Background:
Climate change is impacting plant and animal communities, ultimately reshaping the species, 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 at higher elevations species' ranges could be restricted, 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).

Natural forests are some of the most biodiverse habitats in the United States (White & Miller, 1988) and with climate change, the southeastern forests of Appalachia 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 (Lorimer, 1989; Rentch *et al.*, 2003a,b; Runkle, 1982). 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 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-resistent species such 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).

Though oak species (i.e., *Quercus* genus) are generally fire-resistent, they are also shade-intolerant, thus forest management teams are working to regenerate oaks by establishing gaps in canopies in combination with prescirbed 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 are considered foundation species (Ellison *et al.*, 2005; Mitchell *et al.*, 2019) and greatly influence forest hydrology (Arthur *et al.*, 2012), 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 cricial carbon sinks.

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 increased stress from rising temperatures and decreasing precipitation leading to increased tree mortality from 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 mosiac 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.

Disturbance to canopy trees and the creation of gaps in forests can lead to myriad effects including to 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 outcompete regenerating seedlings and saplings like oaks (Taylor *et al.*, 2017; Mladenoff, 1987). Canopy gaps—especially more northern gaps—with higher soil temperatures have significantly higher total growing season carbon flux then those with lower temperatures and less light availablity (Schatz *et al.*, 2012; Raymond *et al.*, 2006). Thus, identifying microclimatic soil variation in 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.

Reseach Objectives The overall aim of our proposed research is to investigate gap sites in varying size classes 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 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 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 we will examine 10 different woody plant tree and shrub species—with overlapping phylogenies—with 8 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, we will measure a radius of 5m around each tree and record all species present within that circle. With this experiment we 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; 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.

Expected Outcomes and Significance: This experiment will greatly increase forecasts for mixed-forest, mid-elevation sites under climate change. We 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. We also anticipate that mixed-forest, heterogenous 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.

Hypothesis 2: Drought tolerance of the dominant tree species will vary across the gap and closed-canopy sites. Using a phytotron and greenhouse experiment, we will take cuttings from the focal tree individuals in Experiment 1 to test drought tolerance with warming. In the fall of 2021—after budset and before complete leaf drop, we will take 10-16 cuttings of $\tilde{3}$0cm for each individual. Upon delivery to the lab, we will place the cuttings in dormancy conditions of 4°C for 8 weeks, rotating individuals every two weeks to minimize bias from possible phytotron effects. After 8 weeks, we will place the individuals in greenhouse conditions and expose to ambient light and temperature to induce budburst. Once full leafout is reached, we will expose individuals to three levels of drought conditions: (1) control group, (2) little to no precipitation, (3) medium levels of precipitation. Phenology, mortality, soil moisture, soil temperature and nutrient levels will be evaluated. After 8 weeks of drought conditions, we will water half of the treatment groups to evaluate recovery.

Expected Outcomes and Significance: By evaluating inital drought tolerance across the 10 dominant species of the southern Appalacians, we will be able to better predict the effects of climate change on mixed-forest growth. We expect higher interspecific variability in drought tolerance and also low levels of intraspecific variation across the gap size and locations, with individuals from larger, more northern sites having higher levels of drought tolerance than closed-canopy individuals. 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 at the soil surface will increase with increasing sized gaps. Understanding soil microbial community structure is a strong predictor for site response to environmental change. We will record hourly soil temperature at each site using Hobo Loggers buried 7cm below the soil surface. Volumetric soil moisture will be measured monthly using a portable soil moisture probe and throughfall will be recorded for each field season. We 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. We 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, we 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. We 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, we will be able to greatly improve predictive climate models for the region and likely contribute to global modelling systems.

