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## Tree recruitment dynamics in an urban forested natural area

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Tree recruitment dynamics in an urban forested natural area

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Submitted to the Faculty of

Mississippi State University

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for the Degree of Doctor of Philosophy

in Forest Resources

in the Department of Forestry

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Urban forested natural areas (UFNAs) are the remnants of larger forested ecosystems that have become surrounded by urban land development. Due to their proximity to dense population centers, UFNAs provide levels of ecosystem services disproportionate with their small size. However, stressors on UFNAs, such as fragmentation, invasive species, urban heat islands, soil compaction, altered seed predator populations, and disrupted disturbance regimes, can alter species composition and forest structure such that the continued flow of these ecosystem services is unlikely. Further, management of these systems is confounded by their size and location. While most urban forests are managed using individual tree techniques (arboriculture), UFNAs have too many trees for this approach to be tenable. Likewise, traditional forest management techniques (silviculture) may not be possible due to the relatively small size of UFNAs and their proximity to urban population centers where silvicultural strategies may not be politically acceptable. This dissertation helps inform UFNA management strategies by documenting how species composition and forest structure have changed over time as a result of a mostly laissez-faire management strategy in a UFNA of Memphis, Tennessee. Evidence of the negative impacts of this approach on native tree species can be used by managers to build public support for management interventions. This work also examines potential recruitment barriers for the native

overstory species northern red oak (*Quercus rubra* L.) and tulip poplar (*Liriodendron tulipifera* L.) which provide much of the large tree character in this UFNA. These results can guide specific management strategies to promote these important species. Finally, this research elucidates the potential threat to native plant biodiversity in this UFNA posed by a popular non-native ornamental tree species, cherry laurel (*Prunus caroliniana* Aiton). The results can aid in the development of appropriate policies regarding this specific tree and similar species in other UFNAs. Combined these studies provide a glimpse into the complicated dynamics and management of this UFNA. Given the widespread nature of the species examined and the methods used, this research may also provide useful insights into UFNA management in the surrounding regions.

## DEDICATION

To Sara for her unconditional love and support.

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## CHAPTER I

### INTRODUCTION

Urban forests provide a host of benefits to an increasingly urban society. They ameliorate the urban heat island effect (Akbari, 2005), attenuate stormwater flows (Nowak & Dwyer, 2007), and reduce heating and cooling costs (Nowak et al., 2009). Urban forests also improve property values (Bridges et al., 2020) and human health (Kuo & Sullivan, 2001; Wolf et al., 2015). However, as urban areas expand, they often envelope existing forests within the urban land use matrix (Loeb, 2011) creating remnant forest patches, termed urban forested natural areas (UFNAs, Pregitzer et al., 2019a). Park forests and street tree populations are often intentionally planted and managed using traditional arboricultural techniques at an individual tree level (Pregitzer et al., 2019b). In contrast, UFNAs retain many of the characteristics they possessed before urbanization such as large trees, down woody debris, snags, and unmaintained ground layer vegetation (Guldin et al., 1990; Lehvavirta & Rita, 2002; Duinker et al., 2017), thus maintaining ecological and aesthetic attributes, but requiring sustained management to ensure the continued delivery of benefits to society.

However, management of UFNAs is often challenging for municipalities and community partners (Piana et al., 2021b) where opinions on proper management may range from the use of traditional silvicultural techniques to ‘letting nature take its course’ (Loeb, 2011). For instance, some argue that allowing trees to naturally regenerate, i.e., through natural seeding or sprouting rather than by direct planting, is a viable passive management technique in UFNAs that

decreases costs, increases biodiversity, and improves aesthetic and recreational values (Lehvavirta et al., 2014). Tree recruitment dynamics, though, are often altered in UFNAs due to biotic and abiotic urban drivers (Greene et al., 1999; Piana et al., 2019). For example, fragmentation and associated edge environments in UFNAs alter growing conditions, such as light, temperature, and moisture, as well as propagule availability and pollinator behavior (Lehvavirta et al., 2014; Harrison & Winfree, 2015; Duinker et al., 2017). High human recreational use can compact soils, subsequently reducing water infiltration and tree regeneration potential (Lehvavirta, 1999; Lehvavirta et al., 2014). Urban forested natural areas can also host high densities of seed predators and herbivores (Zipperer et al., 1997; Overdyck et al., 2013; Labatore et al., 2016), which can alter seed dispersal, germination, and survival rates. Additionally, introduced invasive species including insects, plants, and pathogens, which are often abundant in UFNAs, can reduce native tree biodiversity, alter plant competitive interactions, and affect seed predator behavior (Nowak et al., 2001; Kowarik, 2005; Aronson et al., 2017).

Consequently, the use of passive management techniques may not lead to desired or predicted outcomes in species composition and forest structure in UFNAs. Also, the application of arboricultural, single tree management techniques may not be appropriate or feasible at the scale of urban woodlands (Duinker et al., 2017). Similarly, the application of traditional silvicultural techniques, may not be feasible at the small scale of UFNAs, nor create the desired species composition or forest structure due to the unique biotic and abiotic conditions found in UFNAs compared to rural forests (e.g., elevated temperatures, increased air pollutants, compacted soils, altered disturbance regimes, changes in species and abundances of seed predators, unique plant species assemblages; Carreiro & Tripler, 2005; Lehvavirta, 2007; Piana

et al., 2021c). Management activity in UFNAs can be further hampered by a lack of public support due to perceived reductions in aesthetic value or to a lack of participatory planning (Johnson & Handel, 2016; Piana et al., 2021b). Therefore, a careful understanding of UFNA dynamics is required to develop appropriate management strategies to preserve desired composition, structure, and function. The current research contributes to the understanding of UFNA dynamics through a series of studies analyzing tree species compositional change, seed germination and seedling emergence, and allelopathic potential of a non-native tree species in the Old Forest State Natural Area (Old Forest), a UFNA in Memphis, Tennessee.

Concerns over the increasing presence of non-native tree species, the loss of foundational tree species (e.g., *Quercus* spp.), and the development of dense sapling and midstory layers are concerns common to both rural and urban forestry (Ellison et al., 2005; Early et al., 2015; Aronson et al., 2017; Hanberry et al., 2020). However, studies examining the extent of these factors in UFNAs are limited. Also, the unique disturbance regimes and environmental stressors in UFNAs may limit our ability to predict their future species composition and stand structure under various management scenarios (Zipperer, 2010). Thus, studies examining species composition and stand structure change over time are valuable for enhancing our understanding of stand dynamics in UFNAs and for the development of management strategies to reduce non-native tree species, retain foundational species, and reduce barriers to regeneration. In Chapter Two, changes in tree species composition and stand structure in the Old Forest between 1987 and 2019 were examined with a particular focus on non-native tree species, loss of oak (*Quercus* spp.), and densification of the sapling and midstory layers. This chapter was formatted for submittal to the Natural Areas Journal.



