per each collection site marker. These values were compared to SPAD values taken at the time of sample digestion to ensure that the storage of samples did not influence the Chl content. Visible degradation of the leaves collected from pre-1 and post-1 was apparent after roughly 5 weeks of storage (Figure 4), however there were no statically significant differences in live and lab SPAD values (Table 3).

Chemical Analysis

For the purpose of this study, Chl content has been selected as a proxy for N and biomass as a proxy for C. Since N is a major component of Chl (Mu, Cheng, 2020), relative Chl levels can be used to indicate N investment. Our sample digestion procedure acts under the postulation that a well controlled but short extraction procedure with repeatable parameters will extract an equal fraction of the total chlorophyll. To eliminate any changes in chlorophyll fluorescence as the result of the discrepancies in the surface area of each leaf, a 1 cm square will be excised from each leaf and used for chlorophyll extraction. Since the veins, especially the midrib vein, interfere with the homogenization of the leaves in practice runs, it is best to locate the square stencil closer to the tip of the leaf and to the side of the midline vein, avoiding other veins or signs of decay if possible. This will be excised after the leaf has been placed in boiling water for one minute. By boiling the leaves, this allows the cytoplasm to also boil which will in turn burst open the cell wall so that nutrients may be extracted and quantified (Hu, Tanaka & Tanaka 2013). To extract the chlorophyll, the homogenized square (Figure 3) will be washed with 100% acetone twice (Hartmut, Lichtenthaler, and Wellburn 1983) and fluorescence will be detected with a Red Tide spectrophotometer (Figure 5 & 6). Oceanview computational software will compute the absorbance at 662 nm. StatPlus software was utilized to compute two-factor ANOVA tests on absorbance at 662 nm divided by the dry weight, in grams of the excised, one cubic centimeter leaf sample.

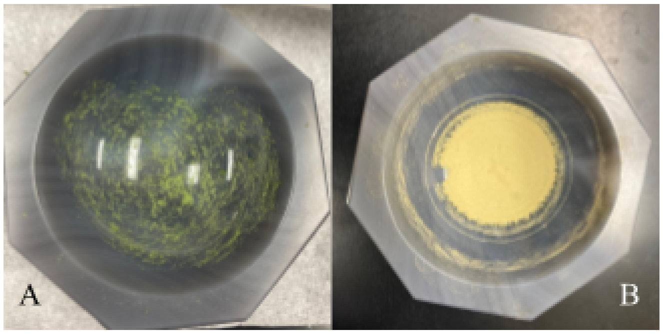

Figure 3. (A) Example of sufficient homogenization of the excised 1x1 cm square after the premiere milling. Visual inspection confirms particles are of the finest powder consistency. Photo was taken prior to the addition of solvent. (B) Photo of sufficiently homogenized whole leaf. Photo was taken after 2 acetone washes. Diminished green color indicates successful extraction of Chl.

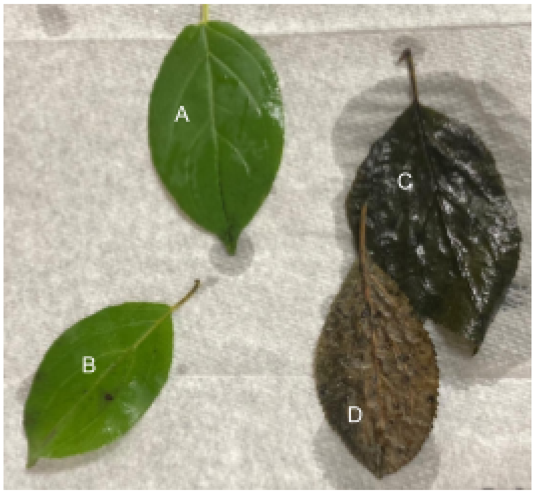

Figure 4. Visual comparison of newly collected leaves (A & B) and leaves collected from before the first graze (C & D). Leaves C and D were collected on July 21st, 2022 from the ungrazed plot and stored at 5°C, wrapped in a damp paper towel and enclosed in a plastic bag. Leaves A and B were collected on September 23rd, 2022 from the ungrazed plot. Photo was taken on September 23rd, 2022 approximately an hour after leaves A and B were collected.

