**Understanding the effects of climate change on forest resilience and carbon storage in southern Appalachia**

Climate change is impacting plant and animal communities, which can ultimately reshape ecosystem services and forest management practices those communities support. Many plant and animal species are under threat from warming and must rapidly adapt through phenological shifts and/or range shifts northward to avoid harsher southern climatic conditions [Parmesan & Yohe, 2003; Schwartz et al., 2006]. There is increasing evidence that climate change is exasperated at higher elevations [Giorgi et al., 1997; Rangwala & Miller, 2012; Pepin et al., 2015] and species’ ranges could be restricted in these areas, potentially leading to regional extinction [Bachelet et al., 2001; Potter et al., 2008]. Thus, through the effects of stress and disturbance from warming, tree species’ migration will be adversely affected, leading to profound impacts on forests and carbon sinks [Opdam & Wascher, 2004].

A tree in a forest

Description automatically generatedNatural forests are some of the most biodiverse habitats in the United States [White & Miller, 1988] and with climate change, the southeastern forests of Appalachia (Figure 1) are predicted to be under threat from increased wildfires and rapid conversion to savanna [Bachelet et al., 2001]. Due to exploitative logging, clearcutting, grazing and wildfires at mid-elevations, these forests have become less complex over time, converted from historically mixed-oak stands to more homogenized stands of yellow poplar or red maple and American beech [Runkle, 1982; Lorimer, 1989; Rentch et al., 2003a, 2003b]. Climate change coupled with rapid land-use change is resulting in the creation of gaps of varying size within forest canopies [Canham et al., 1999]. The effects of these gaps on forest recruitment and resilience---or the ability of a forest to recover after a disturbance---are not fully understood.

Figure 1: Picture from research site in southern Appalachian forest in Asheville, NC.

Climate change is impacting forests in myriad ways—some of which are positive (i.e., increased CO2 fertilization and longer growing seasons)—but many are detrimental such as rising temperatures and decreasing precipitation leading to increased tree mortality from stress and drought [Ayres & Lombardero, 2000; Bachelet et al., 2001; Lloyd & Bunn, 2007; Allen et al., 2010]. Repeated incidence of drought generally leads to increased vulnerability and subsequent decreases in forest resilience [Allen et al., 2010; Anderegg et al., 2020]. Understanding initial drought tolerance is therefore essential to predict future shifts in forest community dynamics. Some species will be more at risk of pests and pathogens following a drought and other habitats will have larger microclimatic variation, leading to a mosaic of drought risk within a forest [Ayres & Lombardero, 2000; Anderegg et al., 2020]. By assessing both inter- and intra-specific variation in drought tolerance, pest damage and microclimatic impact, we can better predict the effects of climate change on temperate forests.

The combined effects of increasing temperatures and decreasing precipitation is impacting tree species differently, with extensive effects on drought-intolerant species leading to northward and westward range shifts [Fei et al., 2017]. Additionally, there is growing evidence that southern Appalachian forests are transitioning to shade-tolerant, fire-resistant species such as red maple and American beech [Fei et al., 2017; Knott et al., 2019] and there is a reduction in foundation species’ regeneration [Izbicki et al., 2020].

Oak species (i.e., Quercus genus) are considered foundation species in many temperate systems [Ellison et al., 2005; Mitchell et al., 2019] and are generally fire-resistant, though they are also shade-intolerant, thus forest management teams are working to regenerate oaks by establishing gaps in canopies in combination with prescribed fires. Recent studies suggest gaps must be large enough for oaks to regenerate successfully and demonstrate significant increases in photosynthetic rates and growing season lengths [Zhang & Yi, 2020]. Oaks greatly influence forest hydrology, nutrient cycling [Arthur et al., 2012] and contribute to increases in biodiversity [Mitchell et al., 2019; Izbicki et al., 2020]. It is therefore essential to understand the effects of climate change on southern Appalachian forest habitats—with a strong focus on oak species—and the cascading impacts to our crucial carbon sinks.