Since UFNAs have a much higher density of trees (Pregitzer et al., 2019b) than other categories of urban forest (e.g., street trees, park trees), managers often rely on natural regeneration for tree recruitment rather than direct planting (Lehvavirta et al., 2014). However, the efficacy of using natural regeneration in UFNAs is uncertain as studies examining recruitment rates in UFNAs, while limited in number, have found native tree recruitment failures across a variety of species and sites (Burton et al., 2005; Piana et al., 2021a). Further, several factors known to impact regeneration in rural forests, such as leaf litter (Facelli & Pickett, 1991), understory vegetation (Royo & Carson, 2006), canopy gaps (Muscolo et al., 2014), and seed predation (Sharik & Buckley, 2002) are not well studied in UFNAs (Piana et al., 2021c). In Chapter Three, results of an experimental study analyzing the effects of seed predation, canopy gaps, leaf litter, and understory vegetation on early establishment of northern red oak (*Quercus rubra*, L.) and tulip poplar (*Liriodendron tulipifera*, L.) in the Old Forest are presented. This chapter was formatted for submittal to the Journal of Forestry.

Due to their proximity to urban areas, UFNAs are often subject to accidental introductions or intentional plantings of horticultural species used in urban landscaping which have been moved well beyond their historic native ranges (Duinker et al., 2017). While exotic invasive plant species (i.e., those moved across continental boundaries) are known to cause negative ecological impacts within their introduced habitat (Richardson et al., 2000), plants that have been moved much smaller distances but outside of their regional context can have similar ecological impacts (Nackley et al., 2017). However, these regional transplants often receive less attention and raise less concern from stakeholders and managers (Berthon et al., 2021) and are rarely studied (Lehvavirta, 2007). Chapter Four examined the potential mechanisms behind the successful invasion of a regionally non-native woody species, cherry laurel (*Prunus caroliniana*

Aiton), in the Old Forest. Cherry laurel is a common horticultural tree that became abundant in the Old Forest between 1987 and 2019. It is known to produce cyanogenic glycosides (Heiling & Gilbert, 2016) which suggests it has allelopathic potential (Cipolloni & Gruner, 2007) *in situ*. Therefore, the current study evaluated the allelopathic potential of cherry laurel by examining the association between cherry laurel and vegetation abundance, diversity, and composition in the Old Forest, by examining the impact of its leaf and root extracts on germination of native tree species, and by examining the impact of its leaf residues on seedling growth of native tree species. This chapter was formatted for submittal to the Journal of the Torrey Botanical Society.

Collectively, the research presented here contributes to an understanding of ecological processes related to changing species composition and stand structure in an urban forested natural area of Memphis, TN. The focus of the research was on the critical early-establishment stage of recruitment, where impacts to future species composition and stand structure are high (Clark et al., 2007). Therefore, the results have potential management implications which are noted in each chapter. To continue expanding the understanding of early-establishment processes in UFNAs, future research needs are also identified within each chapter.

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CHAPTER II  
LONG-TERM TRENDS IN NON-NATIVE TREE SPECIES' INVASION, FOUNDATION  
TREE SPECIES' DECLINE, AND DENSIFICATION OF AN  
URBAN FORESTED NATURAL AREA

**Abstract**

Urban forests provide ecosystem services that are derived from, and dependent upon, tree species composition and structure. Thus, assessing current-day and predicting future forest function requires an understanding of how these forest characteristics change over time. However, factors influencing forest composition and structure have received limited attention in urban forests. This study explored tree species composition, diversity, and structural dynamics in an urban forested natural area (UFNA) over a 32-year period in the Old Forest State Natural Area within Memphis, Tennessee, USA, focusing on the introduction and proliferation of non-native tree species; decline of a foundational genus, specifically oaks (*Quercus* spp.), and densification of the midstory and sapling strata. Tree species composition, density, and diversity were calculated in a 2019 forest inventory and compared to results from a 1987 inventory. We found a significant increase in the relative density of non-native tree species from 0% in 1987 to 14% in 2019 alongside a decrease in native tree abundance and diversity. Oak density and importance value declined in the overstory and seedling categories, while cherry laurel (*Prunus caroliniana* Aiton) and pawpaw (*Asimina triloba* (L.) Dunal) density increased in the midstory and sapling layers. Study results underscore the need for active management and future research

in UFNAs, particularly the necessity of understanding mechanisms and consequences of non-native species invasion, foundation species decline, and densification in urban forests.

## **Introduction**

Urban forests provide a wide variety of societal benefits, in particular urban heat island amelioration, stormwater attenuation, air quality improvements, heating and cooling cost reductions, property value improvements, human health improvements, and biodiversity conservation (Kuo & Sullivan, 2001; Akbari, 2005; Nowak & Dwyer, 2007; Nowak et al., 2009; Donovan & Butry, 2010; Shaffer, 2018; Bridges et al., 2020). Urban forested natural areas (UFNAs) are a subset of urban forests which contain the remnants of larger forested systems that existed prior to urban development (Lehvavirta, 2007; Charlop-Powers et al., 2020). They can deliver high quantities of benefits to the public due to their proximity to urban population centers and the high quantity of trees compared to other urban forest types (Piana et al., 2019; Pregitzer et al., 2019a; Morzillo et al., 2022). Regardless of whether forests are urban or rural, the quantity and quality of benefits they provide depends on their stand structure and species composition (McPherson et al., 1997; Nowak et al., 2008; Kim et al., 2016; Mitchell & Devisscher, 2022). For example, large, healthy trees store more carbon annually than smaller trees (Nowak and Dwyer, 2007), while native tree species are known to provide better habitat for native biodiversity than non-native trees (Berthon et al., 2021). Assuring the continued flow of benefits specifically from UFNAs is difficult as urban stressors (e.g., non-native species introductions, altered disturbance regimes, high degrees of fragmentation) can lead to changes in species composition and structure over time that result in lower quantity and quality of benefits, such as increased presence of non-native plant species, loss of foundational tree species, and densification of midstory and sapling layers (Lehvavirta, 2007; Piana et al., 2019). Therefore,



temporal studies documenting changes in structure and composition of UFNAs are important for the development of management strategies to maximize benefits provided by these systems.

