INVERTEBRATE HERBIVORY OF UNDERSTORY TREES IN THE GEORGIA PIEDMONT IN RESPONSE TO SOIL WARMING

by

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(Under the Direction of Jacqueline E. Mohan)

ABSTRACT

As the global mean surface temperature increases, changes in biogeochemical cycling have the potential to have cascading effects on plant and invertebrate interactions. Previous warming studies have primarily been conducted in recently glaciated, more fertile soils, and the response of plant and invertebrate interactions to warming is unclear in lower latitude, less fertile soils of the Georgia Piedmont. In this study, I examined leaf and soil chemistry (%N, C:N) and herbivore damage (% area consumed) from understory tree seedlings of the Georgia Piedmont. There were species-level foliar carbon and nitrogen content responses to warming, but no warming-induced response of herbivory. Overall, warming did not have an indirect effect on plant-herbivore interactions, which is likely due to Piedmont soils containing less available nitrogen. However, species-level variation in foliar chemistry response to warming has implications for forest composition changes and impacts on nutrients.

INDEX WORDS: Climate change, Global warming, Soil warming, Herbivory, Nitrogen, Carbon:nitrogen ratio, Red maple, *Acer rubrum*, Southern Sugar maple, *Acer floridanum*, Red oak, *Quercus rubra*, White oak, *Quercus alba*, Open-top chamber, Temperate forest

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CHAPTER 1

INTRODUCTION

Global mean surface temperatures are increasing as a result of anthropogenic climate change (IPCC 2014). As of 2019, global mean atmospheric temperatures have increased 0.95℃ above average (NOAA Global Climate Report 2019). Understanding the response of forests to warmer environments is critical in a rapidly changing climate. Forests provide numerous ecosystem functions, e.g. by influencing biodiversity, protecting soil resources by preventing erosion, filtering air and water pollutants, and storing approximately half of terrestrial carbon (Bonan 2008). Increased ecosystem temperature directly impacts biogeochemical processes, including carbon sequestration and nitrogen mineralization (Melillo et al. 2011, Butler et al. 2012, Garten 1993, Rustad et al. 2001). A meta-analysis of 32 ecosystem warming sites showed that 2-9 years of experimental warming increases soil respiration by 20%, and net nitrogen (N) mineralization by 46%, leading to more plant available N and increasing plant productivity by 19% (Rustad et al. 2001). Warming stimulates soil respiration, which can be offset by a warming-induced net gain of carbon (C) in vegetation via increased tree growth (Melillo et al. 2011, Lu et al. 2013)

Soil warming can also induce phytochemical changes in foliar tissue; for example, leaf N content increased and foliar C:N ratios decreased in response to warming in a northern temperate forest warming study (Butler et al. 2012). Through phytochemical changes in foliar tissue, soil warming can potentially impact invertebrate herbivores, which play an important role in plant productivity, nutrient cycling, and trophic dynamics (Carson and Root 1999, Belovsky 2000, Bardgett and Wardle 2003). However, the indirect impacts of soil warming on plant and invertebrate herbivore interactions have not been studied, particularly in field settings (Chung 2013, Cornelissen 2011). Furthermore, experimental soil warming sites are concentrated in northern latitudes with glaciated, less weathered and more fertile soils (Rustad et al. 2001). These sites are expected to differ in response to warming compared to highly weathered, less fertile soils of the Southeast U.S. due to differences in ambient nutrient availability and biogeochemical cycling (Rustad et al. 2001, Crowther et al. 2016, Carey et al. 2016). The goal of the following study was to determine how soil warming will impact soil processes of sub-tropical soils, and the indirect impacts on plant and invertebrate herbivore interactions. This is the only study of which I am aware that examines the response of plant-insect interactions to soil warming in lower latitude, infertile soils.

In this study, I determined plot wide herbivory damage, as well as analyzed foliar C and N of four target species (*Acer rubrum* (red maple), *A. floridanum* (southern sugar maple), *Quercus alba* (white oak), and *Q. rubra* (northern red oak) in an established soil warming experiment in Whitehall Forest, Athens, GA (Figure 2.1). The target species are common southeastern tree species that are abundant at the soil warming site. I predicted that warmed plots would exhibit higher foliar %N, and lower C:N ratios due to increased bioavailable nitrogen. In previous studies, soil warming led to higher bioavailable N through increased N mineralization rates (Peterjohn et al. 1992, Rustad et al. 2001, Melillo et al. 2011). I also predicted that changes in leaf phytochemistry would be associated with changes in invertebrate herbivory. Foliar C and N indicate leaf quality, which correlates with herbivore damage (Mattson 1980, Dudt 1994, Kudo 2003), and have responded to warming in previous studies (Butler et al. 2012, Williams et al. 2003, Dury 1998). To test my predictions, I collected foliar samples in 2018 and 2020 from the four target species, as well as soil samples in 2020, to analyze for carbon and nitrogen. I measured herbivore damage and relative growth rates across nine plots from 2017 to 2020.

CHAPTER 2

INVERTEBRATE HERBIVORY OF UNDERSTORY TREES IN THE GEORGIA PIEDMONT IN RESPONSE TO SOIL WARMING

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Abstract

As the global mean surface temperature increases, changes in biogeochemical cycling have the potential to have cascading effects on plant and invertebrate interactions. In warmer environments, soil processes, such as nitrogen mineralization, can increase and result in more plant available nitrogen. The subsequent changes in leaf chemistry could impact herbivore damage. However, previous warming studies have primarily been conducted in recently glaciated, more fertile soils, and the response of plant and invertebrate interactions to warming is unclear in lower latitude, less fertile soils of the Georgia Piedmont. I collected leaf chemistry (%N, C:N) and herbivore damage (% area consumed) data from understory tree seedlings and saplings at the Whitehall Forest Soil Warming Facility in Athens, GA. There were significant temperature effects on foliar chemistry within species (%N: $p<0.001$, C:N: $p<0.001$). However, herbivory did not respond to warming in any year. Instead, herbivory was best predicted by tree species, relative growth rate, soil moisture, and time. Warming did not have an indirect effect on plant-herbivore interactions via enhanced N mineralization and associated “fertilization effect” for trees. This is likely due to Piedmont soils containing less available nitrogen, and lower intrinsic reaction rates of microbial processes to temperature changes with higher ambient temperatures. There is species-level variation in foliar chemistry response to warming, which has implications for forest composition changes. Future work should consider potential impacts of soil warming on associated plant secondary defensive compounds, as well as investigate possible soil nitrogen and/or carbon limitations to forest soil warming responses.

Introduction

Atmospheric and soil temperatures are increasing, and biogeochemical processes are being altered as a result of anthropogenic climate change (IPCC 2014, Galloway et al. 2009, Melillo et al. 2011, Crowther et al. 2016, Carey et al. 2016, National Climate Assessment 2014, Machmuller et al. 2018). Increased soil temperature directly impacts soil carbon (C) and nitrogen (N) fluxes (Melillo et al. 2011, Butler et al. 2012, Garten 1993, Rustad et al. 2001), which could indirectly impact invertebrate herbivory through changes in host plant chemistry.

A meta-analysis of 32 ecosystem warming sites showed that 2-9 years of experimental warming increases soil respiration by 20% and net nitrogen (N) mineralization by 46%, leading to more plant available N, and increased plant productivity by 19% (Rustad et al. 2001). Warming stimulates net gain of carbon (C) in vegetation via increased tree growth, attributed to increased soil N availability (Melillo et al. 2011). A meta-analysis of ecosystem warming field experiments across biomes found that increased C influx and C storage of plants in response to warming offset the C efflux from increased soil respiration (Lu et al. 2013).

As plant-available soil N increases with warming, leaf N can also increase, with annual and species-level variation in tree responses (Dury et al. 1998, Williams et al. 2003, Butler et al. 2012). Dark-leaved willow (*Salix myrsinifolia*) exhibited increased stem biomass but decreased secondary metabolites in warmed plots (Veteli et al. 2002). Red maple (*Acer rubrum*) has shown increases in foliar N content in warmed plots (a 25% increase in %N), reduction in leaf C:N ratios, and increased relative growth rates, compared to other tree species (Butler et al. 2012). In a greenhouse study, *Quercus robur* had reduced leaf N concentration and increased condensed tannin content at +3°C temperature (Dury et al. 1998). In an ecosystem warming study conducted in Tennessee, warming had no effect on secondary defense compounds of *Acer rubrum*, but did reduce leaf N (Williams et al. 2003), opposite to what was found in studies conducted in New England (Butler et al. 2012, Mellilo et al. 2011).

Warming-induced changes in plant chemistry could impact invertebrate herbivory. Leaf quality affects insect herbivory and is partially determined by leaf N and C content (Mattson 1980, Dudt 1994). Higher quality leaves with lower C:N ratios tend to have more frequent herbivore damage (Kudo 2003). Faster growing plants are typically less defended and experience higher herbivory rates than slower growing species (Coley et al. 1985, Bazzaz 1996, Endara and Coley 2011). However, few studies have examined the impacts of soil warming on plant and invertebrate herbivore interactions (Cornelissen 2011, Chung 2013), particularly in field settings.

Soil warming has been shown to affect soil C and N processes. However, experimental soil warming sites are typically concentrated in northern latitudes with glaciated, less weathered and more fertile soils (Rustad et al. 2001, Crowther et al. 2016, Carey et al. 2016). Our warming experiment at Whitehall Forest is the first and longest running soil warming experiment in lower-latitude locations on highly-weathered clay soils (Ultisols) typified by lower organic matter and low soil nutrients. For example, hardwood stands at Harvard Forest growing at ambient soil temperatures have net nitrogen mineralization rates ranging between 50-100 kg ha^{-1} yr^{-1} and total soil N pools in the upper 50-cm of ~5500 kg/ha (Aber et al. 1989, Melillo et al. 2002) . A +5°C soil warming at Harvard Forest resulted in a 41% increase in N mineralization and N availability to trees (Melillo et al. 2002). However, at Whitehall Forest on the red clay Ultisol soils typical of the southeastern Piedmont, annual net mineralization rates are only 10-30 kg ha^{-1} yr^{-1}, and upper 50 cm N pools are ~2000 kg/ha (Richter et al. 2000, Piatek and Allen 1999). Thus, the northern forest site exhibits a 3-8 fold faster N cycle under ambient temperatures. It remains unclear whether infertile, lower-latitude soils will exhibit any warming-induced increases in soil N or P availability. In addition, regionally varied responses to soil warming are expected. Colder climates generally exhibit stronger warming-induced responses in soil respiration than warmer regions, as temperature sensitivity decreases with higher ambient temperatures (Carey et al. 2016, Machmuller et al. 2018). It remains unclear how soil warming will affect soil processes of highly weathered, sub-tropical soils and how the indirect enhanced N mineralization or "fertilization effect" might impact plant and invertebrate herbivore interactions.

There were two main objectives in this study. First, we wanted to test the impacts of soil warming on soil and foliar C and N content in the highly-weathered soils of the Georgia Piedmont (Schlesinger and Bernhardt 2020, Godfrey 1997). For foliar C and N content, four understory species were used as model organisms: *Acer rubrum* (red maple), *A. floridanum* (southern sugar maple), *Quercus alba* (white oak), and *Q. rubra* (northern red oak). *Acer rubrum* is an opportunistic species that's widespread across the eastern U.S. (Abrams 1998, Mohan et al. 2004, 2007). *A. rubrum* is interesting in that it has high rates of seed dispersal and other characteristics of pioneer species, yet also is a shade-tolerant tree characteristic of mid/later successional taxa (Abrams 1998, Mohan et al. 2004, 2007). Shade tolerant *Acer floridanum* is found in the Coastal Plain and Piedmont of the Southeast U.S. *Quercus alba* is a common co-dominant species and *Quercus rubra* is another frequent co-dominant in the eastern U.S. particularly on moist sites (Little 1979, Godfrey 1997, Weakley 2020). In response to warming, foliar N increased to a varying degree in *Acer rubrum* and *Quercus rubra* in a New England study (Butler et al. 2012), but warming responses of *Q. alba* and *A. floridanum* have not been well studied. Second, my goal was to determine the relationship between soil warming and invertebrate herbivory in the Piedmont forest understory across four years. The main hypotheses were:

H_1: Warmed plots will exhibit decreased foliar and soil [C:N] ratios and increased %N, due to increased microbial and plant-available nitrogen sources.