Disturbance to canopy trees and the creation of gaps in forests can lead to a multitude of effects including increased competition through light availability as well as changes to soil temperature, moisture and microbial community structure. Canopy disturbance often leads to increases in soil nitrogen availability, which can allow for understory species to out-compete regenerating seedlings and saplings like oaks [Mladenoff, 1987; Taylor et al., 2017]. Canopy gaps—especially more northern gaps, where sun angles are lower—with higher soil temperatures have significantly higher total growing season carbon flux than those with lower temperatures and less light availability [Raymond et al., 2006; Schatz et al., 2012]. Thus, identifying microclimatic soil variation among gap and closed-canopy sites is essential for accurate carbon flux forecasting and, by maintaining mixed-forest growth, there is a reduction in risk from the adverse effects of global climate change.

**RESEARCH OBJECTIVES:**

The overall aim of the proposed research is to understand how gap size impacts the reestablishment and maintenance of mixed-forest and regenerative growth. To do this I will investigate gap sites of varying sizes and compare these to closed-canopy sites in the southern Appalachian Mountains to assess (1) forest recruitment of the dominant species and report diversity and richness of shade-tolerant vs shade-intolerant tree and shrub species over time, (2) drought tolerance of the dominant tree species across the gap and closed-canopy sites using a greenhouse and phytotron cutting experiment and (3) soil microbial community structure, variability in soil nutrients, soil temperature, soil moisture and incident PAR of the gap sites versus the closed-canopy sites to understand and predict the impacts of climate change on temperate forest resilience.

**Hypothesis 1:** *The effects of gap size and location will impact species composition, recruitment and phenology.* Using various gap types in comparison to closed-canopy forested sites in the southern Appalachian Mountains, I will examine 10 different woody plant tree and shrub species—with overlapping phylogenies (Figure 2)—with 24 individuals per species: *Acer rubrum*, *Acer saccharum*, *Betula nigra, Corylus cornuta, Carpinus caroliniana, Fagus grandifolia, Hammamelis virginiana, Quercus alba, Quercus montana* and *Quercus rubra*. For each individual, I will measure a radius of 5m around each tree and record all species present within that circle. With this experiment I propose to: evaluate percent herbivory of the focal individual and monitor herbivory over the growing season; quantify and classify the number of seedlings and saplings of each dominant tree species within the site to evaluate recruitment; measure the diameter at breast height (DBH) for all trees and shrubs within the site to understand tree age and growth; monitor early season phenology (i.e., budburst and leafout) of the focal individual and also late season phenology (i.e., leaf drop and budset); and record carbon sequestration measurements.

Figure 2: Phylogenetic tree of the focal tree species.

**Expected Outcomes and Significance:** This experiment will greatly improve forecasts for mixed-forest, mid-elevation sites under climate change by developing equations and metrics of inter- and intraspecific variation in fitness to be used in climate models. I expect sites at the northern edge of large gap sites (i.e., gaps with diameter as larger or larger than the height of the surrounding canopy trees [Raymond et al., 2006]) will have longer growing seasons, warmer soil temperatures and greater carbon flux than closed-canopy sites. I also anticipate that mixed-forest, heterogeneous sites will have larger levels of recruitment and soil nutrients than more homogenized sites. Understanding the effects of warming—and the subsequent risk of disturbances—on temperate forests is essential for informing climate forecast models and determining forest resilience.