A major factor affecting tree species composition and stand structure in forests worldwide is the introduction of **non-native species** (Gavier-Pizarro et al., 2010; Early et al., 2015). The presence of non-native species can alter ecosystem processes, such as increasing fire frequency and intensity (Vitousek et al., 1997; Barnes et al., 2021), disrupting regeneration patterns by inhibiting growth and survival of native plant species (Hartman & McCarthy, 2008; Johnson & Handel, 2016), and ultimately lead to the loss of biodiversity including local extinction of native species (Millennium Ecosystem Assessment, 2005; Pysek et al., 2017). For example, in eastern United States forests, non-native plants, such as *Microstegium vimineum* (Trin.) A. Camus, have been shown to suppress regeneration and growth of native tree and forb species (Johnson et al., 2014; Moyer & Brewer, 2018; Goldsmith et al., 2023) thereby altering successional trajectories. Urban forests are particularly vulnerable to non-native species due to their proximity to urban landscapes where non-native plants are highly utilized (Doroski et al., 2018) and their extensive edge habitat, which can provide avenues for entry of non-native species (Bar-Massada et al., 2014). Indeed, some studies have documented an increased presence of non-native tree species in urban forests over time (Loeb, 2010; Loeb et al., 2011) as well as higher levels of non-native tree species in urban forests when compared to rural forests (Pregitzer et al., 2019b; Piana et al., 2021a). However, few studies have examined the consequences of an increased presence of non-native tree species on diversity, richness, and abundance of native plants in urban forests (Aronson, 2007; Duinker et al., 2017). Understanding how non-native tree species affect forest composition and structure over time is therefore important to ensuring the health and benefits provided by these systems.

Another factor affecting forests worldwide is the **loss of foundational tree species**, that is, those dominant species whose form, abundance, and functional traits have a conspicuous effect on forest structure and dynamics (Dayton, 1972; Ellison et al., 2005). Loss of foundational species can have a cascading effect on ecosystem processes, including altering nutrient fluxes, hydrologic cycles, fire regimes, and successional trajectories, leading to the potential for a series of secondary extinctions (Ebenman & Jonsson, 2005; Ellison et al., 2005). In the eastern United States, the decline of oak (*Quercus* spp.), which was the most abundant genus in these forests prior to European settlement, represents the potential loss of a foundational genus (Hanberry & Nowacki, 2016) with consequences ranging from lower biodiversity via reductions in mast production, insect habitat, and light availability (Gilliam, 2007; McShea et al., 2007; Tallamy & Shropshire, 2009), to modifications of future fire regimes due to changes in leaf litter flammability (McDaniel et al., 2021; Arrington-Thomas & Brewer, 2023) and nutrient and hydrologic cycles due to decreased precipitation throughfall associated with non-oak species (Alexander & Arthur, 2010; Alexander & Arthur, 2014; Siegert et al., 2019; Scavotto et al., 2024). Urban forest studies have documented low levels of oak representation in sapling and midstory layers despite dominance in the overstory (Profous & Loeb, 1984; Hochwender et al., 2016; Piana et al., 2021a). In rural forests, loss of oak is often attributed to disruption of historic fire regimes, subsequent replacement of oaks by more shade-tolerant, fire-sensitive (i.e., mesophytic) species, and crown and leaf litter traits of encroaching mesophytes that reinforce low flammability environments (Nowacki & Abrams, 2008; Alexander et al., 2021). In contrast, the proposed mechanisms of failed oak recruitment in urban areas include stressors such as competition from non-native species, trampling from high usage, and seed predation from elevated densities of small mammals such as eastern gray squirrel (*Sciurus carolinensis*, Sisinni

and Emmerich, 1995; Rudnický and McDonnell, 1989; Zipperer, 2010; Brewer, 2011; Piana et al., 2019). However, studies examining the mechanisms behind oak recruitment in urban forests are scarce. Thus, understanding the changing dynamics of oaks in UFNAs is critical to developing management strategies to maintain this foundational genus.

Along with the introduction of non-native species and loss of foundational species, the **densification** (i.e., increases in trees per hectare) of forest midstories and sapling layers, often associated with changes in historic disturbance regimes, climate, and land use patterns (McEwan et al., 2011; Pederson et al., 2015; Hanberry et al., 2020), has the potential to dramatically modify species composition and stand structure of forests globally (Royo & Carson, 2006; Hanberry & Nowacki, 2016). Densification can lead to the development of a recalcitrant understory tree or shrub layer that substantially limits understory light availability, increases seed predator activity, and increases leaf litter depths (Facelli & Pickett, 1991; Royo & Carson, 2006) leading to altered recruitment dynamics and reductions in native plant diversity (i.e., a homogenization of the herbaceous layer; Facelli & Pickett, 1991; Royo & Carson, 2006; Hanberry & Nowacki, 2016; Sipe, 2023). In the eastern United States, densification of previously open oak-dominated forests has led to an increased prevalence of closed-canopy forests dominated by a different suite of tree species, (i.e., those that are more shade-tolerant and fire-sensitive/opportunistic), along with a reduction in native floral diversity (Royo et al., 2010; Brewer et al., 2015; Hanberry & Nowacki, 2016; Hanberry, 2019; Alexander et al., 2021). While urban forests are generally known to be less dense than surrounding rural forests (Nowak et al., 2007; Pregitzer et al., 2019), densification has been observed in UFNAs (Rudnický & McDonnell, 1989; Piana et al., 2021a). However, this phenomenon remains a relatively little studied aspect in urban forestry. Understanding densification in UFNAs over time is important to

the development of management strategies to ensure native tree recruitment and maintenance of high levels of biodiversity.

To examine the impact of non-native tree species, loss of foundational tree species, and densification of the midstory and sapling layers in a UFNA over time, this research took place within the Old Forest State Natural Area (Old Forest) of Overton Park, Memphis, Tennessee, USA. A detailed forest inventory was conducted in 1987 (Guldin et al., 1990) providing a rare opportunity to evaluate structural and compositional changes in a UFNA. While Guldin et al. (1990) did not find non-native tree species, they did find a lack of oak representation in the understory despite dominance in the overstory and the presence of treefall gaps that provided opportunities for regeneration. They hypothesized that a combination of the presence of non-native shrubs such as Chinese privet (*Ligustrum sinense* Lour.) and vines such as kudzu (*Pueraria montana* (Lour.) Merr.) along with the disruption of the historic fire regime, and presence of a dense understory vegetation layer, particularly pawpaw (*Asimina triloba* (L.) Dunal), were likely contributing factors in the lack of oak recruitment. Current research objectives were to examine the nature of tree species composition and structure change in the Old Forest over 32 years and determine: 1) if non-native tree species have increased in prevalence; 2) if native tree species diversity has declined, 3) patterns of oak recruitment, and 4) levels of tree densification in the midstory and sapling size categories. Study results can be used to develop well-informed urban forest and park management strategies as well as research priorities for the continued supply of societal benefits from this urban forested natural area.