Results

The initial levels of Chl did not significantly vary between the experimental and control plots (p=0.0800, ANOVA with post-hoc T-Test, Figure 1, Table 2). Chl levels within the control plot decreased (p=0.0199, ANOVA with post-hoc T-Test, Figure 2, Table 1) between July 21, 2022 and September 6th, 2022. However, within the experimental plot, Chl levels of the flagged plants did not significantly change (p=0.597, ANOVA with post-hoc T-Test, Figure 2, Table 1) from before the first graze to the time of regrowth in early September. In the control plot, Chl levels continue to significantly decline from early September to late September, just before the second graze (p= 0.192, ANOVA with post-hoc T-Test, Figure 1, Table 1). The experimental plot mimicked this pattern (p=0.00018, ANOVA with post-hoc T-Test, Figure 1, Table 1), yet Chl levels between the control and experimental plots varied significantly at both these times (p=3.59213E-6, p=0.02564 respectively, ANOVA with post-hoc T-Test, Figure 5, Table 2). Chl levels before the second graze, collected September 28th, 2022 were statistically different from the levels after the second graze, collected on November 8th, 2022 within both the experimental and control plots (p=0.192, p=0.0148 respectively, ANOVA with post-hoc T-Test, Figure 7, Table 1). Final levels of Chl between the experimental and control plots show no statistically significant difference (p=0.93299,ANOVA with post-hoc T-Test, Figure 5, Table 2). N investment declines more steeply in the experimental plot than in the control throughout the growing season (p=0.022, ANOVA with post-hoc T-test, Figure 5)

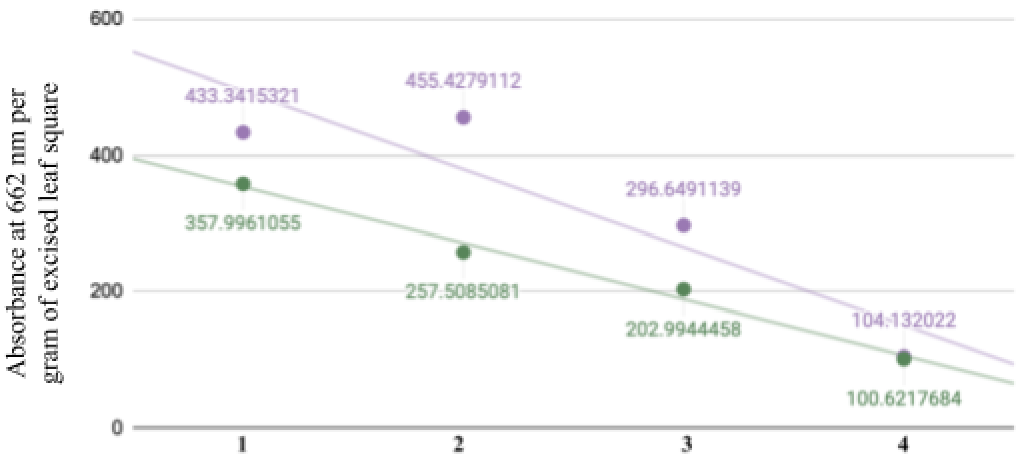

Figure 5. Relative values of Chl levels in the control and grazed plot at each time of sample collection throughout the growing season. The experimental plot is shown in purple ($y=-115*x+494$; $R^2=0.839$), and the control is shown in green ($y=-82.7*x+354$; $R^2=0.987$) Chl levels are reported in absorbance at 662 nm divided per gram of one cubic centimeter of dry leaf. Time 1 represents sample collection before the first graze on July 21st, 2022. Time 2 represents the sample collection that occurred after growth from the first graze on September 6th, 2022. Time 3 signifies sample collection that occurred just prior to the arrival of the goats for the second graze on September 28th, 2022. Time 4 represents sample collection after the second graze on November 8th, 2022, weeks after the departure of the goats to allow for sufficient regrowth.