H_2: Warmed plots will exhibit increased herbivory damage, due to increased leaf quality/leaf nitrogen.

This is the first study to examine herbivory in a lower-latitude forest warming experiment. I hypothesized that trees in warmed plots would have decreased foliar C:N ratios, based on previous literature showing increased plant available N and increased leaf N in several eastern tree species (Rustad 2001, Butler et al. 2012, Mellilo et al. 2011). I further expected that herbivory would increase with increased soil temperature, due to increased leaf quality of the warmed trees. Leaf quality is often positively correlated with herbivore damage (Mattson 1980, Richardson et al., 2002, Cha et al. 2010). Further, leaf N is often negatively correlated with phenolics and other C-rich defense compounds (Mattson 1980, Dudt 1994, Kudo 2003). I expected the potentially less well defended and more nutritious foliage of the warmed plots to experience higher herbivore damage. This study focused on leaf N and C content, rather than secondary metabolites, due to the small leaf sizes from seedlings often less than 10-cm in height. The role of relative growth rate and abiotic variables (soil moisture and light availability) in determining herbivore damage were also considered.

This is the only study of which I am aware that examines the plant-insect interaction response to soil warming in lower latitude, infertile soils. Investigating the effects of leaf quality on insect herbivory in response to soil warming, particularly at under-studied lower latitudes, will improve forecasts of how plant-insect interactions may be altered on a warmer planet.

Methods

Study site

This experiment took place at the Whitehall Forest in Athens, GA (33˚ 53’ 17.92”N, 83˚21’ 41.51” W; 686-ft above sea level). The 840-acre warm temperate forest is composed of both natural and planted pine and hardwood trees. The research site includes 3600 m^2 of deciduous forest dominated by *Quercus alba, Quercus rubra,* and *Acer rubrum* (Weakley 2020). The site is naturally-recruited and has been unmanaged since originating as abandoned farmland in the early-20th century. Precipitation is evenly distributed throughout the year, with a 40-year historical annual mean of 124 cm yr^{-1}(NOAA 2013). Average temperature in January is 9.1°C and 28°C in July. This site is considered representative of deciduous forests in the Piedmont of Georgia and the southeastern U.S. (Edwards et al. 2013, Godfrey 1997).

This experiment is part of a larger study: The Whitehall Forest Soil Warming Facility (WFWF) established in 2009 (see Machmuller et al. 2018 for more details about this site). A randomized complete block design was established in the forest understory. One 18.4-m^2 chambered plot in each of three blocks is heated +3°C above ambient soil temperature, one is +5°C, and two are kept at ambient temperature: one with the open-top chamber design and buried warming cables and one without a chamber enclosure or buried cables (Figure 2.1). Each block contains three open-top chambers and one non-chambered control plot. The chamber walls consist of PVC Poly Grow UVA Tube Suncover 6 mil greenhouse plastic sheeting (A.M. Leonard, Piqua, OH) to exclude deer and most other mammals and provide a small amount (<0.5°C) of aboveground warming dependent upon season and time (warmest in summer afternoons). To heat the soil, every chamber has heating cables manually buried 10cm deep and 20cm apart. The ambient temperature chambers have cables buried, but heat not turned on, and are used as a reference for the other chambers. The “non-chamber” plots (n=3) have neither chambers nor buried warming cables and are used as controls to test for chamber/cable burial effects.

Multiple native tree species (*Pinus virgiana, P. palustris, P. taeda, Quercus rubra, Q. alba, Q. velutina, Q. nigra, Q. virginiana, Q. michauxii, Acer rubrum, A. floridanum, Liriodendron tulipifera, Liquidambar styraciflua, Magnolia virginiana* and *M. grandiflora* (Weakley 2020)) were planted by seed in 2010-2012. The current plots consist of tree seedlings and saplings of both these planted species and species naturally-recruited from adjacent tree seeds. There are approximately 1,500 individuals throughout twelve plots, and every individual tree is ID tagged and has coordinates recorded, in order to relocate individuals each year. There were four main target species for this study: *Quercus alba, Q. rubra, Acer floridanum*, and *A. rubrum* (Weakley 2020). Within each plot the computerized system continuously measures soil and air temperatures, soil moisture, relative humidity, and photosynthetically active radiation (PAR) every 10 minutes and records hourly averages.

Growth

Height data was collected from every individual tree in each plot in 2017, 2018, 2019, and 2020. Beginning and end-of-growing season height data was collected in 2017 and 2018, and over-winter terminal heights were measured in 2019. Height (cm) was measured from soil surface to highest apical bud. Biomass was calculated from height (cm) data using species-specific allometric equations from seedlings growing in the North Carolina Piedmont of Duke Forest (Mohan et al. 2007). Relative growth rates (RGR) were calculated per year as:

$$RGR = [lnBM_2 - lnBM_1]/[t_2 - t_1]$$

where t = elapsed time, and BM_1 and BM_2 are the biomass at time t_1 and t_2, respectively.

Herbivory

Herbivory measurements were collected in May, July, and October of 2017 and 2018 to represent the beginning, middle, and end of the growing season. Mid-season herbivory was also measured in 2019 and 2020. Herbivory was measured as a visual estimate of % foliar area missing for all leaves on a seedling. Visual estimates were recorded across years by the same researcher for consistency. Accuracy of visual estimates was confirmed on a subsample using Leafbyte software (Getman-Pickering, Z. 2020) ($p<0.001$, $R^2=0.902$).

Foliar and soil sampling

There were four main target species for this study: *Quercus alba, Q. rubra, Acer floridanum,* and *A. rubrum* (Weakley 2020). These are the most abundant species in the plots and are found throughout all twelve plots of the ambient, +3°C, +5°C, and non-chamber treatments. Foliar samples from the target species were collected in July 2018 and 2020. Foliar samples for each individual consisted of one mature leaf that was representative in size and invertebrate herbivory damage for all leaves on the individual plant. As we have observed no mucous trails left by terrestrial molluscs (slugs and snails) in this experiment, I believe the invertebrate herbivory corresponds to insect herbivory (Kenna and Mohan, unpublished observations). Three individuals of each species were selected from each plot in a stratified random design. Individuals from varying locations within the plot, and of varying height and herbivore damage were selected. Although the target species are the most abundant, seven of the twelve plots had fewer than three individuals of at least one species. Foliar samples were still collected for all available individuals. In total, there are 204 foliar samples from the 2018 and 2020 growing seasons.

Soil samples from each treatment (ambient, +3°C, +5°C, and non-chamber treatments) were collected in July 2020. Three soil cores (4cm depth) were collected from each plot. Soil samples were collected in a stratified random design, with varying locations within the plots. The litter layer was cleared away before coring.

The foliar and soil samples were oven dried at 60℃ (foliar) or 80℃ (soil) for at least 24 hours, before being ball-milled to less than 250μm particle size according to the standards of the Center for Applied Isotope Studies at UGA. The foliar and soil samples were submitted to the Stable Isotope Ecology Laboratory (SIEL) through the Center for Applied Isotope Studies at UGA. The samples were analyzed for total % N and total % C. Soil sample results were averaged by plot, and foliar sample results were averaged by species per plot.

To check the accuracy of visual estimates of herbivory foliar sub-samples from 2017, 2019, and 2020 were photographed according to the parameters required by Leafbyte (Getman-Pickering, Z. 2020). Leafbyte is an open source software for measuring insect herbivory (https://zoegp.science/leafbyte). Percent area consumed was calculated for each foliar sample using Leafbyte.

Statistical analysis

The data were aggregated by species, plot, block, and temperature treatment for approximately 1,500 individuals. Herbivory variables were logit transformed (Warton and Hui 2011). Leaf quality in 2020 (total %C, %N, and C:N ratio) and 2018 (%C, %N, and C:N ratio) and soil chemical variables, soil moisture, and PAR data met assumptions of normality and were not transformed. Foliar %N in 2018 was natural log transformed. The dependent variables were herbivory, foliar chemistry, and soil chemistry. The independent fixed variables were temperature treatment, species, relative growth rate, soil moisture, and PAR. Block, chamber, and time were treated as random variables. Linear mixed models were used to determine the relationship between herbivory and temperature treatment, as well as species, growth rate, soil moisture, and PAR. Model selection using AIC values was used to determine the best-fit model (Table 2.1). A linear mixed model was also used to determine the relationship between foliar and soil total %N, total %C, and C:N with temperature treatment, species (for foliar chemistry only) and soil moisture. Diagnostic plots satisfied linear modeling assumptions. The Lme4 package in R was used to conduct these analyses (Bates et al. 2015).

Results

Herbivory 2017-2020. The effect of temperature on percent leaf area consumed across all four target species was not significant for any year (p=0.965, Figure 2.3). However, there were marginally significant temperature effects within species (p=0.095). In September 2018, *Quercus alba* in the ambient treatment had significantly higher herbivore damage than *Quercus alba* in the +3°C treatment (p=0.042) and marginally significantly higher herbivore damage in the +5°C treatment (p=0.055, Figure 2.2). In June 2019, *Quercus alba* in the ambient treatment had significantly higher herbivore damage than *Quercus alba* in the +3°C treatment (p=0.0024) and the +5°C treatment (p=0.026, Figure 2.2). In August 2020, *Quercus rubra* in the ambient treatment had significantly lower herbivore damage than *Quercus rubra* in the +5°C (p=0.045) and marginally significantly lower herbivore damage than the +3°C treatments (p=0.089, Figure 2.2). Species had a significant effect on herbivore damage across years: *Quercus rubra* had the highest mean herbivory damage, and *Acer rubrum* and *Acer floridanum* had the lowest mean herbivory damage (p<0.001). Soil moisture was significantly correlated with herbivory (p=0.042), but herbivory did not have a significant relationship with PAR (p=0.260) or relative growth rate (p=0.184). The best-fit linear mixed model was herbivory by species, soil moisture, relative growth rate, and measurement time with interacting effects (AIC: 4933.241, Table 2.1). The next best-fit candidate model included PAR, and the third best-fit model did not include relative growth rate; the fifth best-fit model included temperature treatment (Table 2.1).

Carbon and Nitrogen foliar analyses. Foliar total %N varied significantly with year (p<0.001), species (p<0.001) and temperature treatment (p<0.001, Figure 2.5). In 2018, the foliar total %N of *Acer rubrum* was significantly higher in the ambient treatment versus +3°C treatment (p<0.001). For *Quercus alba*, the +3°C treatment had the lowest foliar N content, and the +5°C treatment had the highest (p<0.001). In 2020, the foliar total %N of *Acer floridanum* was marginally significantly higher in the +3°C treatment versus the ambient (p=0.051). For *Quercus rubra*, the +3°C treatment had significantly higher foliar %N than the ambient treatment (p=0.027) and marginally significantly higher foliar %N than the +5°C treatment (p=0.090).

Foliar total %C varied significantly with year (p<0.001) and species (p<0.0001, Figure 2.6). Temperature as a main effect across all species did not significantly impact foliar total %C, but there were temperature effects within species (p=0.003 for the temperature-by-species interaction). In 2018, *Acer floridanum* had significantly lower foliar %C in the +3°C treatment versus the ambient (p=0.020) and +5°C treatment (p=0.042). In 2020, *Acer rubrum* had significantly higher foliar %C in the +3°C treatment versus the +5°C treatment (p=0.003) and marginally significantly higher foliar %C in the +3°C treatment than the ambient treatment (p=0.058).

Foliar C:N ratio varied significantly by year (p<0.001), species (p<0.001) and temperature treatment (p<0.001, Figure 2.7). In 2018, *Acer rubrum* had significantly higher C:N ratios in the +3°C treatment versus the +5°C treatment (p=0.007). *Quercus alba* had significantly higher C:N ratios in the +3°C treatment versus the ambient treatment (p=0.017) and the +5°C treatment (p<0.001). In 2020, *Acer floridanum* had significantly lower C:N ratios in the +3°C treatment versus the ambient (p=0.041). *Acer rubrum* had significantly higher C:N ratios in the +3°C treatment versus the ambient (p=0.050) and +5°C treatments (p=0.005). *Quercus rubra* had significantly lower C:N ratios in the +3°C treatment versus the ambient (p=0.025) and marginally significantly lower C:N ratios versus the +5°C treatments (p=0.097).