## Methods

### Study area

This research was conducted in the Old Forest State Natural Area (Old Forest) within Overton Park, Memphis, Tennessee (35.145133 N, -89.988562 W), USA. The Old Forest is a 58 hectare (ha) mixed mesophytic forest situated within the boundaries of the 138 ha Overton Park. The Old Forest is owned by the City of Memphis, managed by the nonprofit organization, Overton Park Conservancy, and is under the jurisdiction of the Tennessee Department of Environment and Conservation - Division of Natural Areas. Geographically, the Old Forest lies within the Eastern Deciduous Forest, Western Mesophytic Region, Mississippi Embayment Section (Braun, 1950) and is situated within the Humid Temperate Domain, Subtropical Division, Southeastern Mixed Forest Province (Bailey, 1995). The area is also known as the Brown Loam Bluffs (Hodges, 1995), which is primarily a Loess formation with high water capacity and nutrient content (Sease et al., 1989). Soils in the Old Forest are Memphis Silt Loam with a 2-5% slope, and are a strongly acidic, well-drained Alfisol with a depth to restrictive layer of more than 200 centimeters (cm). The soil is noted as being highly productive for the growth of oaks, tulip poplar (*Liriodendron tulipifera* L.), and other upland hardwoods (Sease et al., 1989). The climate is characterized by mild winters and hot summers with average annual temperatures of 16.8 °C with the highest mean temperature of 27.9 °C occurring in July and lowest mean temperature of 4.8 °C occurring in January (USDC, 2020). The Old Forest is located in the USDA plant hardiness zone 7B defined as having average annual extreme minimum temperatures of -12.2 °C to -15 °C (USDA, 2012). Precipitation averages 134.5 cm per year with the highest monthly average of 14.6 cm occurring in December and the lowest monthly average of 7.3 cm occurring in August (USDC, 2020).

The Old Forest is characterized by a diverse flora consisting of over 330 species of flowering plants including 55 tree species (Heineke, 2009). The site has been noted for its large trees since the property was purchased by the City in 1901 (Guldin, 1987; Bearden, 2004; Heineke, 2009). Specimens as large as 180 cm diameter at 1.3 meters (m) above mean ground level (dbh), 50 m tall, and 189 years old have been recorded (Bridges, 2019). There are examples of tulip poplar, 11 species of oak, cottonwood (*Populus deltoides* W. Bartram ex Marshall), sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), white ash (*Fraxinus americana* L.), and green ash (*Fraxinus pennsylvanica* Marshall) all achieving a dbh of 100 cm and greater (Guldin et al., 1990). The site is also known to contain approximately 80 non-native plant species including at least 16 listed as invasive (Heineke, 2009; Miller et al., 2010) including kudzu, Chinese privet, English ivy (*Hedera helix* L.), tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), mimosa (*Albizia julibrissin* Durazz), paper mulberry (*Broussonetia papyrifera* (L.) L'Her. ex Vent), and princess tree (*Paulownia tomentosa* (Thunb.) Siebold & Zucc. ex Steud).

### **Forest inventory**

In 1987, Guldin et al. (1990) conducted an inventory of the Old Forest using fixed area nested plots in a systematic layout (80.5 m x 80.5 m spacing) to characterize trees by species and size categories (seedling, sapling, midstory, overstory). The current study, conducted in 2019, aimed to closely replicate the 1987 inventory using the same plot spacing and nested plot design (Figure 2.1). The 1987 sample plots were not permanently marked and were thus not remeasured. However, the 2019 plot centers were located with a geographical positioning system (GPS) and marked with steel rebar stakes to facilitate future relocation and resampling. From 1987 to 2019, forest size was reduced from 70.8 ha to 58 ha due to an expansion of the Memphis

Zoo. To provide similar sampling intensities, 74 plots were completed in 1987 and 66 in 2019 providing a sampling intensity of 1.05 and 1.14 plots per ha, respectively. Plot sizes and diameter classes sampled were replicated from Guldin et al. (1990). At each plot center, one 0.081-ha circular subplot (subplot 1) was established within which all trees with dbh > 24.3 cm were inventoried to represent the overstory component. Also, one 0.04-ha circular subplot (subplot 2) was established utilizing the same plot center within which all trees with dbh from 9.1 cm to 24.2 cm were inventoried to represent the midstory component. Two circular 40-m<sup>2</sup> subplots (subplot 3) were located 6 m due north and south, respectively, of plot center within which all trees > 0 cm dbh and < 9.1 cm dbh were inventoried to represent the sapling component. Finally, two circular 4-m<sup>2</sup> (subplot 4) subplots were located similarly north and south of plot center within which all trees between 0.3 m and 1.37 m in height were inventoried to represent the seedling component (Figure 2.1). Species and dbh were recorded for each inventoried tree in subplots one through three with species and count recorded for all trees inventoried in subplot 4. Data were collected from June through August of 2019 using Survey123 software for ArcGIS (ESRI, 2019).

## **Analysis**

All analyses were performed using R Statistical Software (v4.3.2; R Core Team, 2023) and an  $\alpha$  of 0.05. Changes in **species composition** for each size category were evaluated using permutational manovas (PERMANOVA), with robust Aitchison distances (Martino et al., 2019), within the vegan package in R (Oksanen et al., 2022). Primary drivers of species composition change within each size category were identified using constrained analysis of principal coordinates in the capscale function of the vegan package.

Levels of non-native tree species presence, oak recruitment, and densification of the understory were evaluated by comparing changes in plot level averages of the density (trees per ha) of non-native species, oak species, and all species across all size categories using Mann-Whitney  $U$  tests (Mann & Whitney, 1947).

Native and **non-native tree species** richness, diversity, and evenness were examined for each year and size category. Species richness was defined as the number of unique species present on a plot and within a size category. Species diversity was calculated using the Shannon Diversity Index ( $H_{sh}$ ):

$$H_{sh} = - \sum [(p_i) * \ln(p_i)] \quad (2.1)$$

where  $p_i$  equals the ratio of the number of individuals of species  $i$  to the total number of all individuals (Shannon & Weaver, 1949; Magurran, 2004; Gotelli & Chao, 2013; Gotelli & Ellison, 2018). Species evenness was estimated for each sample year by size category and native status with Pielou's  $J$  (Pielou, 1975):

$$J = \frac{H_{sh}}{\ln(S)} \quad (2.2)$$

where  $H_{sh}$  equals the Shannon Diversity Index and  $S$  equals species richness. Species richness for all species and for native species only was analyzed using a Mann-Whitney  $U$  test (Mann & Whitney, 1947) while Shannon Diversity Indices were analyzed by size category, year, and native status using the Hutcheson's  $t$ -test (Hutcheson, 1970) within the `ecolTest` package in R (Salinas & Ramirez-Delgado, 2021).



