Statement of Work:

Introduction/Background:

To grow, survive, and reproduce, perennial plants must assimilate enough carbon per growing season to allocate to these pools and to storage tissue. Physiologically, this entails assimilating more carbon via photosynthesis than they use for respiration, and plants use a variety of different strategies to meet these demands. For example, many temperate tree species regulate their photosynthetic capacity throughout the growing season such that they maintain a near optimal balance between assimilation and respiration rates (Bauerle et al., 2012). However, the strategies that work for adult trees that maintain access to direct sunlight throughout the growing season may not generally work for plants growing in the forest understory.

In temperate deciduous forests, understory plants use two primary strategies for carbon assimilation: shade tolerance and shade avoidance (Henry & Aarssen, 1997). Shade tolerance is well-documented in the literature (Niinemets, 2010), and is generally described as a suite of functional traits allowing plants to persist under low-light conditions (Valladares & Niinemets, 2008). Shade tolerance can be plastic, with some long-lived species growing shade-tolerant leaves in later cohorts (Chabot & Hicks, 1982) or in more-shaded canopy layers (Kull & Niinemets, 1998). For especially long-lived species, such as trees, shade tolerance also differs across ontogeny, with higher shade tolerance for younger, smaller trees (Lusk & Warton, 2007).

Similarly to shade tolerance, shade avoidance also refers to a number of different strategies and traits. Plant height is a shade avoidance trait, with taller plants able to maintain access to full sunlight throughout the growing season. There are many eco-evolutionary adaptations that allow individual plants to avoid shade within their lifespan (Franklin, 2008), such as by growing toward higher quality light sources. More recently, ecologists have highlighted an ecological shade avoidance strategy for plants growing in deciduous forest understories. This strategy, referred to as phenological escape (Jacques et al., 2015), entails understory plant species (including tree seedlings and shrubs) leafing out or emerging up to several weeks before the canopy above them closes, allowing them to assimilate 50-100% of their annual carbon budget within the first few weeks of the growing season (Heberling, Cassidy, et al., 2019; Kwit et al., 2010; Lee & Ibáñez, 2021a). The success of this strategy (i.e., the length of the spring light window), has been mechanistically linked to performance metrics including annual growth, probability of survival, and reproductive success (Augspurger, 2008; Augspurger & Salk, 2017; Ida & Kudo, 2008; Jacques et al., 2015; Lapointe, 1998; Lee & Ibáñez, 2021a; Routhier & Lapointe, 2002; Seiwa, 1998), meaning that phenological escape is extremely likely to affect population-level persistence and demography.

Given its direct relationship with environmental variability, climate change has been implicated in changes in phenological escape success over the coming decades (Heberling, McDonough MacKenzie, et al., 2019; Lee et al., 2022; Lee & Ibáñez, 2021b; Miller et al., 2022), largely in response to spring warming. Most of these studies focus on patterns observed at large geographic scales, generally using historical observational or herbarium datasets (but see Lee & Ibáñez, 2021b). Although this has generated knowledge about how phenological escape has and will vary across space and time, more work is needed to evaluate how variation in phenological escape affects understory plant demographics at the plot or site level.

Research goals/questions:

Here, I propose to investigate whether understory plant communities are shaped by access to spring light using a combination of already-existing datasets collected by my proposed collaborator (Dr. Christy Rollinson) as well as newly collected tree seedling recruitment and demographic data at Morton Arboretum. My specific goals for this project are 1) to investigate the relationship between canopy tree composition and spring light window duration in the understory and 2) to use 5 years of existing understory plant surveys and surface-level environmental data to determine if spring light window duration is related to understory species richness and diversity. Lastly, I will collect vouchers of tree seedlings associated with goal (1) for inclusion in the Morton Herbarium collection, addressing a bias in herbarium collections to later ontogenetic stages of trees.

Methods

Goal 1: Canopy leaf phenology depends primarily on species identity, with different species leafing out at different times throughout the spring in northern temperate deciduous forests. For examples, maples and cherries tend to be some of the earliest to leaf out in spring, whereas oaks and hickories tend to flush in late spring or early summer. The exact timing of leaf-out also depends on environmental drivers such as spring temperature, winter chilling, and photoperiod length, leading to interannual differences in individual phenology.

Regardless, timing of understory light availability in spring is likely to be locally variable and highly dependent on the identity of surrounding canopy trees. For example, locations with higher concentrations of early-leafing tree species will have earlier canopy closure and could potentially preclude understory species that require longer durations of spring light availability to assimilate enough carbon to persist in that location. I have demonstrated evidence for such a mechanism in tree seedlings (Lee & Ibáñez, 2021a, 2021b), but a considerable knowledge gap remains in determining if this mechanism shapes the microsite distribution of spring-active understory plant species more generally.

To explore this idea, I propose to make use of forest stands operated by Morton Arboretum and to census understory plants across a range of canopy tree community composition. I will erect 50 m repeat-census transects in different parts of the forest and record the location of naturally generating plant species in relation to canopy tree species from late March to late May 2023. Understory plant censuses will be conducted at weekly intervals throughout this period, to ensure accurate representation of both early- and late-leafing species. At the time of each census, I will also measure understory light availability using a handheld photosynthetically active radiation (PAR) sensor at 10 m intervals along the transect as well as permanently deployed stationary PAR sensors at 0, 25, and 50 m along each transect, allowing me to compare the relative timing of canopy closure across and along each transect.