Foliar C:N ratios were significantly higher across species in 2020 versus 2018 (p<0.001). Foliar %C (p<0.001) and foliar %N (p<0.001) were significantly lower across species in 2020 versus 2018. Foliar %N and foliar C:N ratios varied significantly by soil moisture and temperature treatment (p=0.004, p=0.033, respectively). Foliar %N and foliar C:N ratios also varied significantly by relative growth rate and temperature treatment (p=0.005, p=0.035, respectively). Herbivory did not significantly correlate with foliar total %N (p=0.282), total %C (p=0.181), or C:N ratio (p=0.478).

Soil chemical properties. Soil total %N, total %C and the C:N ratios did not vary with temperature treatment (p=0.999, p=0.844, p=0.731, respectively; Figures 2.8, 2.9, and 2.10).

Discussion

Ecosystem warming and other landscape scale experiments have documented significant changes in carbon and nitrogen processes in response to temperature, including increased N mineralization and bioavailable soil N (Mohan 2019, Melillo et al. 2011, Butler et al. 2012, Garten 1993, Rustad et al. 2001, Peterjohn et al. 1994). Foliar N content can also be impacted by warming, with spatial and species-level variation (Veteli et al. 2002, Butler et al. 2012, Dury 1998, Williams et al. 2003). Herbivory is often strongly impacted by foliar chemistry, including foliar N (Mattson 1980, Dudt 1994, Cornelissen 2011), and is predicted to be impacted directly and indirectly through ecosystem warming from climate change (Chung 2013, Bale et al. 2002). However, it is not well known how ecosystem warming effects on foliar chemical content will impact herbivory (Zvereva et al. 2006, Chung 2013). I predicted that soil warming would decrease foliar C:N and lead to greater insect herbivory, due to increased leaf quality and leaf N content. In this study, there were species-level responses of leaf chemistry to temperature treatment. Across all individuals, herbivore damage was marginally impacted by temperature treatment within species, but overall there was little response of herbivory to soil warming (Figure 2.2). I also found year-to-year differences in herbivore damage over four years (Figure 2.3).

Foliar C and N concentrations did not have a strong response to warming across all species, but foliar chemistry response to warming varies by species and year. Contrary to my predictions, in *Acer rubrum* and *Quercus alba* in 2018, foliar %N decreased and C:N ratio increased in the +3°C treatment compared to the ambient and +5°C treatments. However, in *Acer floridanum*, *A. rubrum,* and *Quercus rubra* in 2020, foliar %N increased and C:N ratio decreased in the +3°C treatment compared to the ambient and +5°C treatments. Generally, *Acer* species had stronger responses to warming, particularly *Acer floridanum*. At Harvard Forest, MA, soil and air warming decreased foliar C:N of *Acer rubrum* at +3°C, but increased the foliar C:N at +5°C (Lehman, Mohan et al. unpublished data). This is contradictory to the Whitehall Forest study; in 2020 *Acer rubrum* had significantly higher foliar C:N at +3°C, but lower foliar C:N at +5°C (Figure 2.7).

There was also higher total %C, total %N, and lower C:N across all four species in 2018, compared to 2020. Herbivory was also significantly lower across species in 2018 versus 2020 (Figure2.3), which contradicts previous literature findings that higher-quality plants with higher leaf N and lower C:N ratios experienced higher herbivory (Mattson 1980, Richardson et al., 2002, Cha et al., 2010). In our study, herbivory did not correlate with foliar chemistry.

Varying warming responses among species could impact future forest composition, as relative herbivore damage among species can shift with warming-induced changes in phytochemistry. Variance between Northeastern and Southeastern forests emphasizes the need for experimental warming studies across wide latitudinal gradients.

Across all nine chambers, there were no significantly different herbivory responses across the three temperature treatments within or between years This is contradictory to my predictions that herbivory would increase with warming. The lack of response is likely due to lower soil N at the Piedmont site relative to many previous warming experiments and lower reaction rate sensitivity to temperature changes at higher temperatures (Schlesinger and Bernhardt 2020, Mohan 2019). Previous warming studies have been conducted in higher latitude, less weathered, and more fertile soils and demonstrated significant response of soil processes, including N mineralization, to warming (Rustad et al. 2001, Melillo et al. 2011, Crowther et al. 2016, Carey et al. 2016). However, preliminary data suggests warming does not increase N mineralization at WFWF and that June-August soil N is actually immobilized by soil microbes in this forest, at least in 2012 (Mohan 2012, unpublished data). The current study shows there is no change in foliar or soil C and N content in response to warming. One possible reason is related to soil moisture. Soil moisture can be a major limiting factor on rates of soil microbial processes (Peay et al. 2016, Mohan 2019, Schlesinger and Bernhardt 2020), which could constrain responses to soil warming (Machmuller et al. 2018). The +3°C treatment has lower average soil moisture than the other treatments (Figure 2.11), which could influence foliar N and C:N content (Mattson 1980, Huberty and Denno 2004). 2018 had higher soil moisture across all temperature treatments compared to 2020 (Figure 2.11), which could also influence annual variation in soil moisture-driven changes in foliar chemistry. In this study, herbivory was significantly correlated with soil moisture (Figure 2.4). However, the soil moisture of the ambient and +5°C treatments are very similar (Figure 2.11), so any temperature-driven, and not soil moisture driven, response would be expected to be evident between these two treatments.

Increases in plant defense compounds as temperature increases could negate possible increases in plant-available N from warming. Increasing temperature can reduce foliar quality through increasing defense compounds (Dury et al. 1997) and lower leaf N can correlate with higher phenolics and other herbivore defense compounds (Kudo 2003, Bauerfield 2013). An increase in plant available soil N with warming may not always be reflected in foliar % N, but foliar defense compounds might have increased. However, I did not detect a response of herbivory to warming, which one would expect with a warming-induced changed in defense compounds. I also did not detect a response of soil N or soil C:N to warming. Therefore, low soil N availability is the most likely limiting factor to temperature response.

Relative growth rate also did not have a significant impact on herbivory, which is contradictory to previous studies that have demonstrated faster growing, less defended plants experience higher herbivore damage (Coley et al. 1985, Endara and Coley 2011). *Acer* species may have higher relative growth rates and experience higher herbivore damage than *Quercus* species in similar habitats (Mohan et al. 2007, Butler et al. 2012). However, in the current study herbivory was typically lower in the *Acer* species, particularly *Acer rubrum*, than in the *Quercus* species. The lower herbivore damage could be due to higher leaf N of *Acer* species, as shown in 2020 foliar analyses, which could lower herbivory by increasing the proportion of leaf N per unit mass consumed.

The non-chambered plots did show significant differences in responses of herbivore damage and soil N compared to the other treatments (Figure 2.1A and 2.1D, Figure 2.8). In August 2020 and September 2018, the non-chambers had significantly higher herbivore damage.

This is possibly due to the PVC lining around the open-top chambers as opposed to chicken wire surrounding the non-chambered control plots decreasing insect accessibility to the chambered plots, as have been observed in previous studies (Mohan 2019, Chapter 3). In 2020, the soil %N was higher in the non-chambers compared to the ambient and +3°C chambers. This might suggest the initial disturbance from the manually buried warming cables, at least in the ambient and +5°C chambers, had a long-term impact on soil processes via disturbance-mediated aeration of soils which could enhance soil N cycling rates (Schlesinger and Bernhardt 2020).

Conclusions and future directions

Anthropogenic climate change is expected to directly impact soil C and N processes (IPCC 2014, Mohan 2019, Schlesinger and Bernhardt 2020), which may affect herbivory indirectly through plant physiological chemical changes due to enhanced soil N bioavailability. In previous, higher latitude studies, warming induced increased nitrogen mineralization and plant available N (Peterjohn et al. 1994, Rustad et al. 2001, Melillo et al. 2011), and leaf N was altered in response to temperature (Dury 1998, Williams et al. 2003, Butler et al. 2012). The indirect effects of warming-induced leaf chemistry changes on insect herbivory are not well studied, particularly under field conditions (Chung 2013, Cornelissen 2011). Furthermore, previous warming studies have been concentrated in higher latitude sites with glaciated, more fertile and N-rich soils. This study is the first I am aware of that investigated warming-induced indirect response of insect herbivores in lower latitude, subtropical highly weathered soils. There was some species-level response of foliar chemistry to temperature treatment, but no response in soil chemistry. There was no warming-induced response of insect herbivory for any species. Therefore, I conclude warming does not have an indirect effect via fertilization on herbivory and phytochemistry at this site. There was some variation in species response to warming, which indicates potential for warming-induced forest compositional changes. Future work should consider potential impacts of soil warming on associated plant secondary defensive compounds, as well as investigate possible soil nitrogen limitations to plant soil warming responses.

References

Aber, J. D., Magill, A., Mcnulty, S. G., Boone, R. D., Nadelhoffer, K. J., Downs, M., & Hallett, R. (1995). Forest biogeochemistry and primary production altered by nitrogen saturation. *Water, Air, and Soil Pollution, 85*(3), 1665-1670.

Abrams, M. D. (1998). The Red Maple Paradox. *BioScience*, *48*(5), 355–364.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., *et al.* (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16.

Bardgett, R. D., & Wardle, D. A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology, 84*(9), 2258-2268.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bazzaz, F. A., & Bazzaz, F. (1996). *Plants in changing environments: linking physiological, population, and community ecology*. Cambridge University Press.

Bauerfeind, S. S., & Fischer, K. (2013). Increased temperature reduces herbivore host-plant quality. *Global Change Biology*, *19*(11), 3272–3282.

Belovsky, G. E., & Slade, J. B. (2000). Insect herbivory accelerates nutrient cycling and increases plant production. *Proceedings of the National Academy of Sciences*, *97*(26), 14412-14417.

Bonan, G. B. (2008). Forests and Climate Change: forcings, feedbacks, and the climate benefits of forests, *Science, 320*(5882): 1444–1450.

Butler, S. M., Melillo, J. M., Johnson, J. E., Mohan, J., Steudler, P. A., Lux, H., Burrows, E., Smith, R.M., Vario, C.L., Scott, L., Hill, T.D., Aponte, N., & Bowles, F. (2012). Soil warming alters nitrogen cycling in a New England forest: Implications for ecosystem function and structure. *Oecologia*, *168*(3), 819–828.

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., *et al.* (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy of Sciences*, *113*(48), 13797-13802.

Carson, W. P., & Root, R. B. (1999). Top-down effects of insect herbivores during early succession: influence on biomass and plant dominance. *Oecologia*, *121*(2), 260-272.

Cha, D. H., Appel, H. M., Frost, C. J., Schultz, J. C., & Steiner, K. C. (2010). Forest Ecology and Management Red oak responses to nitrogen addition depend on herbivory type, tree family, and site. *Forest Ecology and Management*, *259*(10), 1930–1937.

Chung, H., Muraoka, H., Nakamura, M., Han, S., Muller, O., & Son, Y. (2013). Experimental warming studies on tree species and forest ecosystems: A literature review. *Journal of Plant Research*, *126*(4), 447–460.

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, *230*(4728), 895-899.

Cornelissen, T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology*, *40*(2), 155–163.

Crowther, T., Todd-Brown, K., Rowe, C. *et al.* (2016) Quantifying global soil carbon losses in response to warming. *Nature* 540**,** 104–108.

Dudt, J. F., & Shure, D. J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, *75*(1), 86–98.

Dury, S. J., Good, J. E. G., Perrins, C. M., Buse, A., & Kaye, T. (1998). The effects of increasing CO2 and temperature on oak leaf palatability and the implications for herbivorous insects. *Global Change Biology*, *4*(1), 55–61.

Edwards, Leslie, Jonathon Ambrose, and L. Katherine Kirkman. (2013). The Natural Communities of Georgia. University of Georgia Press Athens, GA. 675 pp.

Endara, M. J., & Coley, P. D. (2011). The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology*, *25*(2), 389–398.

Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, *85*(5), 1383-1398.

Garten, C. T. (1993). Variation in foliar ^{15}N abundance and the availability of soil nitrogen on walker branch watershed. *Ecology*, *74*(7), 2098–2113.

Getman-Pickering, Z. L., Campbell, A., Aflitto, N., Grele, A., Davis, J. K., & Ugine, T. A. (2020). LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. *Methods in Ecology and Evolutio*n, 2041–210X.13340.

Godfrey, M.A. 1997. A Field Guide to the Piedmont: The Natural Habitats of America's Most Lived-In Region, from New York to Montgomery, Alabama. The University of North Carolina Press. Chapel Hill, NC. 524 pp.