## Results

PERMANOVA results indicated significant changes in tree species composition for all size categories between 1987 and 2019 (Table 2.1), while constrained analysis of principal components (capscale) identified the species primarily responsible for these changes within each size category. Capscale scores, density changes, and importance value (defined as the average of relative density and relative dominance) changes for all species are detailed in Table 2.2. The highest density changes by species and size category are detailed in Figure 2.2. In the overstory, oak species were strongly associated with the 1987 inventory while boxelder (*Acer negundo* L.) and tulip poplar were strongly associated with 2019. In the midstory, flowering dogwood (*Cornus florida* L.) was associated with the 1987 inventory while boxelder and pawpaw were strongly associated with 2019. No species had a particularly strong association with the 1987 saplings; however, pawpaw and cherry laurel (*Prunus caroliniana* Aiton) were strongly associated with the 2019 saplings. In the seedling layer, pawpaw was more associated with the 1987 inventory while cherry laurel and green ash were more associated with 2019.

Density of non-native tree species was significantly greater in 2019 for all size categories except the overstory which did not change (Table 2.3). The majority (81%) of increases occurred in the seedling size category. Species richness was significantly higher in 2019 for all species and for native species in all size categories except seedlings where richness was significantly lower (Table 2.4). Native tree species diversity, as measured by the Shannon Diversity Index, was significantly lower in 2019 for the midstory, sapling, and seedling size categories (Table 2.4). When including native and non-native species and evaluating across all size categories, tree species diversity was significantly higher in 2019. Tree species evenness, as calculated by Pielou's  $J$ , decreased overall and for all size categories except seedlings (Table 2.4).

Density of oak species was significantly lower in the 2019 overstory and seedling size categories and not significantly different in the midstory and sapling size categories (Table 2.3). Specifically, overstory oak density declined from 28 to 13 trees per ha while the importance value dropped from 45 to 22. The largest change was in cherrybark oak (*Quercus pagoda* Raf.) which declined in density from 9 to 4 trees per ha and from 16 to 6 in importance value. In the seedling layer, oak density declined from 806 to 243 trees per ha and from 9 to 3 in importance value with white oak (*Quercus alba* L., -184 trees per ha), black oak (*Quercus velutina* Lam., -152 trees per ha), and cherrybark oak (-142 trees per ha) accounting for 85% of those changes. See Figure 2.3 for changes in density for the six most abundant oak species.

Tree density in the midstory and sapling size categories was significantly higher in 2019 than 1987. Specifically, midstory density increased by 96% from 105 to 206 trees per ha while sapling density increased by 244% from 857 to 2,945 trees per ha (Table 2.3). Three species accounted for 90% of increased density in the midstory and sapling size categories, [i.e., pawpaw (1,285 trees per ha), cherry laurel (413 trees per ha), and boxelder (177 trees per ha)].

## Discussion

Results of the current study are indicative of changes to species composition and stand structure being experienced in forests worldwide, albeit within an urban context. In particular, an increasing presence of non-native species (Alvey, 2006; Aronson et al., 2017), reductions in foundation species (Ellison et al., 2005; Hochwender et al., 2016), and densification of the midstory and sapling layers (Hanberry & Nowacki, 2016; Pregitzer et al., 2019b) were of primary interest and found to exist in the Old Forest. Specifically, non-native tree species became more abundant while the density (trees per ha) of native tree species, including oaks, declined. Non-native tree species increased from zero to 14% of the relative density (stems per

hectare of non-native trees divided by the total stems per hectare of all trees) with most remaining in the sapling and seedling layers. Alongside the increase in non-native tree density, native tree species diversity, as measured by the Shannon Diversity Index, declined. Further, the relative density of oaks declined from 8.0% to 2.5% with most declines occurring in the overstory and seedling categories. Evidence of densification was found with significant density increases in the midstory (101 trees per ha increase) and sapling (2,088 trees per ha increase) size classes. Without management interventions the buildup of non-native tree species, loss of foundational oak species, and densification of the midstory and sapling layer will likely lead to reduced compositional and structural diversity and the subsequently loss of ecosystem services in this urban forest.

### **Non-native tree species**

The study exemplifies the well-known phenomenon of non-native species becoming established in an urban forest over time (Zipperer & Guntenspergen, 2009; Loeb, 2010; Loeb et al., 2011; Duinker et al., 2017). Specifically, seven non-native tree species were introduced in the Old Forest over a 32-year period accounting for 13% of the total species composition. Others have documented similar findings. For instance, Zipperer (2002) noted seven non-native introductions accounting for 17% of the species composition in urban forested natural areas of Syracuse, NY, although they were not able to document an exact period over which these introductions occurred. Also, Aronson (2007) noted four non-native tree introductions making up approximately 20% of the species composition in the Hutcheson Memorial Forest, a UFNA in New Jersey, over a 53-year period. Thus, while a general increase in non-native species abundance was expected, the increase of 1,524 trees per ha alongside a decrease in native stem density (from 9,609 to 9,129 trees per ha) documented in the current study was higher than

anticipated. In particular, the non-native cherry laurel had the highest density increase (1,349 trees per ha) of any species in the study and became the second most abundant tree species in the Old Forest. While others have documented increased non-native tree species density over time in urban forested natural areas, none have reported increases of the magnitude observed in the current study, nor an accompanying decrease in native tree species density (Zipperer, 2002; Bertin et al., 2005; Zipperer & Guntenspergen, 2009; Pregitzer et al., 2019b; Piana et al., 2021b).

The current study also documented 99% of the increased non-native stem density in the seedling and sapling size categories while the overstory remained composed of mostly native species. This finding was similar to others demonstrating the UFNA overstories typically maintain a high proportion of native trees (Hochwender et al., 2016; Pregitzer et al., 2019a; Piana et al., 2021a). For instance, Pregitzer et al. (2019a) found that the overstory of New York City's (NYC) UFNAs are composed of 82% native species. While the number of non-native tree species was limited to seven and their position was limited primarily to the understory, high density suggests non-native tree species are poised to move into the larger size categories and further reduce the presence of native tree species in the Old Forest.