Although I have not found any published information showing the relative difference in timing of canopy close in different canopy tree communities, I do have some pilot data from several years ago, collected in deciduous forests in the Great Lakes region (southeast Michigan). Preliminary analysis of these data show that areas with a higher proportion of canopy trees in the species *Acer* (maples, characteristically early leaf-out phenology) tend to close earlier in the spring than areas more dominated by canopy trees in the genus *Quercus* (oaks, characteristically late leaf-out phenology), as measured using a densiometer (**Fig 1**).

For this project, I will collect similar data as illustrated in Fig. 1 to link relative canopy close timing to local canopy tree species composition. I will then use hierarchical Bayesian models to assess how transect-level understory plant community metrics (e.g., species richness, presence and/or abundance of early- vs. late-leafing species) are affected both directly by canopy tree community composition and indirectly by the timing of canopy close. I expect that early-leafing understory species will be more abundant (and thus overall understory species richness greater) in transects with later relative canopy close phenology.

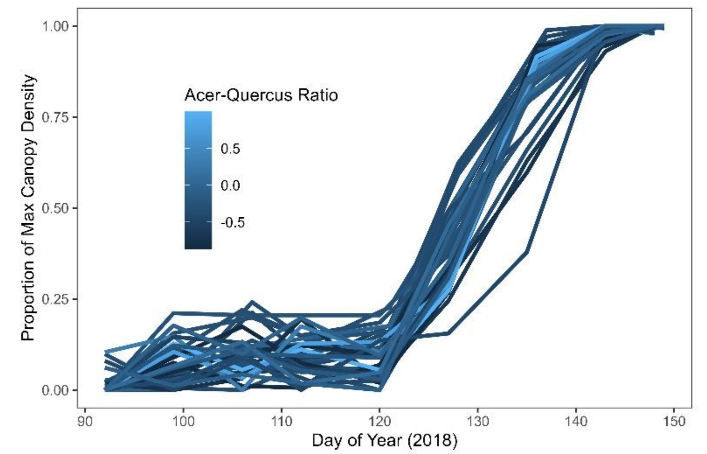

Fig. 1: Plot-level canopy proportional density (0 = open, 1 = closed canopy) over the course of spring 2018 in southeastern Michigan, with each line representing a single plot. Lines with positive color-scale values (light blue) are more heavily dominated by *Acer* genus species whereas negative values (dark blue) indicate plots dominated more by *Quercus*. Relative dominance is calculated using basal area of trees within 15 m of the center of the plot.

Goal 2: I will also work with existing data sets collected by my proposed collaborator, Dr. Christy Rollinson, to answer related questions. Dr. Rollinson has been collecting phenological data in repeat-census plots at Morton Arboretum, spanning 5 years. At the center of each plot (n = 16 [4 quadrats X 4 plots]; plot design illustrated in **Fig. 2**), her group has been collecting continuous meteorological data, including understory light levels. I will map canopy trees located within 15 m of all quadrats at each plot and then use the existing data to model how canopy composition and early spring light availability affect understory plant communities (as described in *Goal 1*, above).

Additionally, the multi-year aspect of these data will allow me to construct additional models aimed more at assessing the importance of interannual variation in spring light availability. For example, I intend to test whether understory plant community characteristics are affected by interannual differences in canopy closure timing (e.g., do we see higher plot-level abundance or diversity of spring ephemeral wildflowers when canopy close is relatively later in the year?).

Herbarium Collections: Finally, as somebody who currently works at the Carnegie Museum of Natural Resources herbarium, I am keenly aware of the importance of collecting plant vouchers during ecological research, and so I have made it part of my research protocol to do so. As a scientist with an interest in temperate tree recruitment, and thus tree seedlings, I am also keenly aware of the lack of tree seedlings in herbarium collections. Herbaria are often directed to maximize species diversity and spatial distribution of species within their collections. While these emphases are wonderful for systematists and even species distribution modelers, they also create systematic biases in herbarium collections (Daru et al., 2018), such as specimens more likely to be collected in highly-populated areas or near to major roads.

One such bias for trees is an over-representation of specimens that include fruit or flowers, as these organs often help in species identification. However, this naturally biases against non-reproductive individuals and especially tree seedlings. While systematics and phylogenetics research is likely unaffected by this, use of herbarium collections in ecological and trait-based research is growing (Heberling, 2022). Trees often exhibit strong shifts in traits (especially leaf traits and phenology) across ontogeny (Cavender-Bares & Bazzaz, 2000; Lee &

Ibáñez, 2021a), meaning there is an important gap in our ability to use herbarium collections in studies of recruitment and forest regeneration. Additionally, tree roots are rarely collected and preserved in herbaria, due to the effort needed to collect representative roots from mature trees. Collecting tree seedlings, due to the ability to fit an entire seedling with intact roots on one herbarium page, would thus also alleviate this gap in herbarium collections.