A picture containing indoor, room, small, flower

Description automatically generated**Hypothesis 2:** *Drought tolerance of the dominant tree species will vary across the gap and closed-canopy sites.* Using a phytotron and greenhouse experiment, I will take cuttings from the focal tree individuals in Experiment 1 to test drought tolerance coupled with warming nighttime temperatures. Nighttime temperatures are increasing at a faster rate than daytime temperatures with warming [Fu et al., 2016] and impact individual phenologies differently than daytime temperatures. There is also evidence for increased stress on plants from nighttime temperatures [Grinevich et al., 2019]. We will additionally expose control and treatment individuals to varying increases in nighttime temperatures in the early season and during drought conditions. In the fall of 2021—after budset and before complete leaf drop, I will take 10-16 cuttings of approximately 30cm for each individual. Upon delivery to the lab, I will place the cuttings in dormancy conditions of 4°C for 10 weeks (Figure 3), rotating individuals every two weeks to minimize bias from possible phytotron effects. After 10 weeks, I will place the individuals in phytotron conditions and expose to 8 hour photoperiod and 15°C daytime temperatures and 10°C, 12°C and 15°C nighttime temperatures until leafout. Once full leafout is achieved, I will expose individuals to three levels of drought conditions: (1) control group, (2) little to no precipitation, (3) medium levels of precipitation, coupled with a full factorial experiment of increased nighttime temperatures (+0°C, +2°C and +5°C) for each drought group for a total of 9 treatments. Phenology, mortality, soil moisture, soil temperature and nutrient levels will be evaluated. After 8 weeks of drought conditions and increased nighttime temperatures, we will water half of the treatment groups to evaluate recovery by observing end of season growth through phenological measurements, canopy development, mortality and shoot apical meristem damage [Burgess, 2006; Blackman et al., 2009; Brodribb & Cochard, 2009].

Figure 3: Example of phytotron experiment

**Expected Outcomes and Significance:** By evaluating initial drought tolerance across the 10 dominant species of southern Appalachia coupled with nighttime warming, I will be able to better predict the effects of increasing temperatures and decreasing precipitation from climate change on mixed-forest growth. I expect higher interspecific variability in drought tolerance and also low levels of intraspecific variation across the gap size and locations, with individuals from larger gaps having higher levels of drought tolerance than closed-canopy individuals due to increased heat tolerance. In addition, I expect individuals exposed to higher nighttime temperatures will demonstrate increased mortality and decreased recovery from drought. These findings are critical for forecasts as stress and disturbance are predicted to increase with warming.

**Hypothesis 3:** *The variability in soil temperature, soil moisture and soil nutrients will increase with increased gap size.* Understanding soil microbial community structure is a strong predictor for site response to environmental change. I will record hourly soil temperature at each site using Hobo Loggers buried 5cm below the soil surface and evaluate light availability using hemispherical canopy photos and then analyzing photos using Gap Analyzer software [Burton et al, 2014, Canham, 1990, Forrester et al., 2014]. Volumetric soil moisture will be measured monthly using a portable soil moisture probe and throughfall will be recorded for each field season. I will collect soil cores from 0-10cm and 10-20cm for each field season and compare to soil cores collected at the same or similar sites from 2017 to compare soil microbial functional groups and nutrient content. I will then submit the soil cores to NCSU Soil Lab for standard nutrient analysis and to Microbial ID lab (Newark, DE) for PLFA analysis. Using structural equation modeling, I will evaluate the relationship of vertical and horizontal structure and soil microbial community structure.

**Expected Outcomes and Significance:** Through the interactive effects of climate change and rapid land-use change, gap size and location will influence soil microclimatic conditions as well as nutrient availability. I expect light availability and soil temperatures to be greatest in the northern portion of the gap, while maximum soil moisture will occur in the southern portion of the gap [Schatz et al., 2012; Raymond et al., 2006]. By examining belowground responses to canopy gaps through soil moisture, temperature and nutrient composition, I will be able to greatly improve predictive climate models for the region and likely contribute to global modelling systems.

**JUSTIFICATION OF SPONSORING SCIENTIST AND HOST INSTITUTION:**

The proposed project and broader impact programs will be carried out in the lab of Dr. Zakiya Leggett in the College of Natural Resources at North Carolina State University. This project will allow me to transition to career independence and help broaden my skills from phenology observations and modelling [Chamberlain et al., 2020; Chamberlain et al., 2019; Ettinger et al., 2020; Furze et al., 2020] to studying ecosystem-scale processes---with a focus on both above and belowground systems---and forest resilience. In addition to developing my scientific skills, I will also leverage my leadership, professional development and diversity and inclusion initiatives. By joining the Leggett Lab, I will be able to further develop these skills while maintaining an interface with the nonprofit sector but I will also gain new research skills in soil carbon and nutrient cycling measurements. I have chosen to work with Dr. Leggett because my career goals are to work with BIPOC communities and, in the Leggett lab, I will have the opportunity to learn new, valuable teaching and mentoring skills---with a focus on BIPOC training---as well as expand my research and scientific toolkit to be more field based and incorporate more intensive field measurement skills.