IPCC, 2014: *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Kudo, G. (2003). Variations in leaf traits and susceptibility to insect herbivory within a Salix miyabeana population under field conditions. *Plant Ecology*, *169*(1), 61–69.

Lehman, F, Mohan, J.E., Melillo, J.M., Clark, J. & Salk, C. (2011). Climate warming effect on Acer rubrum seedling susceptibility to foliar herbivory.

Little, E. L. (1979). *Checklist of United States trees (native and naturalized)* (No. 541). DC: Forest Service, US Department of Agriculture.

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., *et al.* (2013). Responses of ecosystem carbon cycle to experimental warming: A meta-analysis. *Ecology*, *94*(3), 726–738.

Machmuller, M. B., Ballantyne, F., Markewitz, D., Thompson, A., Wurzburger, N., Frankson, P. T., & Mohan, J. E. (2018). Temperature sensitivity of soil respiration in a low-latitude forest ecosystem varies by season and habitat but is unaffected by experimental warming. *Biogeochemistry*, *141*(1), 63–73.

Mattson, W. J. (1980). Herbivory in Relation to Plant Nitrogen Content. *Annual Review of Ecology and Systematics*, *11*(1), 119–161.

Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., *et al.* (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, *298*(5601), 2173-2176.

Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., *et al.* (2011). Soil warming, carbon–nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences*, *108*(23), 9508-9512.

Mohan, J. E., Clark, J. S., & Schlesinger, W. H. (2004). Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO2. *Global Change Biology*, *10*(2), 233-247.

Mohan, J. E., Clark, J. S., & Schlesinger, W. H. (2007). Long-term CO2 enrichment of a forest ecosystem: implications for forest regeneration and succession. *Ecological Applications, 17*(4), 1198-1212.

Mohan, J. E. (Ed.). (2019). *Ecosystem Consequences of Soil Warming: Microbes, Vegetation, Fauna and Soil Biogeochemistry*. Academic Press.

NOAA (2013) National oceanic and atmospheric administration. National climate data center, Athens, Georgia, Ben Epps Airport Climate Station

Nottingham, A. T., Meir, P., Velasquez, E., & Turner, B. L. (2020). Soil carbon loss by experimental warming in a tropical forest. *Nature*, *584*(7820), 234-237.

Peay, K.G., Kennedy, P.G., Talbot, J.M., 2016. Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology 14*, 434.

Pérez-Sánchez, R. M., Jurado, E., Chapa-Vargas, L., & Flores, J. (2011). Seed germination of Southern Chihuahuan Desert plants in response to elevated temperatures. *Journal of Arid Environments*, *75*(10), 978–980.

Peterjohn, W. T., Melillo, J. M., Steudler, P. A., Newkirk, K. M., Bowles, F. P., & Aber, J. D. (1994). Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. *Ecological applications*, *4*(3), 617-625.

Piatek, K. B., & Allen, H. L. (1999). Nitrogen mineralization in a pine plantation fifteen years after harvesting and site preparation. *Soil Science Society of America Journal*, *63*(4), 990-998.

Richardson, S. J., Press, M. C., Parsons, A. N., & Hartley, S. E. (2002). How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. *Journal of Ecology*, *90*(3), 544–556.

Richter, D. D., Markewitz, D., Heine, P. R., Jin, V., Raikes, J., Tian, K., & Wells, C. G. (2000). Legacies of agriculture and forest regrowth in the nitrogen of old-field soils. *Forest Ecology and Management*, *138*(1-3), 233-248.

Rustad, L. E. J. L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A, et al. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*(4), 543-562.

Schlesinger, W. H. and Bernhardt, E., (4th Ed.). (2020). *Biogeochemistry*. Elsevier.

Veteli, T. O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H., & Tahvanainen, J. (2002). Effects of elevated CO2 and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology*, *8*(12), 1240–1252.

Warton, D., & Hui, F. (2011). The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, *92*(1), 3–10.

Weakley, A. S. (2020). *Flora of the Southeastern United States*. University of North Carolina Herbarium, North Carolina Botanical Garden, University of North Carolina, Chapel Hill,1848 pp.

Williams, R. S., Lincoln, D. E., & Norby, R. J. (2003). Development of gypsy moth larvae feeding on red maple saplings at elevated CO2 and temperature. *Oecologia*, *137*(1), 114–122.

Zvereva, E. L., & Kozlov, M. V. (2006). Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: A metaanalysis. *Global Change Biology*, *12*(1), 27–41.

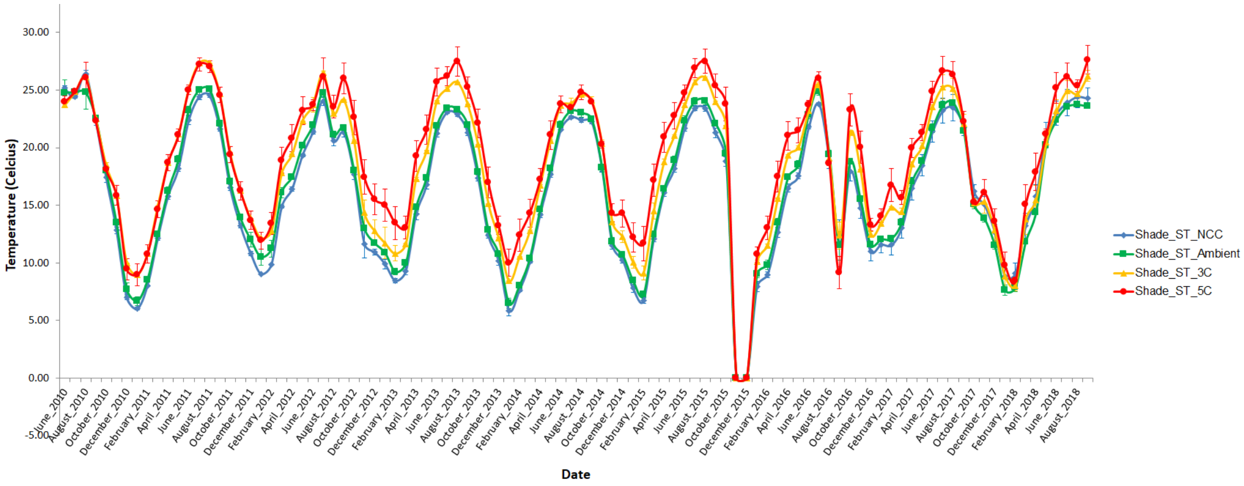

Figure 2.1: Soil temperatures (°C) by temperature treatment from June 2010 to August 2018. Values were averaged by plot. Error bars represent ±1 standard error. Buried warming cables were successful in heating the chambers to approximately +3°C and +5°C above ambient.

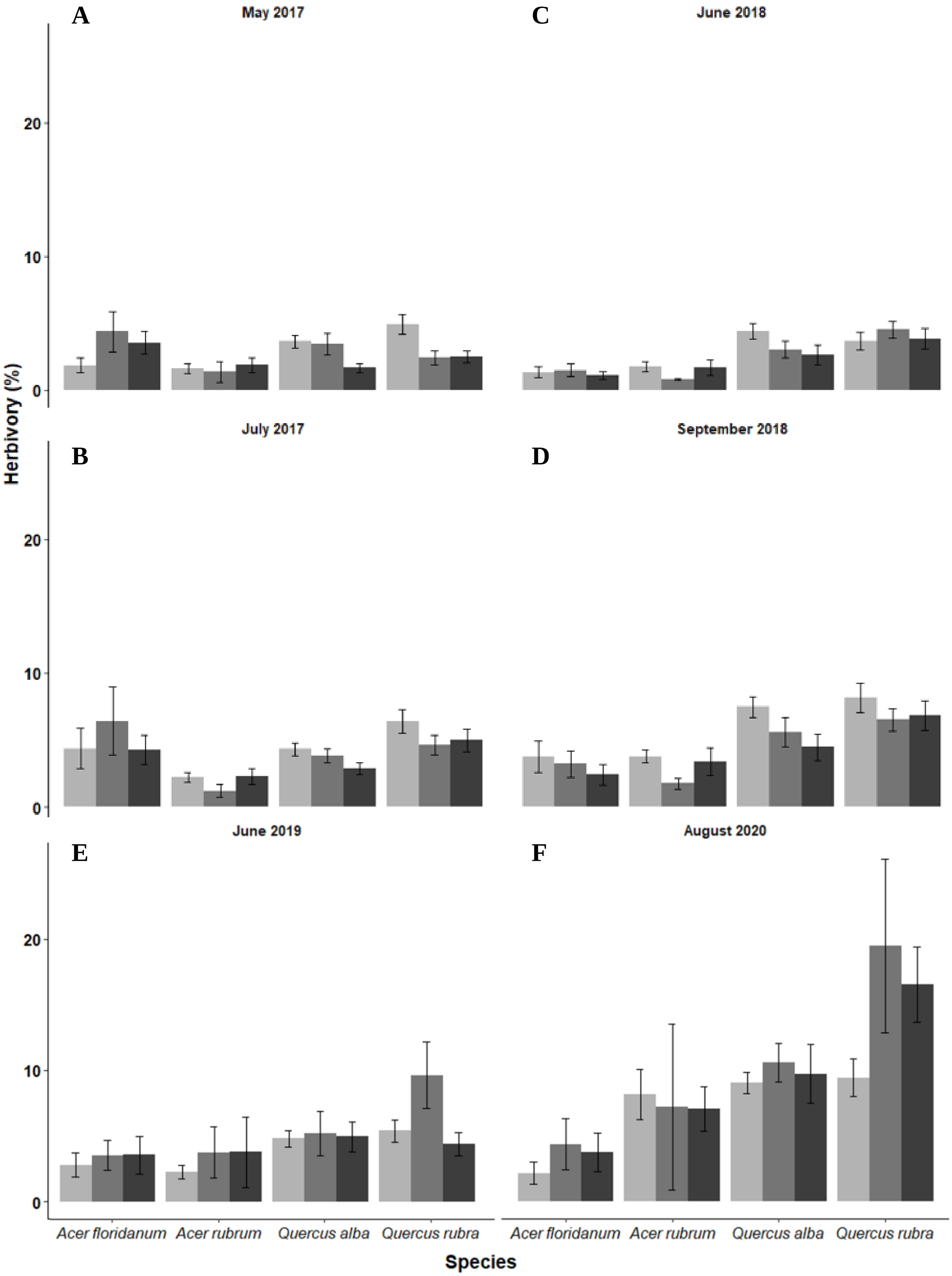
A
May 2017
C
June 2018
B
July 2017
D
September 2018
E
June 2019
F
August 2020
Herbivory (%)
0
10
20
Acer floridanum
Acer rubrum
Quercus alba
Quercus rubra
Species

Figure 2.2 Warming effect by species on leaf herbivory among years. Data is across all nine plots and four target species (*Acer floridanum, A. rubrum, Quercus alba, Q. rubra*) from May 2017 (A), July 2017(B), June 2018(C), September 2018(D), June 2019(E), and August 2020(F). Herbivory represents percent leaf area consumed. Leaf herbivory data was logit-transformed and analyzed using linear mixed effect models. Temperature treatment, species and year were fixed effects and block, chamber, and time were used as random effects. The y-axis was back-transformed to percent. The error bars represent ±1 standard error. In September 2018, *Quercus alba* in the ambient treatment had significantly higher herbivore damage than *Quercus alba* in the +3℃ treatment (p=0.042) and marginally significantly higher herbivore damage in the +5℃ treatment (p=0.055). In June 2019, *Quercus alba* in the ambient treatment had significantly higher herbivore damage than *Quercus alba* in the +3℃ treatment (p=0.0024) and the +5℃ treatment (p=0.026). In August 2020, *Quercus rubra* in the ambient treatment had significantly lower herbivore damage than *Quercus rubra* in the +5℃ (p=0.045) and marginally significantly lower herbivore damage than the +3℃ treatments (p=0.089). Species had a significant effect on herbivore damage across years: *Quercus rubra* had the highest mean herbivory damage, and *Acer rubrum* and *Acer floridanum* had the lowest mean herbivory damage (p<0.001).