Accompanying the increase in non-native tree species density, the diversity (i.e., Shannon Diversity Index) of native tree species has declined in the Old Forest. The most substantial decrease was found in the sapling size category where native tree diversity declined from a Shannon index value of 2.2 to 1.6. The reduction in native tree diversity appears to be driven largely by decreased species evenness, as measured by Pielou's  $J$ . Thus, even as native species richness increased in the sapling and midstory size categories, native species diversity declined. Decreased evenness could be attributed to the high density of non-native species, particularly in the sapling category, and may be indicative of the homogenization of biodiversity often

attributed to non-native species (McKinney, 2006; Petsch et al., 2022). Others have found similar results of decreased native diversity alongside increased non-native abundance in UFNAs (Aronson, 2007; Pregitzer et al., 2019b). However, those studies used species richness as the metric for diversity and incorporated shrubs, vines, and understory vegetation in their data collection. While Airola & Buchholz (1982) found that both native species richness and evenness declined over a 31-year period within the oak forests of the Greenbrook Sanctuary, a UFNA in New Jersey, they did not note the presence of non-native tree species.

Density and distribution of non-native tree species in the Old Forest presents an opportune moment for management as early control efforts, including mechanical and chemical methods, are more cost-effective than after populations have become entrenched (Reaser et al., 2020). Also, having 99% of non-native tree stems in the understory presents the opportunity to engage the local community in mechanical removal efforts (e.g., uprooting). However, the density of understory non-natives also suggests that species are disseminating into the forest through wind, avian, or human dissemination (Duinker et al., 2017) rather than by overstory seed sources within the forest. The popularity of non-native plants in urban landscapes may be providing this propagule pressure (Doroski et al., 2018). Indeed, during the inventory, non-native plantings were frequently observed within the park and surrounding neighborhoods just outside the Old Forest boundaries. This implies frequent monitoring will be necessary to identify new introductions even after containment of the existing populations. Thus, while non-native tree species appear to be impacting native species diversity, management efforts could still be effective at reducing their prevalence (Johnson & Handel, 2016).

## Loss of oak

The overall trend for oaks in the Old Forest mirrored trends found in forests across the eastern US where oak density and dominance has been declining (Fei et al., 2011). In the current study, overstory oaks dropped from 36 to 12% relative density, and oak seedlings declined from 9 to 3% relative density from 1987 to 2019. There was a moderate increase in sapling oak density, but this change was not significant. Reduction of oak density and dominance in the overstory was anticipated due to the lack of midstory oaks noted in 1987 (Guldin et al., 1990) and was consistent with other findings (Airola & Buchholz, 1982; Fahey et al., 2012; Piana et al., 2021a) where remnant oaks remain in the overstory albeit with gradually decreasing dominance. For instance, Fahey et al. (2012) documented a decreasing oak dominance (from 41 to 7% between 1820-2010) across the urban forests of the Chicago metropolitan region where oaks had historically been the dominant genus. Similarly, Airola & Buchholz (1982) documented a 31% decrease in red oak importance values within the Greenbrook Sanctuary, a New Jersey UFNA, between 1949 and 1980. Despite density reduction in the current study, as a group, oaks held the third highest importance value (IV = 14) in 2019 behind pawpaw (IV = 30) and tulip poplar (IV = 15) and made up 26% of the 2019 basal area. Thus, the remaining overstory oaks make up an important, albeit dwindling component of the site's large-tree character.

Reduction of oak seedling density in the current study was also consistent with other findings (Stalter, 1981; Rudnicky & McDonnell, 1989; Piana et al., 2021a). For example, Stalter (1981) found little oak reproduction in the understory of a NYC UFNA (Alley Park) over a 39-year period despite continued dominance of oaks in the overstory. Similarly, Rudnicky and McDonnell (1989) found oak retained its dominance alongside eastern hemlock (*Tsuga canadensis* L.) in the New York Botanical Garden Forest, a NYC UFNA, between 1937 and

1985, but was failing to regenerate. The current and above-referenced studies relied on seedling data collected from a single year and therefore may not represent average annual seedling abundances. However, Piana et al. (2021a) measured seedling data over three consecutive years and found oak seedling densities were significantly lower (2 stems per square meter) in the NYC UFNAs when compared to the rural forests (seven stems per square meter) surrounding the city.

In rural forests, the pattern of oak dominance in the overstory accompanied by lack of recruitment in the understory has often been attributed to disruption of historic fire regimes and subsequent replacement of fire-tolerant oaks by mesophytic and opportunistic species (Nowacki & Abrams, 2008; Hanberry & Nowacki, 2016; Alexander et al., 2021). However, in urban forests, other stressors such as non-native species (competition, reduced light levels, and allelopathy), heavy public use (trampling of the understory), and high densities of seed predators have been proposed as potential mechanisms limiting oak recruitment and eventually leading to replacement by opportunistic species (Sisinni & Emmerich, 1995; Rudnicky & McDonnell, 1989; Zipperer, 2010; Piana et al., 2019). Experimental studies examining the impact of urban stressors on oak recruitment would be helpful in the development of urban silvicultural strategies to prevent the loss of this foundational genus.

## **Densification**

While the increased midstory and sapling density found in the current study is reflective of a larger trend of eastern U.S. forests becoming more dense especially in these size categories (Nowacki & Abrams, 2008; Hanberry et al., 2020; Alexander et al., 2021), the extant literature on urban forest densification is scant. In the Old Forest, tree density increased by approximately seven percent (962 trees per ha) overall with the most substantive increases occurring in the midstory and sapling layers where density increased by 96 and 244%, respectively. Changes in

seedling density were not significant. While the current study results were consistent with Rudnicky and McDonnell (1989) who documented increased density in the midstory of a UFNA in NYC between 1937 and 1985, they were inconsistent with Airola and Buchholz (1982) who found reduced sapling density in a New Jersey UFNA between 1953 and 1980. Although not reporting change over time, Piana et al. (2021a) found higher sapling densities of NYC UFNAs when compared to surrounding rural forests. Factors often attributed to densification in rural forests included altered disturbance and browsing regimes, typically decreased fire and increased deer (*Odocoileus virginianus*) browse (Nowacki & Abrams, 2008; Hanberry, 2019; Alexander et al., 2021). However, urban forests face additional factors such as increased prevalence of non-native species and intentional plantings, which may promote densification, and heavy use and trampling, which may inhibit densification (Hamberg et al., 2011; Lehvavirta et al., 2014). Thus, further study is needed to clarify densification in UFNAs.