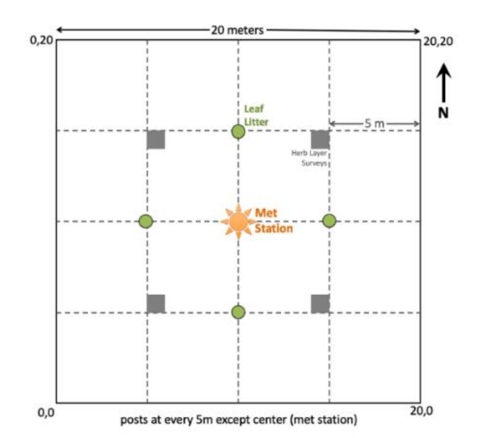

Fig. 2: Experimental plot design for Dr. Rollinson's repeat-census phenology experiment.

Thus, with the Arboretum's permission, I intend to collect research vouchers of all species of tree seedlings represented in our understory plant censuses. I will collect vouchers the first census they are observed, thereby creating a collection that is useful for studies of both biodiversity and phenology. I will also collect specimens of our target understory wildflower species. All specimens will be collected in triplicate (when possible) for the Morton Arboretum Herbarium, the Field Museum Herbarium, and the herbarium at the Carnegie Museum of Natural History.

Fellowship Timeline:

	2023			2024
Quarter of year:	Q2	Q3	Q4	Q1
Goal (1) – Establish tree seedling transects; set out understory light sensors	■			
Goal (1) – Tree seedling species, presence/absence, and height data collection	■	■		
Herbarium Collections – Collect seedling vouchers for Morton Arb Herbarium	■	■		
Goal (1) – Data entry; Statistical Analysis			■	
Goal (2) – Data cleaning; statistical analysis		■	■	
Goals (1&2) – Writing; submit interim report to Morton Arboretum			■	■
Goals (1&2) – Submit manuscripts for peer review/publication				■
Tree Talk seminar presentation at Morton Arboretum			■	■

Deliverables:

- Tree seedling vouchers (for Morton Arboretum Herbarium)
- Understory light availability dataset
- Herbarium spring ephemeral flowering phenology dataset
- Interim findings report presented to Morton Arboretum before end of fellowship year
- Manuscript(s) of final findings submitted for peer review (possible target journals: *Journal of Ecology*, *Tree physiology*, *Ecology*, *Functional Ecology*, and *American Journal of Botany*)
- Conference presentation (aiming for 2023 or 2024 ESA meeting, depending on status of analyses)
- Tree Talk seminar at Morton Arboretum (targeted for late 2023, early 2024)

Alignment with Center for Tree Science Mission:

Recent research suggests that phenological escape in spring is critical to tree recruitment, but it remains to be tested whether and how spatial variation in spring light window duration affects seedling recruitment dynamics. The proposed research will provide needed information about how this process, thereby providing new scientific knowledge necessary to understand and sustain temperate deciduous tree species. Additionally, I will collect tree seedling specimens to be included in the Morton Arboretum, Field Museum, and Carnegie Museum of Natural History herbaria. Tree herbarium collections are generally focused on the leaves, flowers, and fruit, of adult specimens, but rarely include root tissue or younger ontogenetic stages. I propose to work toward filling this gap by collecting representative species vouchers for tree seedlings found recruiting in Morton Arboretum.

Budget:

Amount	Category	Justification/Description
$400	Supplies	$95 x 8 HOBO PAR/temp sensors + shipping costs (https://www.onsetcomp.com/products/data-loggers/ua-002-64) to be stationed at 0 and 50 m intervals along understory phenology/census transects. Light at 25 m will be collected by existing meteorological stations.
$150	Supplies	HOBO base station required to deploy HOBO PAR pendants and to download the data at end of the study (https://www.onsetcomp.com/products/communications/base-u-4)
$0	Stipend	*See note below
$700	Travel	Two round-trip flights at $350/flight between Pittsburgh and Chicago. Not counting the trip to present results at the Arboretum, I plan to travel to the Arboretum twice in 2023 to help set up PAR sensors and research transects and to help train undergraduate field assistants.
$500	Travel	Car rental for spring visits to Morton Arboretum
$550	Travel	Costs for meals during 2 weeks of visits.
$1,200	Travel	$100/night x 6 nights/visit x 2 visits for hotel stays during research trips
$4,000	Professional Services	Remaining stipend to be used to hire a field tech (likely an undergraduate student from nearby universities) for the 2.5-month duration of data collection (late March-late May)
$7,500	**TOTAL**	

*As a current NSF Postdoctoral Research Fellow in Biology, I am unable to request any of the funding to be allocated to personal stipend. As such, I request that this stipend be allocated to a combination of field assistance (undergraduate researchers) and research equipment, with the remainder set aside for my travel to and from Morton Arboretum.

Preferred Payment Schedule: The standard three payment schedule works for me, although the costs associated with hiring a field tech in March-May will need to be early to be fair to the student hired. I am happy to cover all other fees for later reimbursement if necessary.

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