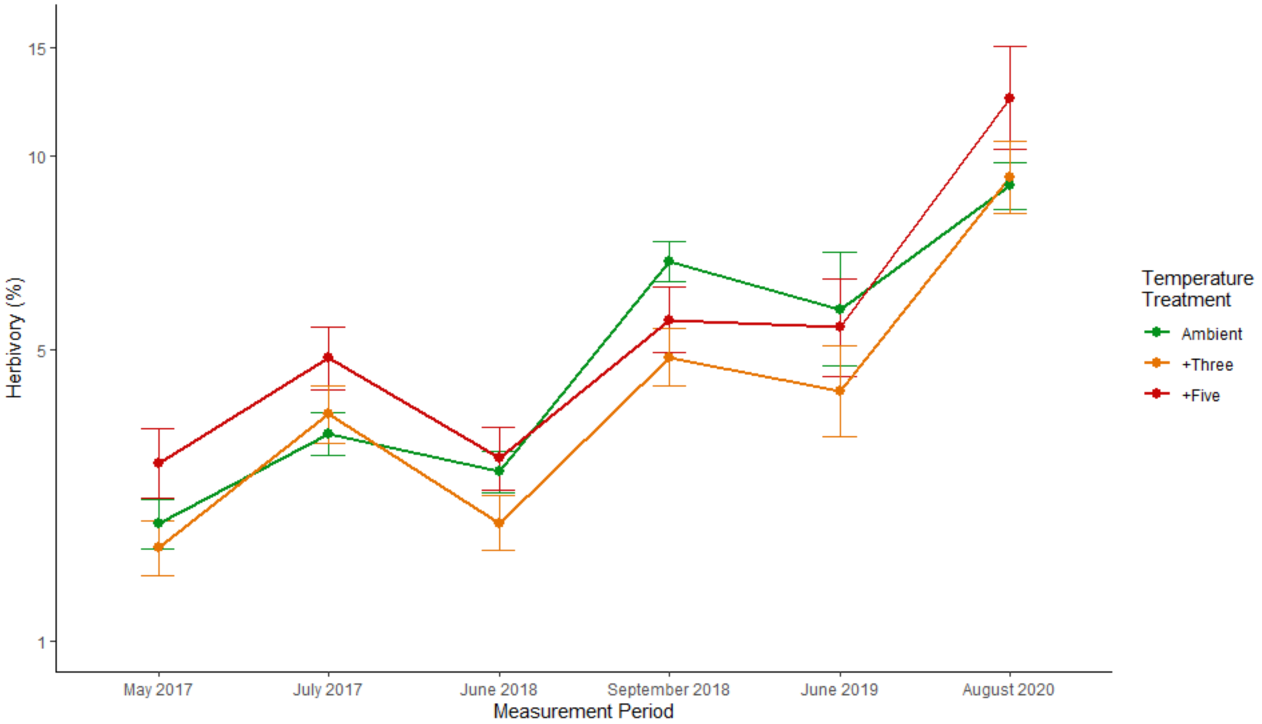

Figure 2.3: Warming effect on mid-season leaf herbivory across six measurement periods: May 2017, July 2017, June 2018, September 2018, June 2019, and August 2020 for the ambient (green), +3℃ (orange) and +5℃ (red) treatments. Leaf herbivory data represents leaf area consumed (back-transformed) and was analyzed over four years with a repeated measures linear mixed model. Block, chamber, and species were used as random effects. The error bars represent ±1 standard error. The effect of temperature on percent leaf area consumed across all four target species was not significant for any year (p=0.965). There is annual variation in mean herbivory.

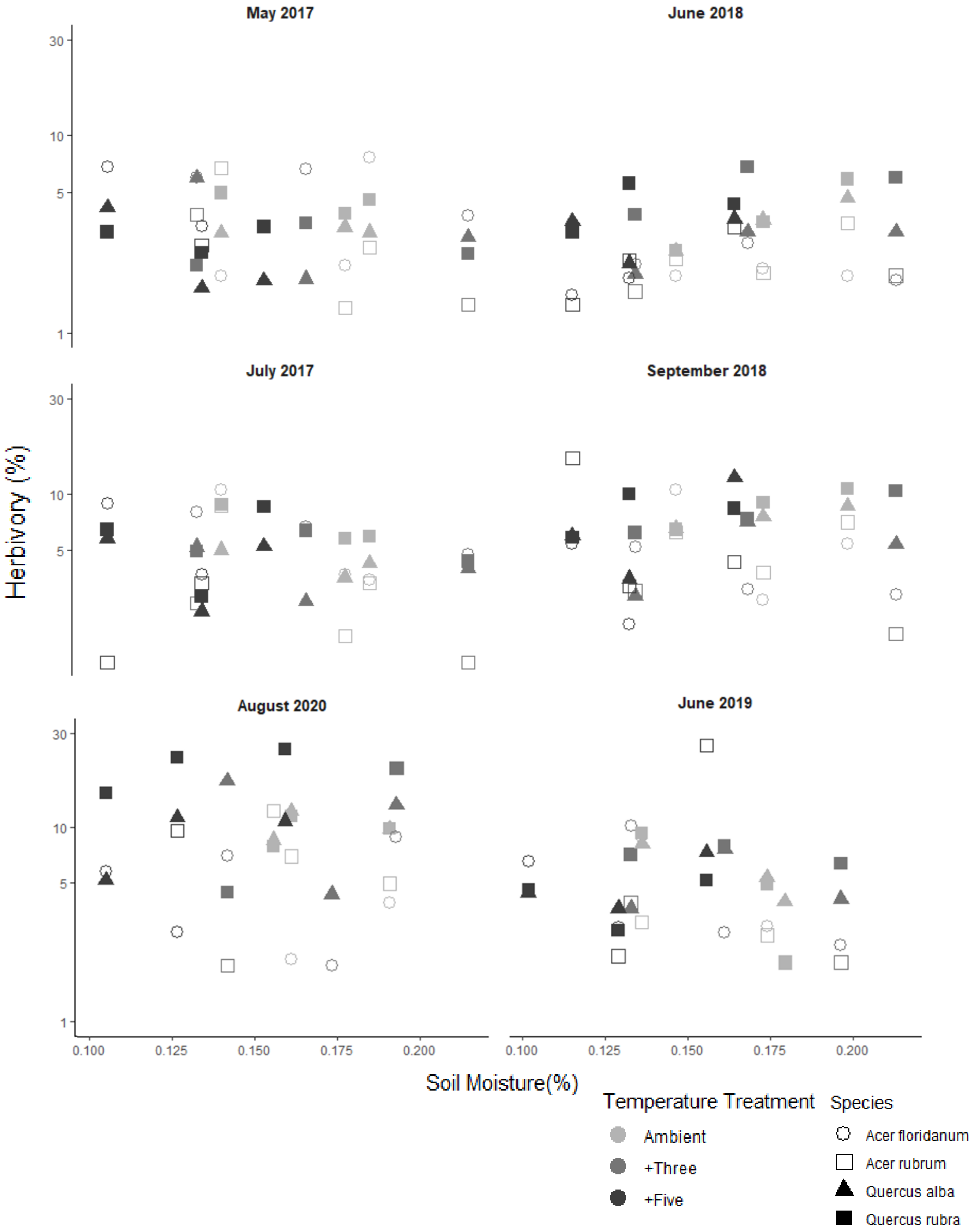
May 2017
June 2018
July 2017
September 2018
August 2020
June 2019
Herbivory (%)
30
10
5
1
Soil Moisture(%)
0.100
0.125
0.150
0.175
0.200
Temperature Treatment
Ambient
+Three
+Five
Species
Acer floridanum
Acer rubrum
Quercus alba
Quercus rubra

Figure 2.4: Herbivory (%) by soil moisture for each measurement period. Data is across all nine plots and four target species (*Acer floridanum, A. rubrum*, *Quercus alba*, *Q. rubra*) from May 2017, July 2017, June 2018, September 2018, June 2019, and August 2020. Data was aggregated by chamber and species. Temperature treatment is indicated by color, and species is indicated by shape. Leaf herbivory data was logit-transformed and analyzed using linear mixed effect models. Soil moisture, temperature treatment, species and year were fixed effects and block, chamber, and time were used as random effects. The y-axis was back-transformed to percent. Soil moisture was significantly correlated with herbivory (p=0.042) and the best fit linear mixed model was herbivory by species, soil moisture, relative growth rate, and measurement time with interacting effects (AIC: 4933.241).

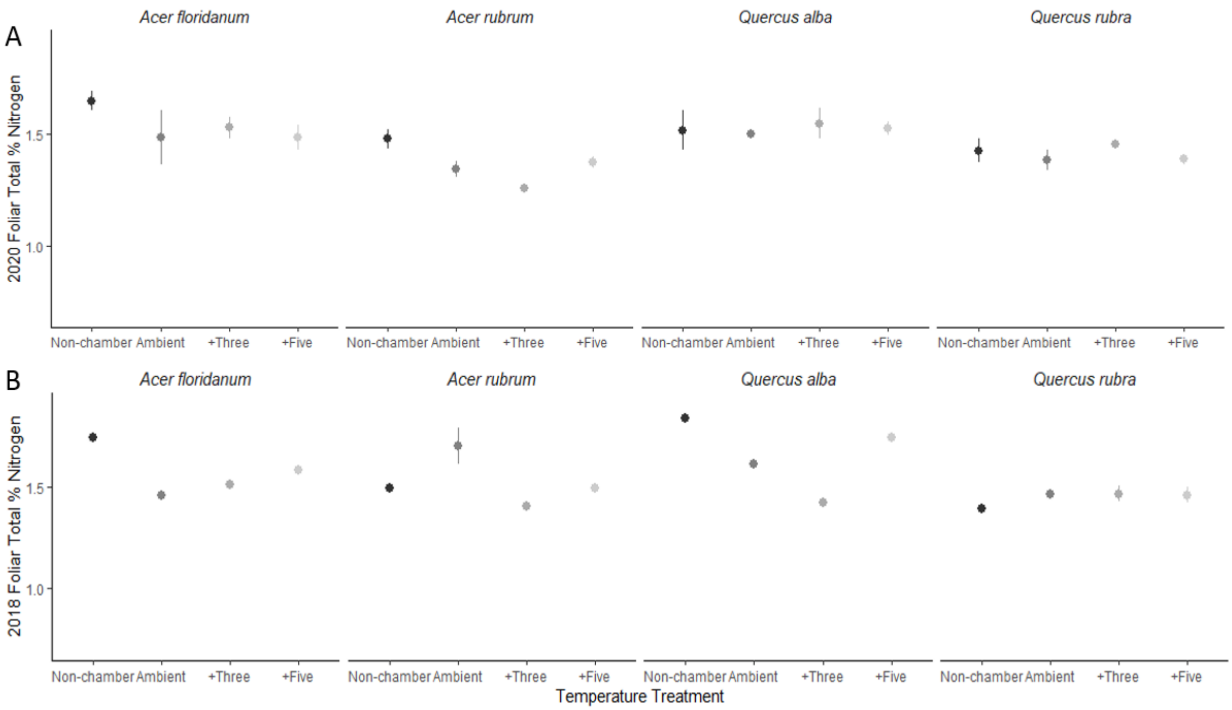

Figure 2.5: Warming effect on foliar % nitrogen across species. Foliar total % Nitrogen from 2020 (A) and 2018 (B) by temperature treatment (Non-chamber, ambient, +3℃, +5℃) for each target species (*Acer floridanum*, *A. rubrum*, *Quercus alba*, *Q. rubra*). Foliar total % N represents the proportion of nitrogen of dry mass of a single leaf sample from each individual tree. Three individual trees per species per plot were averaged and analyzed as plot-based means (n=3). Total % N values were averaged by species per plot. Points represent mean per temperature treatment by species, and error bars represent ±1 standard error. Foliar total %N varied significantly with year ($p<0.001$), species ($p<0.001$) and temperature treatment ($p<0.001$). In 2018, the foliar total %N of *Acer rubrum* was significantly higher in the ambient treatment versus +3℃ treatment ($p<0.001$). For *Quercus alba*, the +3℃ treatment had the lowest foliar N content, and the +5℃ treatment had the highest ($p<0.001$). In 2020, the foliar total %N of *Acer floridanum* was marginally significantly higher in the +3℃ treatment versus the ambient ($p=0.051$). For *Quercus rubra*, the +3℃ treatment had significantly higher foliar %N than the ambient treatment ($p=0.027$) and marginally significantly higher foliar %N than the +5℃ treatment ($p=0.090$).