Proposed Research Timeline:

Year	Season	Research	Broader Impacts
2021	Summer	Identify focal individuals and plots	Advertise course
2021	Summer	Record observations for **Exp 1 & 3**	Collect resources
2021	Fall	Record phenology; take cuttings for **Exp 2**	Make course available
2021	Winter	Set up **Exp 2**	Develop website
2022	Winter	Begin drought treatments for **Exp 2**	Begin teaching
2022	Spring	Record phenology for **Exp 1**	Begin webinar series
2022	Summer	Record observations for **Exp 1 & 3**	Continue webinar series
2022	Fall	Record phenology; Begin analyses	Update course materials
2023	Winter	Prepare manuscripts for submission	Offer the course again

References

Allen, C.D., Macalady, A.K., Chenchouni, H., Bachelet, D., McDowell, N., Vennetier, M., Kitzberger, T., Rigling, A., Breshears, D.D., Hogg, E.T., Gonzalez, P., Fensham, R., Zhang, Z., Castro, J., Demidova, N., Lim, J.H., Allard, G., Running, S.W., Semerci, A. & Cobb, N. (2010) A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *Forest Ecology and Management* **259**, 660 – 684, adaptation of Forests and Forest Management to Changing Climate.

Anderegg, W.R.L., Trugman, A.T., Badgley, G., Konings, A.G. & Shaw, J. (2020) Divergent forest sensitivity to repeated extreme droughts. *Nature Climate Change* .

Arthur, M.A., Alexander, H.D., Dey, D.C., Schweitzer, C.J. & Loftis, D.L. (2012) Refining the OakâĂŘFire Hypothesis for Management of Oak-Dominated Forests of the Eastern United States. *Journal of Forestry* **110**, 257–266.

Ayres, M.P. & Lombardero, M.J. (2000) Assessing the consequences of global change for forest disturbance from herbivores and pathogens. *Science of The Total Environment* **262**, 263–286.

Bachelet, D., Neilson, R.P., Lenihan, J.M. & Drapek, R.J. (2001) Climate change effects on vegetation distribution and carbon budget in the united states. *Ecosystems* **4**, 164–185.

Canham, C.D., Kobe, R.K., Latty, E.F. & Chazdon, R.L. (1999) Interspecific and intraspecific variation in tree seedling survival: effects of allocation to roots versus carbohydrate reserves. *Oecologia* **121**, 1–11.

Ellison, A.M., Bank, M.S., Clinton, B.D., Colburn, E.A., Elliott, K., Ford, C.R., Foster, D.R., Kloeppel, B.D., Knoepp, J.D., Lovett, G.M., Mohan, J., Orwig, D.A., Rodenhouse, N.L., Sobczak, W.V., Stinson, K.A., Stone, J.K., Swan, C.M., Thompson, J., Von Holle, B. & Webster, J.R. (2005) Loss of foundation species: consequences for the structure and dynamics of forested ecosystems. *Frontiers in Ecology and the Environment* **3**, 479–486.

Fei, S., Desprez, J.M., Potter, K.M., Jo, I., Knott, J.A. & Oswalt, C.M. (2017) Divergence of species responses to climate change. *Science Advances* **3**.

Giorgi, F., Hurrell, J.W., Marinucci, M.R. & Beniston, M. (1997) Elevation dependency of the surface climate change signal: a model study. *Journal of Climate* **10**, 288–296

Izbicki, B.J., Alexander, H.D., Paulson, A.K., Frey, B.R., McEwan, R.W. & Berry, A.I. (2020) Prescribed fire and natural canopy gap disturbances: Impacts on upland oak regeneration. *Forest Ecology and Management* **465**, 118107.

Knott, J.A., Desprez, J.M., Oswalt, C.M. & Fei, S. (2019) Shifts in forest composition in the eastern united states. *Forest Ecology and Management* **433**, 176–183.

Lloyd, A.H. & Bunn, A.G. (2007) Responses of the circumpolar boreal forest to 20th century climate variability. *Environmental Research Letters* **2**, 045013.

Lorimer, C.G. (1989) Relative effects of small and large disturbances on temperate hardwood forest structure. *Ecology* **70**, 565–567.