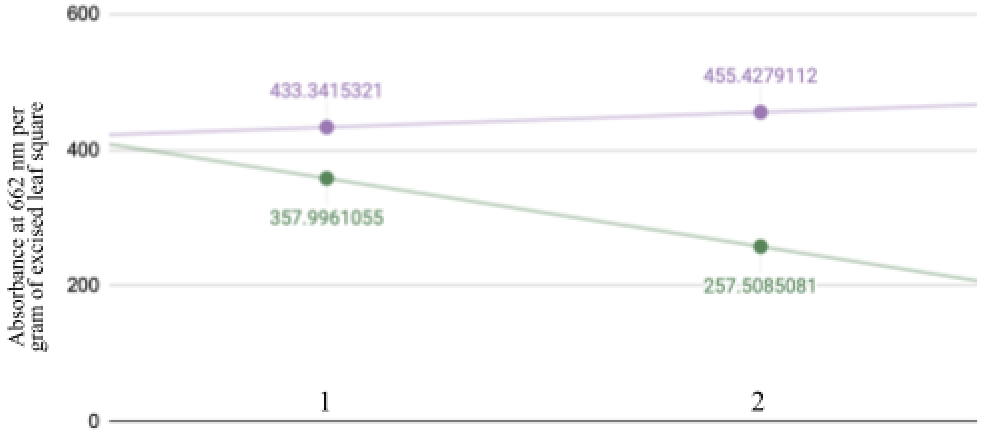

Figure 6. A comparison of the Chl levels between the control and experimental plots before and after the first graze. Time 1 represents sample collection before the first graze on July 21st, 2022. Time 2 represents the sample collection that occurred after growth from the first graze on September 6th, 2022. Chl levels are reported in absorbance at 662 nm divided per gram of one cubic centimeter of dry leaf. Experimental plot is shown in purple, and the control plot is shown in green

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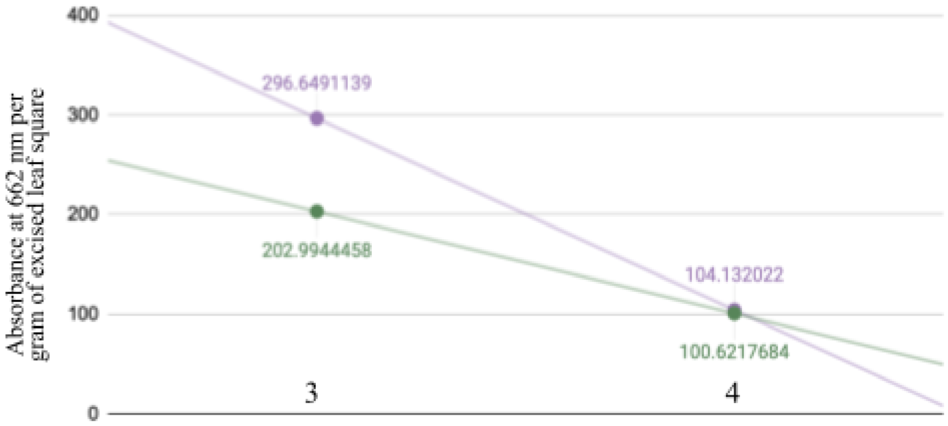

Figure 7. A comparison of the Chl levels between the control and experimental plots before and after the second graze. Chl levels are reported in absorbance at 662 nm divided per gram of one cubic centimeter of dry leaf. Time 3 signifies sample collection that occurred just prior to the arrival of the goats for the second graze on September 28th, 2022. Time 4 represents sample collection after the second graze on November 8th, 2022, weeks after the departure of the goats to allow for sufficient regrowth The experimental plot is shown in purple, and the control plot shown in green.

Discussion

N reallocation

The steady decline of Chl as seen in the control plants confirms prior research that woody species invest N early in the growing season to prepare the plant with the necessary organs and active leaves to maximize development in the warm and sunny weather (Qaderi, Martel, Dixon 2019). However this is the opposite trend from the grazed plots. From the pre/post time frame surrounding the first graze,relative Chl levels increased in the experimental plot (Figure 1 & Figure 3). Following the re-growth period between the first and second grazing time point, relative Chl levels decreased as expected in both control and treatment plants. However, the slope of the decline was steeper in the grazed plants compared to the ungrazed plants (Figure 2 & 3). This overall trend of a steeper loss of relative Chl continues to the end of the growing season wherein there was no difference in Chl levels in the two plots (Figure 2).

This evidence illustrates the first graze, occurring early in the growing season, is crucial since only during the early months of the growing season did the changing Chl levels not reflect that of the control plot (Figure 1 & 2). During this period of herbivory threat, *R. cathartica* responded to the loss of photosynthetically active leaves and the destruction of vital tissue by diverting N to the Chl biosynthesis pathway. Releasing stored N or increasing the uptake of N serves the plant to maintain the efficiency of the photosynthetic apparatus as well as to support cell division to repair damaged tissues or organs (Liu, Ren, White, Cong, & Lu 2018). It’s unclear where exactly the trade off for N is. Water-soluble proteins are readily available to be degraded to free amino acids in the cytosol, which then are recycled as a source for N. However, enzymes involved in metabolism, such as Rubisco, are not optimal sources for N, since their decrease would negatively affect the photosynthetic apparatus (Gruber & Feiz 2018). Previous research suggests that the rate and direction of N allocation to the photosynthetic apparatus is under the control of N sink-source interactions, and competition among sinks could potentially hinder the plants ability to effectively respond to herbivory attack (Field, Mooney 1986).