In rural forests, a dense sapling layer composed of small-maturing (< 15 m tall height at maturity) shade-tolerant trees has been shown to reduce tree regeneration and species diversity both directly through deeply reduced light levels under the canopy, and indirectly by providing protection for seed and seedling predators (Royo & Carson, 2008; Kain et al., 2011). In the current study, 80% of the 2019 sapling density was composed of small-maturing trees (Gilman, 1997). In particular, pawpaw made up 56% of the 2019 sapling density while the non-native cherry laurel made up 14%. While Baumer and Runkle (2010) recorded 63% lower tree regeneration under pawpaw canopy than outside of the canopy in a mature hardwood forest of Ohio, this effect had not been tested in urban forests. However, given that pawpaw canopy has been shown to reduce available light to less than 0.5% of full sunlight (Sipe, 2023), it is likely



that tree regeneration in the Old Forest is inhibited by a dense understory of small-statured trees such as pawpaw and cherry laurel.

Thus, the structure and composition of the Old Forest State Natural Area (i.e., the high midstory and sapling density and the high percentage of small-statured species) make it likely that reductions in species diversity will manifest over time. In fact, a reduction in midstory and sapling species diversity has already been noted in the current study. Also, given that dense understories have been shown to present obstacles to oak recruitment, continued decline of oak prevalence is also likely in the Old Forest (Lorimer et al., 1994; Nowacki & Abrams, 2008). Future research should examine mechanisms behind densification in urban forested natural areas and evaluate effectiveness of urban silvicultural strategies designed to reduce its negative impacts.

Given the potential consequences to species composition and stand structure in urban forested natural areas, management interventions should focus on reducing non-native species prevalence, preventing the loss of foundation species, and reducing the level of densification by small-statured species. However, given that urban silviculture is a nascent field (Johnson et al., 2021; Piana et al., 2021b), further research is also warranted, particularly research that examines the mechanisms behind these phenomena (i.e., non-native species invasions, oak recruitment failure, densification). Study results can be used to develop management and research priorities into the key aspects of urban forested natural area dynamics in Memphis and throughout the region.

## Tables

Table 2.1 Permanova results comparing species composition by size category between 1987 and 2019 in the Old Forest State Natural Area, Memphis, TN, USA.

		<b>df</b>	<b>SS</b>	<b>F</b>	<b>p-value</b>
<b>Overstory</b>	<i>Year</i>	1	2.15	2.476	0.013
	<i>Residual</i>	138	119.64		
<b>Midstory</b>	<i>Year</i>	1	2.65	3.696	0.001
	<i>Residual</i>	134	96.16		
<b>Sapling</b>	<i>Year</i>	1	43.66	23.175	0.001
	<i>Residual</i>	128	241.15		
<b>Seedling</b>	<i>Year</i>	1	13.59	4.664	0.001
	<i>Residual</i>	124	361.22		

Overstory = trees > 24.3 cm dbh; midstory = trees 9.1 cm < 24.3 cm dbh;  
 saplings = trees 0 cm < 9.1 cm dbh; seedling = trees 0.3 < 1.37 m tall.

Distance metric = robust Aitchison.

Table 2.2 Capscale scores, density change (trees per ha), and importance value (IV) change between 1987 and 2019 for tree species with an importance value of at least 1.0 in one inventory year in the Old Forest State Natural Area, Memphis, TN, USA.

species	capscale score				density change	IV change
	overstory	midstory	sapling	seedling		
<i>Acer negundo</i>	0.98	1.26	0.17	0.11	452.9	4.8
<i>Acer rubrum</i>	0.17	0.20	-0.05	-0.02	-72.5	-0.3
<i>Acer saccharum</i>	0.03	0.05	0.01	-0.01	-27.0	0.0
<i>Albizia julibrissin</i> *	0.00	0.01	0.00	0.06	150.1	0.7
<i>Asimina triloba</i>	0.00	0.77	1.50	-0.65	-598.4	-3.4
<i>Carpinus caroliniana</i>	0.00	0.04	-0.02	-0.04	-108.8	-0.6
<i>Carya cordiformis</i>	0.45	0.65	0.02	0.02	95.0	1.3
<i>Carya tomentosa</i>	0.11	0.14	-0.02	0.00	-2.6	0.3
<i>Celtis laevigata</i>	0.13	0.20	0.07	0.02	100.5	1.0
<i>Cornus florida</i>	0.00	-0.32	-0.05	-0.04	-140.5	-1.1
<i>Fraxinus americana</i>	-0.26	-0.07	0.01	-0.07	-174.5	-2.2
<i>Fraxinus pennsylvanica</i>	0.26	-0.01	0.11	0.24	691.3	4.5
<i>Liquidambar styraciflua</i>	0.26	-0.12	-0.01	0.00	-14.0	-0.4
<i>Liriodendron tulipifera</i>	0.59	-0.18	0.00	0.01	25.3	3.9
<i>Ostrya virginiana</i>	0.07	0.12	0.00	-0.01	-17.6	0.0
<i>Prunus caroliniana</i> *	0.00	0.10	0.51	0.37	1,349.0	6.8
<i>Prunus serotina</i>	-0.08	-0.13	0.00	0.04	88.5	-0.3
<i>Quercus alba</i>	-0.01	0.00	0.00	-0.07	-182.2	-0.5
<i>Quercus falcata</i>	-0.33	0.00	0.00	-0.02	-65.0	-3.5
<i>Quercus pagoda</i>	-0.44	-0.04	0.00	-0.05	-146.7	-6.0
<i>Quercus rubra</i>	-0.22	0.00	0.00	-0.01	-17.5	-1.5
<i>Quercus shumardii</i>	0.00	0.01	0.00	0.00	1.5	0.3
<i>Quercus velutina</i>	-0.12	-0.01	0.00	-0.06	-155.4	-1.9
<i>Sassafras albidum</i>	0.28	-0.09	-0.01	-0.19	-499.0	-2.2
<i>Ulmus alata</i>	0.06	0.17	0.05	0.02	87.2	0.5
<i>Ulmus americana</i>	0.17	0.36	0.04	0.00	49.8	0.8
<i>Ulmus rubra</i>	0.06	-0.06	0.04	0.00	31.6	0.1

\* Indicates non-native species. Positive capscale scores indicate higher association with 2019 inventory, negative capscale scores indicate higher association with 1987 inventory.

Table 2.3 Density by strata and year for all species, native species, non-native species and oak (*Quercus* species) in the Old Forest State Natural Area, Memphis, TN, USA.