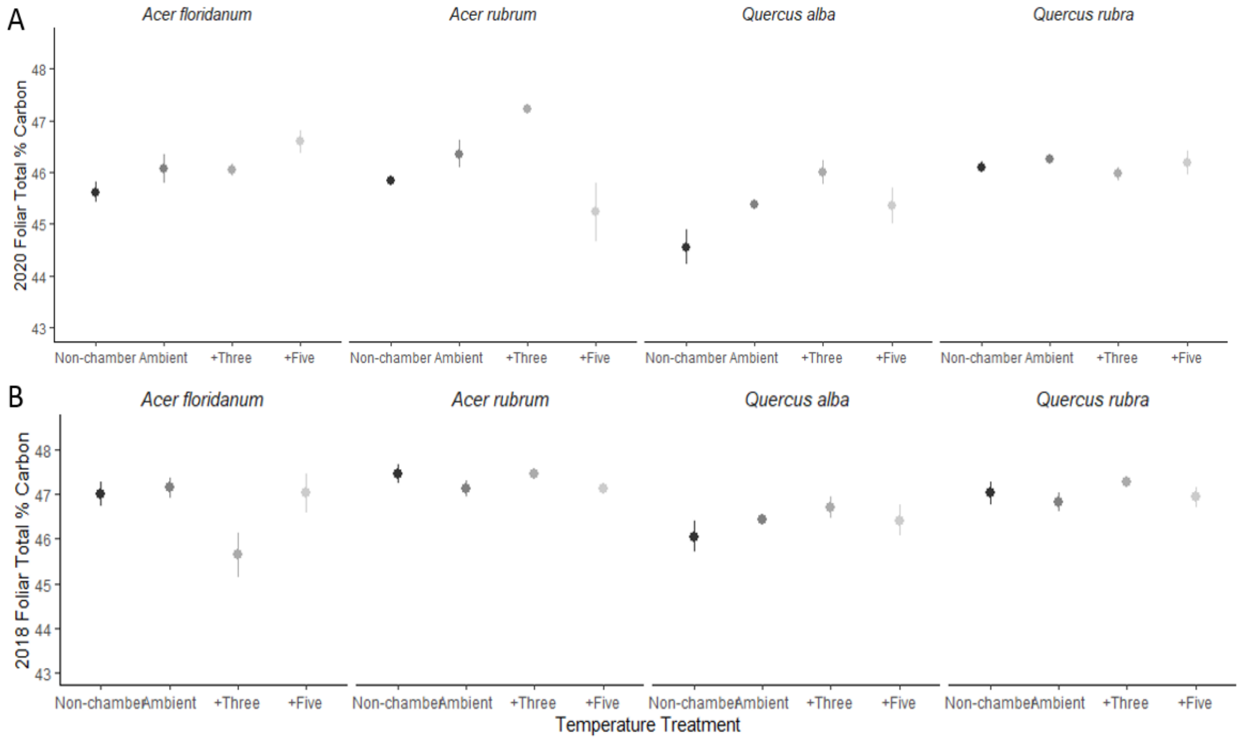

Figure 2.6: Warming effect on foliar % carbon across species. Foliar total % Carbon from 2020 (A) and 2018 (B) by temperature treatment (Non-chamber, ambient, +3℃, +5℃) for each target species (*Acer floridanum*, *A. rubrum*, *Quercus alba*, *Q. rubra*). Foliar total % C represents proportion of carbon of dry mass of a single leaf sample from each individual tree. Three individual trees per species per plot were analyzed. Total % C values were averaged by species per plot (n=3). Points represent mean per temperature treatment by species, and error bars represent ±1 standard error. Foliar total %C varied significantly with year ($p<0.001$) and species ($p<0.0001$). Temperature as a main effect across all species did not significantly correlated with foliar total %C, but there were temperature effects within species ($p=0.003$). In 2018, *Acer floridanum* had significantly lower foliar %C in the +3℃ treatment versus the ambient ($p=0.020$) and +5℃ treatment ($p=0.042$). In 2020, *Acer rubrum* had significantly higher foliar %C in the +3℃ treatment versus the +5℃ treatment ($p=0.003$) and marginally significantly higher foliar %C in the +3℃ treatment than the ambient treatment ($p=0.058$).

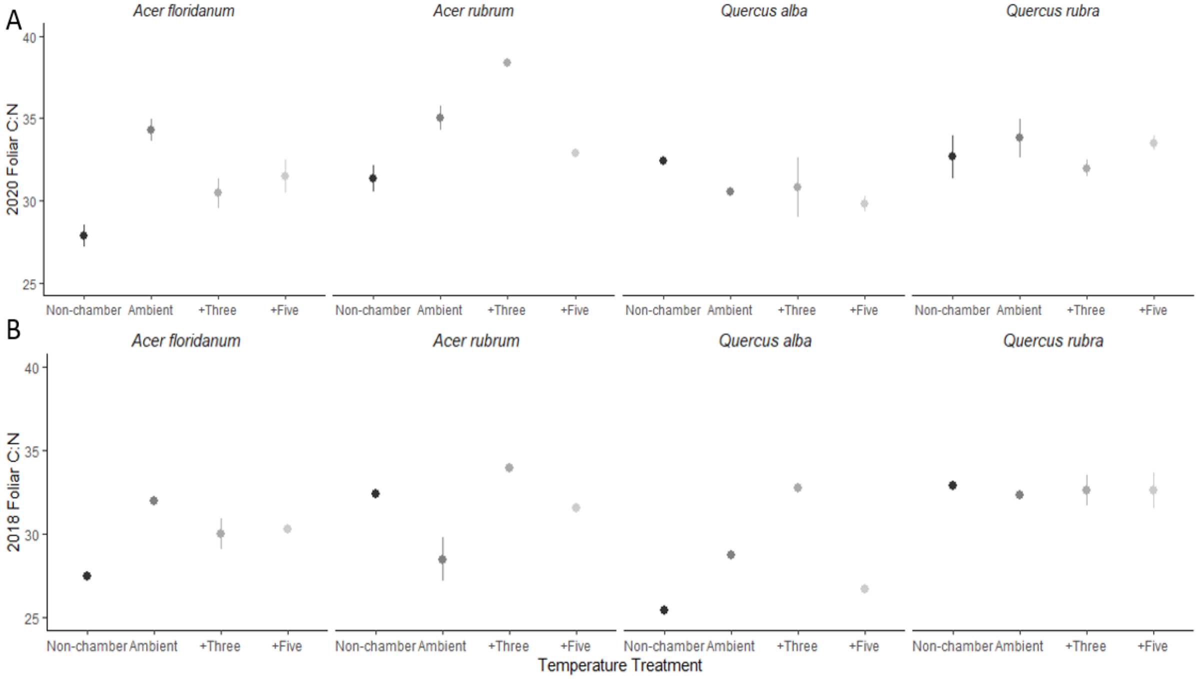

Figure 2.7: Warming effect on foliar C:N ratios across species. Foliar C:N ratio from 2020 (A) and 2018 (B) by temperature treatment (Non-chamber, ambient, +3℃, +5℃) for each target species (*Acer floridanum*, *A. rubrum*, *Quercus alba*, *Q. rubra*). Foliar C:N represents ratio of total % carbon to total % nitrogen of dry mass of a single leaf sample from each individual tree. Three individual trees per species per plot were analyzed. C:N values were averaged by species per plot. Points represent mean per temperature treatment by species, and error bars represent ±1 standard error. Foliar C:N ratio varied significantly with year (p<0.001), species (p<0.001) and temperature treatment (p<0.001). In 2018, *Acer rubrum* had significantly higher C:N ratios in the +3℃ treatment versus the +5℃ treatment (p=0.007). *Quercus alba* had significantly higher C:N ratios in the +3℃ treatment versus the ambient treatment (p=0.017) and the +5℃ treatment (p<0.001). In 2020, *Acer floridanum* had significantly lower C:N ratios in the +3℃ treatment versus the ambient (p=0.041). *Acer rubrum* had significantly higher C:N ratios in the +3℃ treatment versus the ambient (p=0.050) and +5℃ treatments (p=0.005). *Quercus rubra* had significantly lower C:N ratios in the +3℃ treatment versus the ambient (p=0.025) and marginally significantly lower C:N ratios versus the +5℃ treatments (p=0.097).

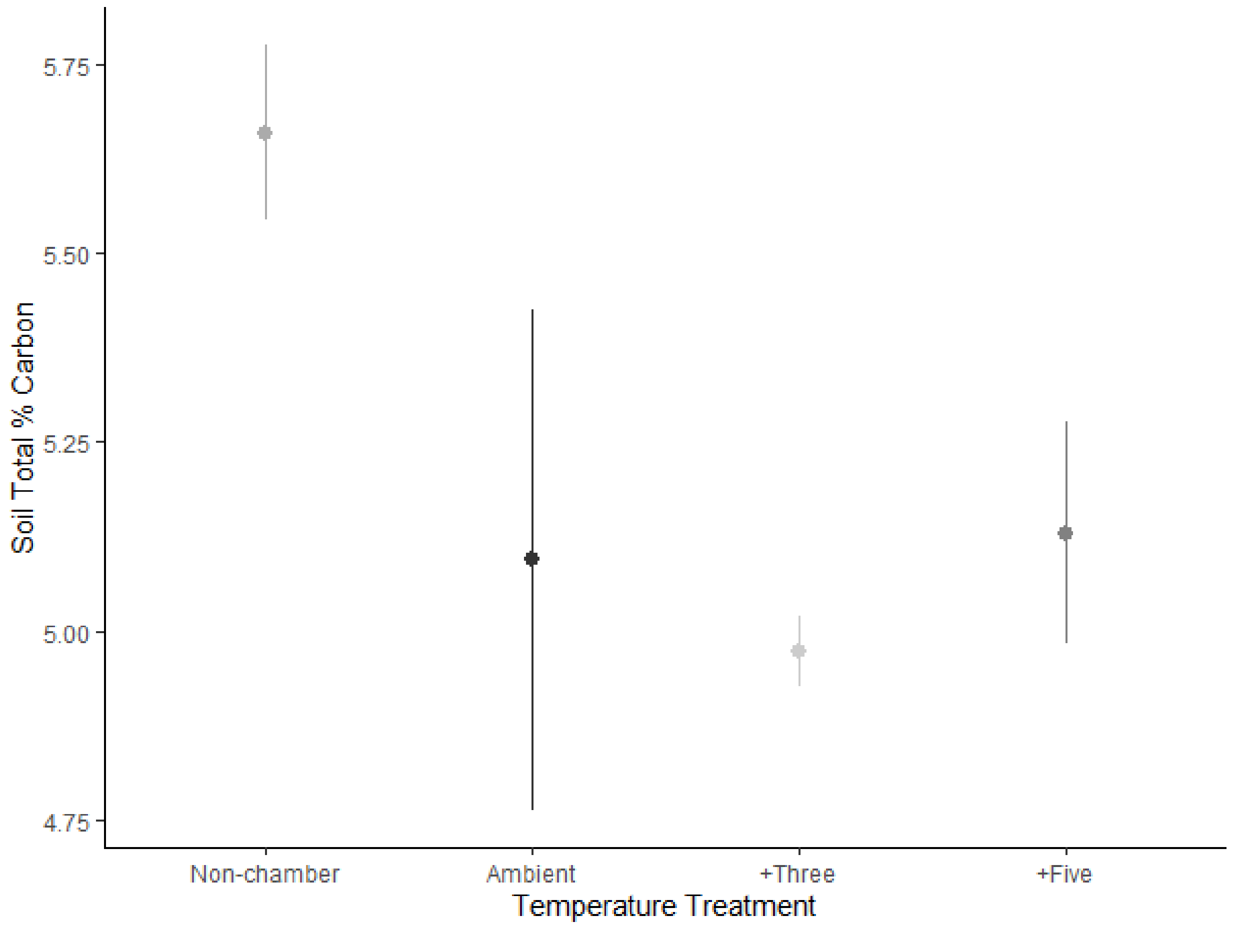

Figure 2.8: Warming effect on soil % carbon in 2020. Soil % C represents the total carbon dry mass proportion of the soil sample. Three 4cm by 4cm soil cores were collected from each plot and analyzed. Total % C values were averaged by plot. Points represent mean per temperature treatment (n=3), and error bars represent ±1 standard error. Soil total % C did not vary with temperature treatment (p=0.844).