It's plausible then, that the initial goat grazing was the catalyst for *R. cathartica* to reinvest N to resynthesize active leaf tissue. This increase in N is short lived, however, since the final Chl levels between the experimental and control plots are the same. Since initial levels of Chl were equivalent in the two plots and only elevated in the treatment plot after the first round of grazing, and they later reached the same relative value by the end of the growing season suggests that by forcing *R. cathartica* to reinvest N early in the growing season there is less available N to respond to the second session of herbivory attack. Therefore, there was a steeper decline of Chl over the growing season than I observed in the control plots. Extending growing season into the fall is an advantage for buckthorn over other native species, but the steeper decline in Chl levels at the end of the growing season suggests that goat grazing lessens that advantage. The lower Chl levels reduces plant efficiency during a time where the plant wants to maximize uptake to prepare for the approaching cold season. With successive grazing, I would expect growth in the fall season to decline, since earlier grazing looks to deplete resources necessary for the reproduction of *R. cathartica*. Understanding how herbivore injury affects the photosynthetic physiology of *R. cathartica* may help us to understand the physiological mechanisms underlying *R. cathartica's* tolerance to herbivory attack, and offer insights into how to best combat this invasive species. This research reveals that *R. cathartica*, when under pressure due to herbivory attack, will re-invest more N when grazed early in the growing season. This suggests a compensatory mechanism of reallocation to foster greater photosynthetic capacity early in the growing season in the grazed plants. It also suggests a potential limitation for buckthorn, in that there is perhaps less N available at the end of the growing season, which could limit its end-of-the-season competitive advantage with continued grazing pressure over time.

Limitations and Further Explorations

Ultimately, Chl fluorescence only offers relative values of total N content in the samples. For a direct comparison of total concentration of C and N, I would suggest the Kjeldahl titration methods to determine total N content, and a combination of wet- and dry- combustion methods to determine total C content. Additionally, the storage of samples collected before and after the first graze resulted in visible degradation of the leaf tissue (Figure 4). Errors in absorbance at 662 nm may have occurred as the result of the growth of molds on the stored sample. This growth began after roughly 5 weeks of storage in a damp and refrigerated place. In repetitions of this experiment, I suggest that sample digestion occur the same day as sample collection to limit the growth of potentially fluorescence-interfering molds. Utilization of an adaptation period after the homogenization of samples, in which samples are placed in the dark for a controlled amount of time, may limit premature re-emission of light by Chl at varying degrees across the sample to reduce inconsistency in the photoreception of the leaves.

Additionally, it was difficult to maintain consistency in the number of days after the departure of the goats for the second and fourth sample collection. For the second sample collection, it took just two weeks for sufficient growth of the selected *R. cathartica* plants in the 30 cm-1.5m height range. For the fourth sample collection, sufficient regrowth was not apparent until 3 weeks after the departure of the goats and was considerably less than the regrowth after the initial graze. Insufficient regrowth, given the decline in daylight and temperature, was a concern for the final sample collection. Of the leaves that re-emerged from the second graze, they were in general smaller, more yellow, and lower in abundance than the re-emerged leaves from the initial grazing session. Yet, this concern demonstrates that the combination of the grazing sessions ultimately reduces the competitive advantage of *R. cathartica* by maintaining photosynthetic efficiency into the late end of the growing season. To further understand this compensatory mechanism of N investment, I suggest monitoring C:N ratio throughout the growing season to determine: (1) at what point during each grazing session does the plant begin to invest N, (2) for how long after each grazing will the plant continue to increase Chl, (3) overall efficiency of *R. cathartica* in its response to the external stress of herbivore attack. determine at what point during each grazing session does the plant begin to invest N and for how long after each grazing will the plant continue to increase Chl levels.

Huang's research team in Zhengjiang, China studied the effects of *P. solenopsis* infestation at varying degrees of density on tomato leaves and the relative changes to Chl content as a result of herbivory attack. Their results confirm a mechanism of compensatory photosynthesis in tomato leaves under condition of low-density infestation, and suggest the degree of infestation results in varied physiological responses, including photosynthetic efficiency (Huange 2013). Seemingly minor variations in experimental design often generate contrasting results in studies that seek to assess the impacts of herbivory attacks (Fernandez-Lugo et al. 2013). As a result we are generally unable to predict the directionality of resource flow in all but a few very specific cases. In other words, varied intensities of goat grazing may have altered effects on *R. cathartica's* metabolic response to herbivory attack. It would be interesting to explore if the number of goats grazing in the grazed plot has significant effects on the direction and magnitude of N reallocation. It's seemingly plausible that controlling for the number of herbivores may reveal optimal grazing conditions that would maximize the detrimental effects on *R. cathartica* to combat this invasive species(Popay, Field 1996).