**BROADER IMPACTS**

**Teaching:** I will further sharpen my skills in meeting facilitation for ecology and natural resources by co­-mentoring graduate students in the College of Education focused on a project entitled Natural Resources Diversity Curriculum Integration. The main project goal is to review entry level courses at North Carolina State University and develop modules to incorporate diversity and inclusion into these courses since they impact a large body of students early in their college experience. I will also co-­facilitate working groups with an NSF funded RCN - The Undergraduate Network for Increasing Diversity of Ecologists (UNIDE). The project aims to build a sustainable and interdisciplinary network of ecologists, educators and social scientists to address how cultural and social barriers impact human diversity in ecology and environmental disciplines (EE). These two opportunities will help develop skills in the area of cross-sector engagement and meeting facilitation.

**Mentoring:** I propose to establish a mentorship pipeline for BIPOC individuals within the National Needs Fellowship (NNF) Program and the Doris Duke Conservation Scholars (DDCS) Program—where Dr.Leggett is lead PI and campus director. Both the NNF and DDCS programs have goals of recruiting and increasing diverse students in the field of ecology. Under the pipeline program I plan to develop, I will train graduate students, who will train undergraduate students who will in turn train high school students, with the goal of increasing diversity and interest in ecology at all levels. By providing this training. Dr. Leggett is developing/building upon an existing training developed for the Doris Duke Conservation Scholars Program for mentors that have never advised BIPOC students for her NNF program, which will be used for the pipeline training program.

**Career Development and Community-level Outreach:** In addition to my work with increasing diversity and inclusion at all levels, I intend to attend webinars and workshops offered by North Carolina State University designed for postdocs to hone project development and grant writing skills. I will also assist with grant writing to develop a collaborative project with The Nature Conservancy, which is working towards a project to evaluate forest carbon sequestration and increasing diversity and inclusion in program participants. I will also help Dr. Leggett develop research and education grants including expanding the National Needs Fellowship Program to include more students and developing a funding source for a dual degree program she has developed with Tuskegee University and North Carolina State University. Finally, I will work alongside Dr. Leggett, Dr. Asia Dowtin and Dr. Caren Cooper in the development and implementation of a large scale citizen science project which will involve students and communities across the nation that focuses on urban forestry/tree cover and BIPOC communities. Research has shown that BIPOC students are drawn to scientific pursuits that help local communities but deterred from disciplines that promote science as an isolating field [Puritty et al., 2017]. Therefore this citizen science project will provide an opportunity for these students and the participating public to nurture their interest in science while bringing awareness to their communities.

**PROPOSED RESEARCH TIMELINE:**

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| **Year** | **Semester** | **Research** | **Broader Impacts** |
| 2021 | Summer | Identify focal individuals and plots  Deploy hobo loggers  Record observations for Exp 1 & 3 | Develop IDP  Recruit students for pipeline program  Assist with citizen science study  Co-facilitate UNIDE working group |
| 2021 | Fall | Record phenology; take cuttings for Exp 2 | Begin mentoring and training students |
| 2021 | Spring | Set up Exp 2  Begin drought treatments for Exp 2 | Maintain mentorship program |
| 2022 | Summer | Record phenology for Exp 1  Record observations for Exp 1 & 3 | Train students in field skills  Supervise/advise undergraduates |
| 2022 | Fall | Record phenology & Begin analyses |  |
| 2023 | Spring | Prepare manuscripts for submission | Maintain pipeline program |