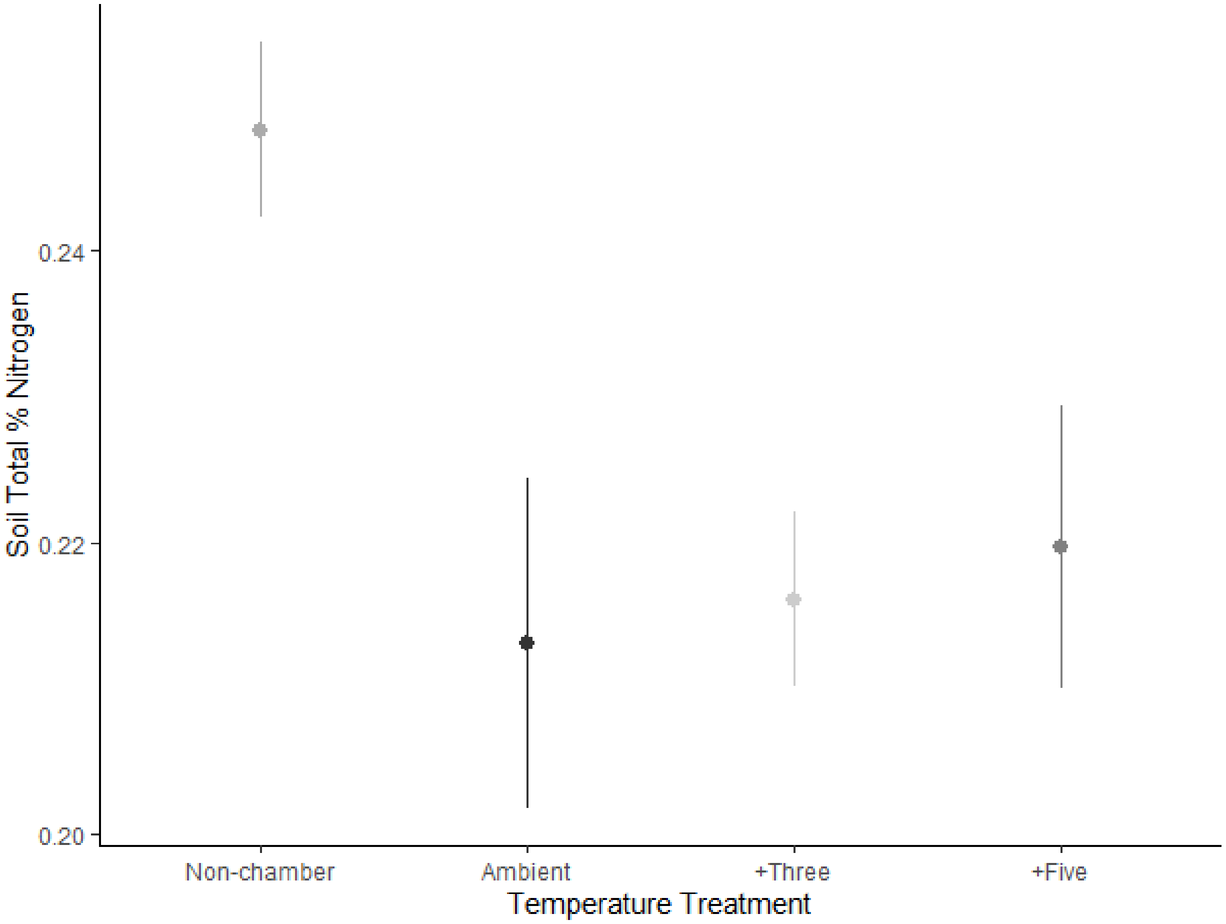

Figure 2.9: Warming effect on soil % nitrogen in 2020. Soil % N represents the total nitrogen dry mass proportion of the soil sample. Three 4cm by 4cm soil cores were collected from each plot and analyzed. Total % N values were averaged by plot. Points represent mean per temperature treatment(n=3), and error bars represent ±1 standard error. Soil total % N did not vary with temperature treatment (p=0.999).

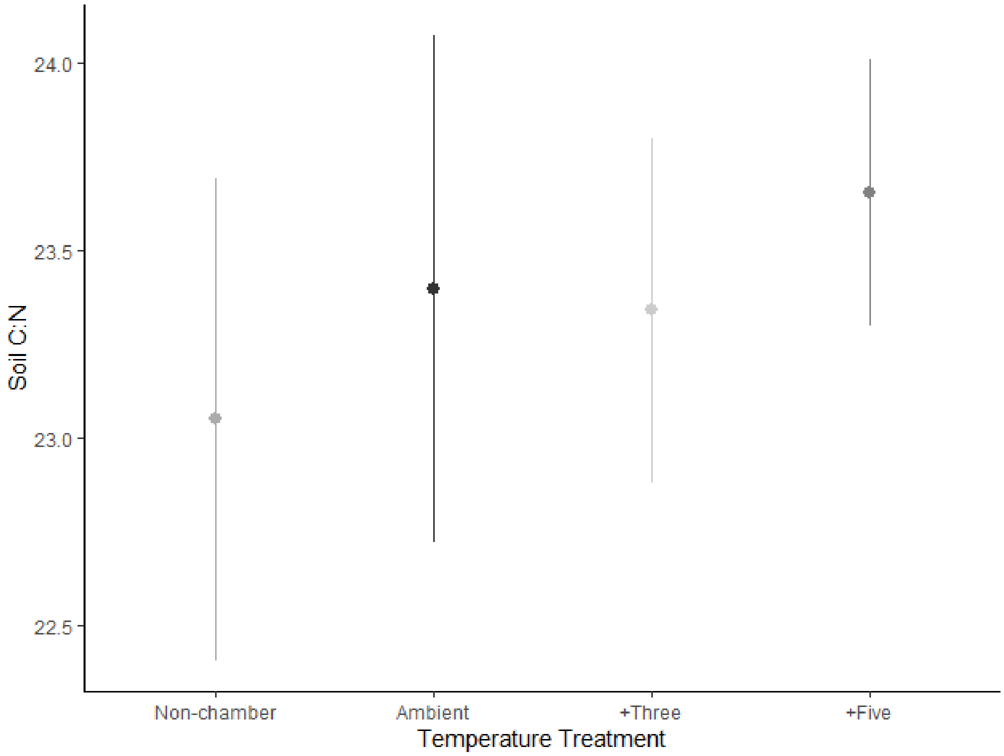

Figure 2.10: Warming effect on soil C:N ratios in 2020. Soil C:N represents the total % carbon to total % nitrogen ratios of each soil sample. Three 4cm by 4cm soil cores were collected from each plot and analyzed. C:N ratios were averaged by plot. Points represent mean per temperature treatment by species, and error bars represent ±1 standard error. Soil C:N ratios did not vary with temperature treatment (p=0.731).

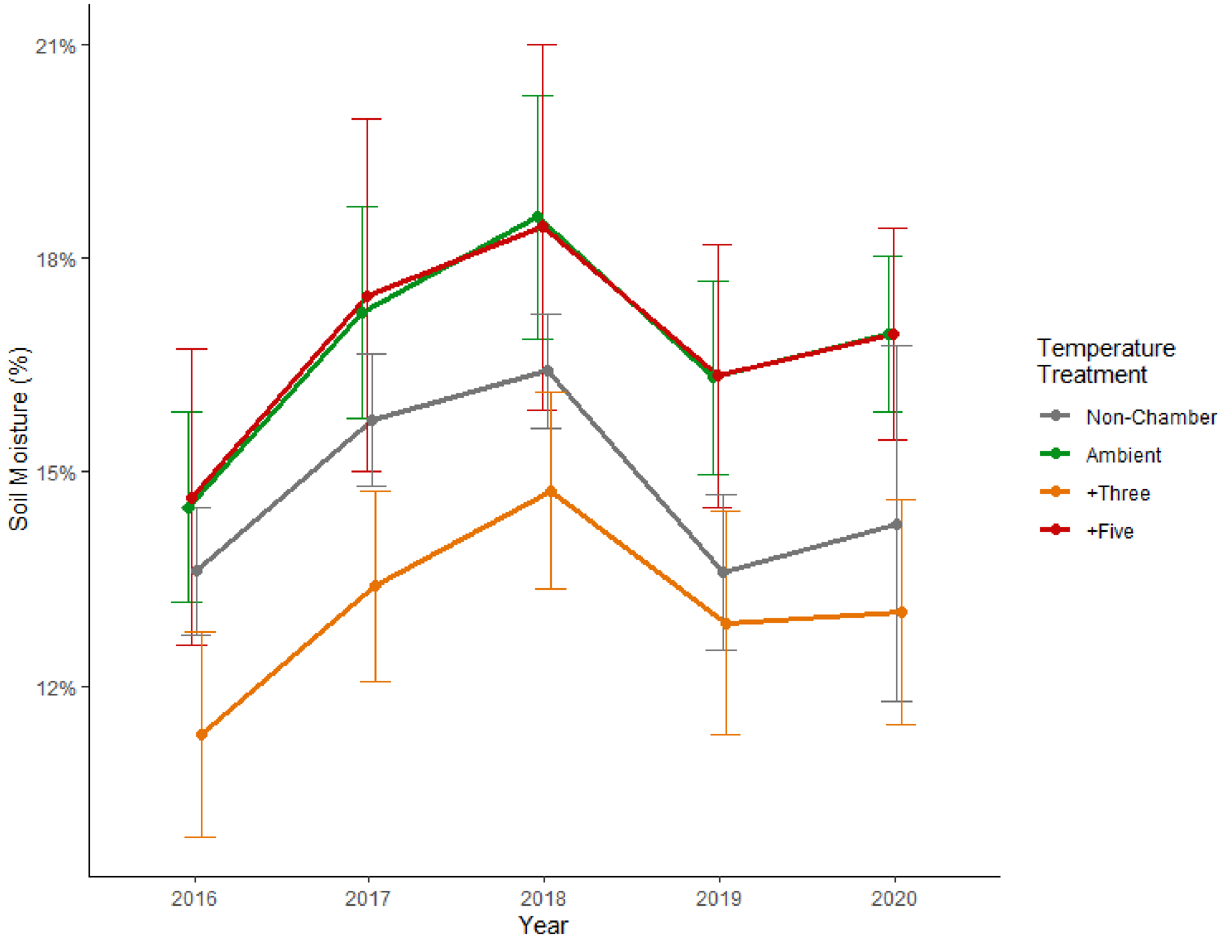

Figure 2.11: Soil moisture (%) by temperature treatment from 2016 to 2020. Growing season (May through September) averages of soil moisture per plot were grouped by temperature treatment. Error bars represent ±1 standard error. The non-chamber and +3°C treatment had significantly lower soil moisture than the ambient and +5°C treatments ($p<0.001$).

Table 2.1: Best-fit linear mixed model for herbivory. Candidate models are the global model, then the five best fit models for explaining percent herbivory. The Akaike information criterion (AIC) indicates the fit of the model; lower AIC values indicate a better fit.

Candidate Models (for %Herbivory)	**AIC**
Global: Temperature Treatment x Species x Soil Moisture x Time x Relative Growth Rate + PAR	5196.005
Species x Soil Moisture x Time x Relative Growth Rate	4933.241
Species x Soil Moisture x Time x Relative Growth Rate + PAR	4946.726
Species x Soil Moisture x Time	5061.060
Species x Soil Moisture x Time + Relative Growth Rate	5067.388
Temperature Treatment x Species x Soil Moisture x Relative Growth Rate	5073.611

CHAPTER 3

CONCLUSIONS

Forests provide numerous ecosystem functions through influencing biodiversity, protecting soil resources, and storing approximately half of terrestrial carbon (Bonan 2008). As atmospheric and soil temperatures increase due to anthropogenic climate change (IPCC 2014), the response of forests to warmer environments is critical to understand. Increased ecosystem temperature directly impacts biogeochemical processes, including carbon sequestration and nitrogen mineralization (Mohan 2019, Melillo et al. 2011, Butler et al. 2012, Garten 1993, Rustad et al. 2001). A meta-analysis of 32 ecosystem warming sites showed that 2-9 years of experimental warming increases soil respiration by 20%, net nitrogen (N) mineralization by 46%, leading to more plant available N, and increased plant productivity by 19% (Rustad et al. 2001). Soil warming induced phytochemical changes in foliar tissue: leaf nitrogen content increased, and foliar C:N ratios decreased in response to warming in a temperate forest warming study (Butler et al. 2012). Through phytochemical changes in foliar tissue, soil warming can impact invertebrate herbivores, which play an important role in plant productivity, nutrient cycling, and trophic dynamics (Carson 1999, Belovsky and Slade 2000, Bardgett and Wardle 2003). However, few studies have examined the impacts of soil warming on plant and invertebrate herbivore interactions particularly in field settings (Chung 2013, Cornelissen 2011). Furthermore, experimental soil warming sites are concentrated in northern latitudes with glaciated, less weathered and more fertile soils, that are expected to differ in response to warming versus highly weathered, less fertile soils of the Southeast U.S. (Rustad et al. 2001, Crowther et al. 2016, Carey et al. 2016). The goal of this study was to determine how soil warming will impact soil processes of highly weathered, sub-tropical soils and the indirect impacts on plant and invertebrate herbivore interactions. This is the only study of which I am aware that examines the plant-insect interaction response to soil warming in lower latitude, infertile soils.