To further understand the effects of the induced reinvestment in N mid-season on plant fitness, more research needs to be conducted to determine if goat grazing ultimately decreases the reproductive rates of *R. cathartica* through modifications to berry production. A decline in berry production as the result of the induced stress may indicate that goat grazing is effective at controlling the spread of the invasive species *R. cathartica*. Since this research represents an initial look at how N in the invasive *R. cathartica* responds to grazing pressure, it would be interesting to explore how each enzyme involved in the biogenesis of Chl is affected by the warranted increase in photosynthetic capacity. Increased enzyme activity within this pathway would corroborate the directionality of N reallocation, and may offer insight on the signaling mechanism that enables *R. cathartica* to respond to sessions of herbivory attack. Additionally, it would be interesting to test if parallel pathways such as heme formation is affected by the upregulation of Chl synthesis. Investigations in such pathways could provide better understanding and a greater awareness of the mechanisms that facilitate refoliation after grazing. These questions can contribute to a broader understanding of mechanisms that may reduce *R. cathartica's* competitive advantage in deciduous forests when experiencing grazing pressure.

Conclusions

In this research, I sought to determine if the invasive species *R. cathartica* will alter their C:N ratio in response to pressure from goat grazing. I ascertained that *R. cathartica* will invest more N to the photosynthetic pathway in response to herbivory attack that occurred early in the growing season. By forcing *R. cathartica* to either increase N uptake or reallocate N pools, it looks to deplete the organism of available N to continue photosynthesis into the late end of the growing season. This reduction in resources at the end of the season, may disrupt the phenological advantage of *R. cathartica* of extending its growing season past that of native species. This research enabled for a better understanding of how herbivores may impact biochemical processes in *R. cathartica*. More research must be conducted to determine if these impacts effectively reduce plant-fitness and ultimately mitigate the spread of the invasive species *R. cathartica.*

Acknowledgements

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Appendix

Table 1. Results obtained from a two factor ANOVA T-test computed by StatPlus computational software. P-values are taken from the Fisher LSD test on the differences in Chl

levels over time for the grazed and control plots separately. Time 1 represents sample collection before the first graze on July 21st, 2022. Time 2 represents the sample collection that occurred after growth from the first graze on September 6th, 2022. Time 3 signifies sample collection that occurred just prior to the arrival of the goats for the second graze on September 28th, 2022. Time 4 represents sample collection after the second graze on November 8th, 2022, weeks after the departure of the goats to allow for sufficient regrowth.

Plot	Time	P-value	Test statistic	Significance
Control	1 vs 2	0.01985	2.34529	Yes
Control	2 vs 3	0.19243	1.30717	No
Control	3 vs 4	0.01482	2.45476	Yes
Grazed	1 vs 2	0.59689	0.52960	No
Grazed	2 vs 3	0.00018	3.80730	Yes
Grazed	3 vs 4	6.43040E-6	4.61629	Yes

Table 2. Results obtained from a two factor ANOVA T-test computed by StatPlus computational software. P-values are taken from the Fisher LSD test on the differences in Chl levels in grazed vs ungrazed plants at times 1-4 of sample collection. Time 1 represents sample collection before the first graze on July 21st, 2022. Time 2 represents the sample collection that occurred after growth from the first graze on September 6th, 2022. Time 3 signifies sample collection that occurred just prior to the arrival of the goats for the second graze on September 28th, 2022. Time 4 represents sample collection after the second graze on November 8th, 2022, weeks after the departure of the goats to allow for sufficient regrowth.

Time	P-value	Test statistic	Significance
1	0.07995	1.75849	No
2	3.59213E-6	4.74583	Yes
3	0.02564	2.24571	Yes
4	0.93299	0.08417	No

Table 3. Results obtained from a two factor ANOVA T-test computed by StatPlus computational software. P-values are taken from the Fisher LSD test on differences in live SPAD values vs SPAD values taken just before chemical analysis. Time 2 represents the sample collection that occurred after growth from the first graze on September 6th, 2022. Time 3 signifies sample collection that occurred just prior to the arrival of the goats for the second graze on September 28th, 2022. Time 4 represents sample collection after the second graze on November 8th, 2022, weeks after the departure of the goats to allow for sufficient regrowth.

Time	p-value	Test Statistic	Significant
2	0.04619	2.01528	No
3	0.32197	0.99467	No
4	0.87166	0.1619	No

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