Mitchell, R.J., Bellamy, P.E., Ellis, C.J., Hewison, R.L., Hodgetts, N.G., Iason, G.R., Littlewood, N.A., Newey, S., Stockan, J.A. & Taylor, A.F.S. (2019) Collapsing foundations: The ecology of the british oak, implications of its decline and mitigation options. *Biological Conservation* **233**, 316–327.

Mladenoff, D.J. (1987) Dynamics of nitrogen mineralization and nitrification in hemlock and hardwood treefall gaps. *Ecology* **68**, 1171–1180.

Opdam, P. & Wascher, D. (2004) Climate change meets habitat fragmentation: linking landscape and biogeographical scale levels in research and conservation. *Biological conservation* **117**, 285–297.

Parmesan, C. & Yohe, G. (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* **421**, 37.

Pepin, N., Bradley, R.S., Diaz, H.F., Baraer, M., Caceres, E.B., Forsythe, N., Fowler, H., Greenwood, G., Hashmi, M.Z., Liu, X.D., Miller, J.R., Ning, L., Ohmura, A., Palazzi, E., Rangwala, I., Schöner, W., Severskiy, I., Shahgedanova, M., Wang, M.B., Williamson, S.N., Yang, D.Q. & Group, M.R.I.E.W. (2015) Elevation-dependent warming in mountain regions of the world. *Nature Climate Change* **5**, 424–430.

Potter, K.M., Frampton, J., Josserand, S.A. & Nelson, C.D. (2008) Genetic variation and population structure in fraser fir (abies fraseri): a microsatellite assessment of young trees. *Canadian Journal of Forest Research* **38**, 2128–2137.

Rangwala, I. & Miller, J.R. (2012) Climate change in mountains: a review of elevation-dependent warming and its possible causes. *Climatic Change* **114**, 527–547.

Raymond, P., Munson, A.D., Ruel, J.C. & Coates, K.D. (2006) Spatial patterns of soil microclimate, light, regeneration, and growth within silvicultural gaps of mixed tolerant hardwood ÂŰwhite pine stands. *Canadian Journal of Forest Research* **36**, 639–651.

Rentch, J.S., Fajvan, M.A. & Hicks, Ray R., J. (2003a) Spatial and Temporal Disturbance Characteristics of Oak-Dominated Old-Growth Stands in the Central Hardwood Forest Region. *Forest Science* **49**, 778–789.

Rentch, J.S., Fajvan, M.A. & Hicks, R.R. (2003b) Oak establishment and canopy accession strategies in five old-growth stands in the central hardwood forest region. *Forest Ecology and Management* **184**, 285 – 297.

Runkle, J.R. (1982) Patterns of disturbance in some old-growth mesic forests of eastern north america. *Ecology* **63**, 1533–1546.

Schatz, J.D., Forrester, J.A. & Mladenoff, D.J. (2012) Spatial patterns of soil surface c flux in experimental canopy gaps. *Ecosystems* **15**, 616–623.

Schwartz, M.D., Ahas, R. & Aasa, A. (2006) Onset of spring starting earlier across the Northern Hemisphere. *Global Change Biology* **12**, 343–351.

Taylor, B.N., Patterson, A.E., Ajayi, M., Arkebauer, R., Bao, K., Bray, N., Elliott, R.M., Gauthier, P.P., Gersony, J., Gibson, R., Guerin, M., Lavenhar, S., Leland, C., Lemordant, L., Liao, W., Melillo, J., Oliver, R., Prager, C.M., Schuster, W., Schwartz, N.B., Shen, C., Terlizzi, K.P. & Griffin, K.L. (2017) Growth and physiology of a dominant understory shrub, hamamelis virginiana, following canopy disturbance in a temperate hardwood forest. *Canadian Journal of Forest Research* **47**, 193–202.

White, P.S. & Miller, R.I. (1988) Topographic models of vascular plant richness in the southern appalachian high peaks **76**, 192–199.

Zhang, M. & Yi, X. (2020) Seedling recruitment in response to artificial gaps: predicting the ecological consequence of forest disturbance. *Plant Ecology* **1**.