Leaf herbivory did not directly respond to temperature or C and N content, but responded to interactive effects of species, growth rate, and soil moisture. Foliar C and N content varied in response to temperature by species. Soil C and N content did not have a strong response to warming. This is contradictory to my predictions that herbivory would increase with warming, through warming-induced changes in carbon and nitrogen. Herbivory can be strongly impacted by foliar chemistry, including foliar N (Mattson 1980, Dudt 1994, Cornelissen 2011), but there was not a strong relationship between %N and C:N ratios and herbivore damage in this study. Herbivory is also predicted to be impacted indirectly through ecosystem warming from climate change (Chung 2013, Bale et al. 2002), but in this study there were no warming-induced responses to herbivory. There were species-level responses to herbivory, as well as within-species temperature effects. Unexpectedly, herbivory was typically lower in the *Acer* species, particularly *Acer rubrum*, than in the *Quercus* species.

The lack of response to warming is likely due to lower soil N in the Piedmont relative to many previous warming experiments (Schlesinger and Bernhardt 2020, Mohan 2019). Preliminary data suggests warming does not increase N mineralization at WFWF in the Piedmont (Mohan 2012, unpublished data), contradictory to previous studies. This study shows there is no change in foliar or soil C and N content or invertebrate herbivory in response to warming. Therefore, I conclude warming does not have an indirect effect via fertilization on herbivory and phytochemistry. This work highlights the latitudinal variation in warming response of plant and invertebrate interactions. Future research must be done in lower latitudes and varying soil types, as there is a bias towards higher latitude, fertile soils in the literature (Rustad et al. 2001). Furthermore, future work should consider potential impacts of soil warming on associated plant secondary defensive compounds, as well as investigate possible soil nitrogen limitations to soil warming response. The effect of climate change on tree seedlings and invertebrate interactions has potential to impact future forest dynamics and composition, as species respond differently to warmer environments.

REFERENCES

Aber, J. D., Magill, A., Mcnulty, S. G., Boone, R. D., Nadelhoffer, K. J., Downs, M., & Hallett, R. (1995). Forest biogeochemistry and primary production altered by nitrogen saturation. *Water, Air, and Soil Pollution, 85*(3), 1665-1670.

Abrams, M. D. (1998). The Red Maple Paradox. *BioScience, 48*(5), 355–364.

Bale, J. S., Masters, G. J., Hodkinson, I. D., Awmack, C., Bezemer, T. M., *et al.* (2002). Herbivory in global climate change research: direct effects of rising temperature on insect herbivores. *Global Change Biology*, *8*(1), 1–16.

Bardgett, R. D., & Wardle, D. A. (2003). Herbivore-mediated linkages between aboveground and belowground communities. *Ecology*, *84*(9), 2258-2268.

Bates, D., Maechler, M., Bolker, B., Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.

Bazzaz, F. A., & Bazzaz, F. (1996). *Plants in changing environments: linking physiological, population, and community ecology*. Cambridge University Press.

Bauerfeind, S. S., & Fischer, K. (2013). Increased temperature reduces herbivore host-plant quality. *Global Change Biology*, *19*(11), 3272–3282.

Belovsky, G. E., & Slade, J. B. (2000). Insect herbivory accelerates nutrient cycling and increases plant production. *Proceedings of the National Academy of Sciences*, *97*(26), 14412-14417.

Bonan, G. B. (2008). Forests and Climate Change: forcings, feedbacks, and the climate benefits of forests, *Science*, *320*(5882): 1444–1450.

Butler, S. M., Melillo, J. M., Johnson, J. E., Mohan, J., Steudler, P. A., Lux, H., Burrows, E., Smith, R.M., Vario, C.L., Scott, L., Hill, T.D., Aponte, N., & Bowles, F. (2012). Soil warming alters nitrogen cycling in a New England forest: Implications for ecosystem function and structure. *Oecologia*, *168*(3), 819–828.

Carey, J. C., Tang, J., Templer, P. H., Kroeger, K. D., Crowther, T. W., *et al.* (2016). Temperature response of soil respiration largely unaltered with experimental warming. *Proceedings of the National Academy of Sciences, 113*(48), 13797-13802.

Carson, W. P., & Root, R. B. (1999). Top-down effects of insect herbivores during early succession: influence on biomass and plant dominance. *Oecologia*, *121*(2), 260-272.

Cha, D. H., Appel, H. M., Frost, C. J., Schultz, J. C., & Steiner, K. C. (2010). Forest Ecology and Management Red oak responses to nitrogen addition depend on herbivory type, tree family, and site. *Forest Ecology and Management*, *259*(10), 1930–1937.

Chung, H., Muraoka, H., Nakamura, M., Han, S., Muller, O., & Son, Y. (2013). Experimental warming studies on tree species and forest ecosystems: A literature review. *Journal of Plant Research*, *126*(4), 447–460.

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, *230*(4728), 895-899.

Cornelissen, T. (2011). Climate change and its effects on terrestrial insects and herbivory patterns. *Neotropical Entomology*, *40*(2), 155–163.

Crowther, T., Todd-Brown, K., Rowe, C. *et al.* (2016) Quantifying global soil carbon losses in response to warming. *Nature* 540**,** 104–108.

Dudt, J. F., & Shure, D. J. (1994). The influence of light and nutrients on foliar phenolics and insect herbivory. *Ecology*, *75*(1), 86–98.

Dury, S. J., Good, J. E. G., Perrins, C. M., Buse, A., & Kaye, T. (1998). The effects of increasing CO2 and temperature on oak leaf palatability and the implications for herbivorous insects. *Global Change Biology*, *4*(1), 55–61.

Edwards, Leslie, Jonathon Ambrose, and L. Katherine Kirkman. (2013). The Natural Communities of Georgia. University of Georgia Press Athens, GA. 675 pp.

Endara, M. J., & Coley, P. D. (2011). The resource availability hypothesis revisited: A meta-analysis. *Functional Ecology*, *25*(2), 389–398.

Huberty, A. F., & Denno, R. F. (2004). Plant water stress and its consequences for herbivorous insects: a new synthesis. *Ecology*, *85*(5), 1383-1398.

Garten, C. T. (1993). Variation in foliar ^{15}N abundance and the availability of soil nitrogen on walker branch watershed. *Ecology*, *74*(7), 2098–2113.

Getman-Pickering, Z. L., Campbell, A., Aflitto, N., Grele, A., Davis, J. K., & Ugine, T. A. (2020). LeafByte: A mobile application that measures leaf area and herbivory quickly and accurately. *Methods in Ecology and Evolutio*n, 2041–210X.13340.

Godfrey, M.A. 1997. A Field Guide to the Piedmont: The Natural Habitats of America's Most Lived-In Region, from New York to Montgomery, Alabama. The University of North Carolina Press. Chapel Hill, NC. 524 pp.

IPCC, 2014: *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland, 151 pp.

Kudo, G. (2003). Variations in leaf traits and susceptibility to insect herbivory within a Salix miyabeana population under field conditions. *Plant Ecology*, *169*(1), 61–69.

Lehman, F, Mohan, J.E., Melillo, J.M., Clark, J. & Salk, C. (2011). Climate warming effect on Acer rubrum seedling susceptibility to foliar herbivory.

Little, E. L. (1979). *Checklist of United States trees (native and naturalized)* (No. 541). DC: Forest Service, US Department of Agriculture.

Lu, M., Zhou, X., Yang, Q., Li, H., Luo, Y., Fang, C., *et al.* (2013). Responses of ecosystem carbon cycle to experimental warming: A meta-analysis. *Ecology*, *94*(3), 726–738.

Machmuller, M. B., Ballantyne, F., Markewitz, D., Thompson, A., Wurzburger, N., Frankson, P. T., & Mohan, J. E. (2018). Temperature sensitivity of soil respiration in a low-latitude forest ecosystem varies by season and habitat but is unaffected by experimental warming. *Biogeochemistry*, *141*(1), 63–73.

Mattson, W. J. (1980). Herbivory in Relation to Plant Nitrogen Content. *Annual Review of Ecology and Systematics*, *11*(1), 119–161.

Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., Bowles, F. P., *et al.* (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, *298*(5601), 2173-2176.

Melillo, J. M., Butler, S., Johnson, J., Mohan, J., Steudler, P., Lux, H., *et al.* (2011). Soil warming, carbon–nitrogen interactions, and forest carbon budgets. *Proceedings of the National Academy of Sciences*, *108*(23), 9508-9512.

Mohan, J. E., Clark, J. S., & Schlesinger, W. H. (2004). Genetic variation in germination, growth, and survivorship of red maple in response to subambient through elevated atmospheric CO2. *Global Change Biology*, *10*(2), 233-247.

Mohan, J. E., Clark, J. S., & Schlesinger, W. H. (2007). Long-term CO2 enrichment of a forest ecosystem: implications for forest regeneration and succession. *Ecological Applications*, *17*(4), 1198-1212.

Mohan, J. E. (Ed.). (2019). *Ecosystem Consequences of Soil Warming: Microbes, Vegetation, Fauna and Soil Biogeochemistry*. Academic Press.

NOAA (2013) National oceanic and atmospheric administration. National climate data center, Athens, Georgia, Ben Epps Airport Climate Station

Nottingham, A. T., Meir, P., Velasquez, E., & Turner, B. L. (2020). Soil carbon loss by experimental warming in a tropical forest. *Nature*, *584*(7820), 234-237.

Peay, K.G., Kennedy, P.G., Talbot, J.M., 2016. Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology 14*, 434.

Pérez-Sánchez, R. M., Jurado, E., Chapa-Vargas, L., & Flores, J. (2011). Seed germination of Southern Chihuahuan Desert plants in response to elevated temperatures. *Journal of Arid Environments*, *75*(10), 978–980.

Peterjohn, W. T., Melillo, J. M., Steudler, P. A., Newkirk, K. M., Bowles, F. P., & Aber, J. D. (1994). Responses of trace gas fluxes and N availability to experimentally elevated soil temperatures. *Ecological applications*, *4*(3), 617-625.

Piatek, K. B., & Allen, H. L. (1999). Nitrogen mineralization in a pine plantation fifteen years after harvesting and site preparation. *Soil Science Society of America Journal*, *63*(4), 990-998.

Richardson, S. J., Press, M. C., Parsons, A. N., & Hartley, S. E. (2002). How do nutrients and warming impact on plant communities and their insect herbivores? A 9-year study from a sub-Arctic heath. *Journal of Ecology*, *90*(3), 544–556.

Richter, D. D., Markewitz, D., Heine, P. R., Jin, V., Raikes, J., Tian, K., & Wells, C. G. (2000). Legacies of agriculture and forest regrowth in the nitrogen of old-field soils. *Forest Ecology and Management*, *138*(1-3), 233-248.

Rustad, L. E. J. L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A, et al. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, *126*(4), 543-562.

Schlesinger, W. H. and Bernhardt, E., (4th Ed.). (2020). *Biogeochemistry*. Elsevier.

Veteli, T. O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, H., & Tahvanainen, J. (2002). Effects of elevated CO2 and temperature on plant growth and herbivore defensive chemistry. *Global Change Biology*, *8*(12), 1240–1252.

Warton, D., & Hui, F. (2011). The arcsine is asinine: the analysis of proportions in ecology. *Ecology*, *92*(1), 3–10.

Weakley, A. S. (2020). *Flora of the Southeastern United States*. University of North Carolina Herbarium, North Carolina Botanical Garden, University of North Carolina, Chapel Hill,1848 pp.

Williams, R. S., Lincoln, D. E., & Norby, R. J. (2003). Development of gypsy moth larvae feeding on red maple saplings at elevated CO2 and temperature. *Oecologia*, *137*(1), 114–122.

Zvereva, E. L., & Kozlov, M. V. (2006). Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: A metaanalysis. *Global Change Biology*, *12*(1), 27–41.