Strata	Year	Density			
		All species	Native species	Non-native species	<i>Quercus</i> species
Overstory	2019	<b>107.5 ± 4.8</b>	<b>106.3 ± 4.9</b>	1.1 ± 1.0	<b>12.7 ± 1.6</b>
	1987	<b>77.6 ± 3.6</b>	<b>77.6 ± 3.6</b>	-	<b>28.0 ± 2.7</b>
Midstory	2019	<b>206.3 ± 10.8</b>	<b>199.6 ± 10.7</b>	<b>6.7 ± 2.0</b>	0.7 ± 0.5
	1987	<b>105.2 ± 7.8</b>	<b>105.2 ± 7.8</b>	-	1.7 ± 0.7
Sapling	2019	<b>2,944.6 ± 169.2</b>	<b>2,514.1 ± 152.0</b>	<b>430.6 ± 62.8</b>	13.1 ± 5.4
	1987	<b>856.5 ± 89.8</b>	<b>856.5 ± 89.8</b>	-	3.3 ± 2.3
Seedling	2019	7,394.4 ± 861.7	6,308.6 ± 721.3	<b>1,085.8 ± 259.3</b>	<b>243.4 ± 113.9</b>
	1987	8,921.6 ± 1,058.5	8,921.6 ± 1,058.5	-	<b>805.6 ± 313.4</b>

Values are mean ± standard error. Density = trees per hectare. Overstory = trees >24.3 cm dbh; midstory = trees 9.1 cm < 24.3 cm dbh; sapling = trees 0 cm < 9.1 cm dbh; seedlings = trees 0.3 m < 1.37 m tall. Boldface type indicates significant difference between 1987 and 2019 (P<0.05 Mann-Whitney U test [Mann & Whitney, 1947]).

Table 2.4 Richness, Shannon Diversity Index, and evenness by size category and native status in the Old Forest State Natural Area, Memphis, TN, USA.

		<u>Richness</u>		<u>Diversity (H')</u>		<u>Evenness (J)</u>	
		1987	2019	1987	2019	1987	2019
All strata	All species	<b>9.6 ± 0.4</b>	<b>11.9 ± 0.4</b>	<b>1.715</b>	<b>1.848</b>	0.451	0.461
	Native only	<b>9.6 ± 0.4</b>	<b>10.9 ± 0.4</b>	<b>1.715</b>	<b>1.608</b>	0.451	0.415
Overstory	All species	<b>3.9 ± 0.2</b>	<b>4.9 ± 0.2</b>	2.557	2.711	0.759	0.757
	Native only	<b>3.9 ± 0.2</b>	<b>4.9 ± 0.2</b>	2.557	2.676	0.759	0.759
Midstory	All species	<b>3.0 ± 0.2</b>	<b>4.6 ± 0.2</b>	2.815	2.692	0.864	0.757
	Native only	<b>3.0 ± 0.2</b>	<b>4.4 ± 0.2</b>	<b>2.815</b>	<b>2.587</b>	0.864	0.768
Sapling	All species	<b>3.0 ± 0.2</b>	<b>5.6 ± 0.3</b>	<b>2.234</b>	<b>1.819</b>	0.678	0.493
	Native only	<b>3.0 ± 0.2</b>	<b>4.7 ± 0.3</b>	<b>2.234</b>	<b>1.607</b>	0.678	0.445
Seedling	All species	<b>3.5 ± 0.3</b>	<b>2.4 ± 0.2</b>	<b>1.551</b>	<b>1.684</b>	0.447	0.553
	Native only	<b>3.5 ± 0.3</b>	<b>1.9 ± 0.2</b>	<b>1.551</b>	<b>1.416</b>	0.447	0.481

Richness values are mean ± standard error from inventory plots. Diversity = Shannon Diversity Index ( $H'$ ), evenness = Pielou's  $J$ . Boldface type indicates significant difference between 1987 and 2019 ( $p < 0.05$ , Richness evaluated with Mann-Whitney  $U$  test (Mann & Whitney, 1947), diversity evaluated with Hutcheson's  $t$ -test).

## Figures

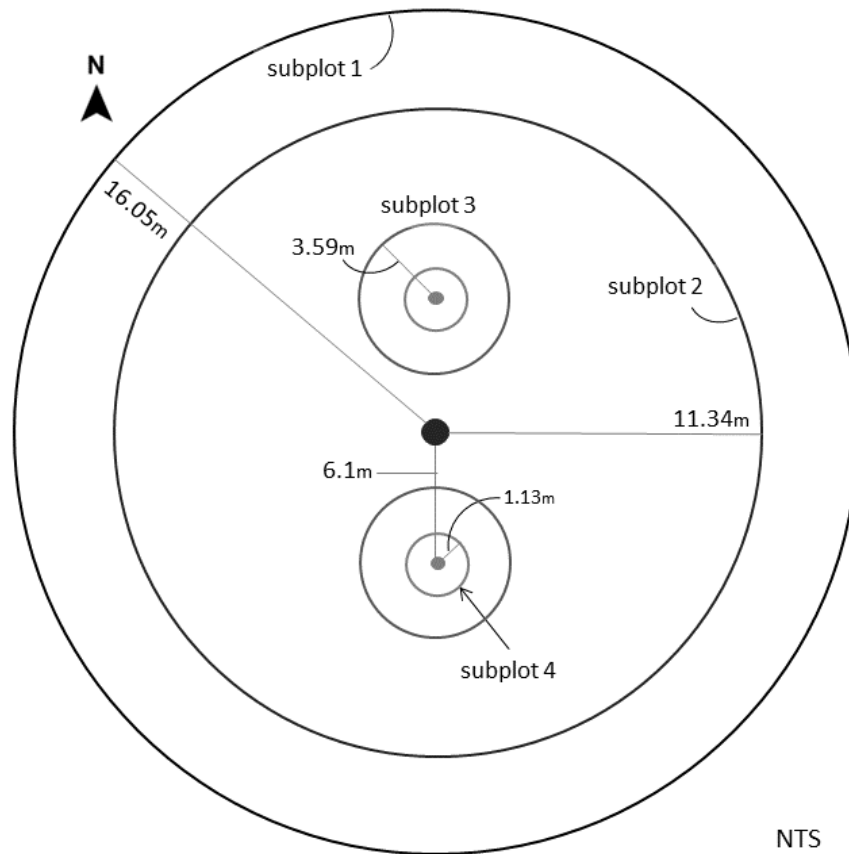


Figure 2.1 Nested plot design implemented in 1987 and 2019 in the Old Forest State Natural Area, Overton Park, Memphis, TN, USA.

Overstory trees (>24.3 cm dbh) were measured in subplot 1, midstory trees (9.1 cm < 24.3 cm dbh) in subplot 2, saplings (0 cm < 9.1 cm dbh) in subplot 3, and seedlings (trees 0.3 m < 1.37 m tall) in subplot 4. NTS = not to scale.

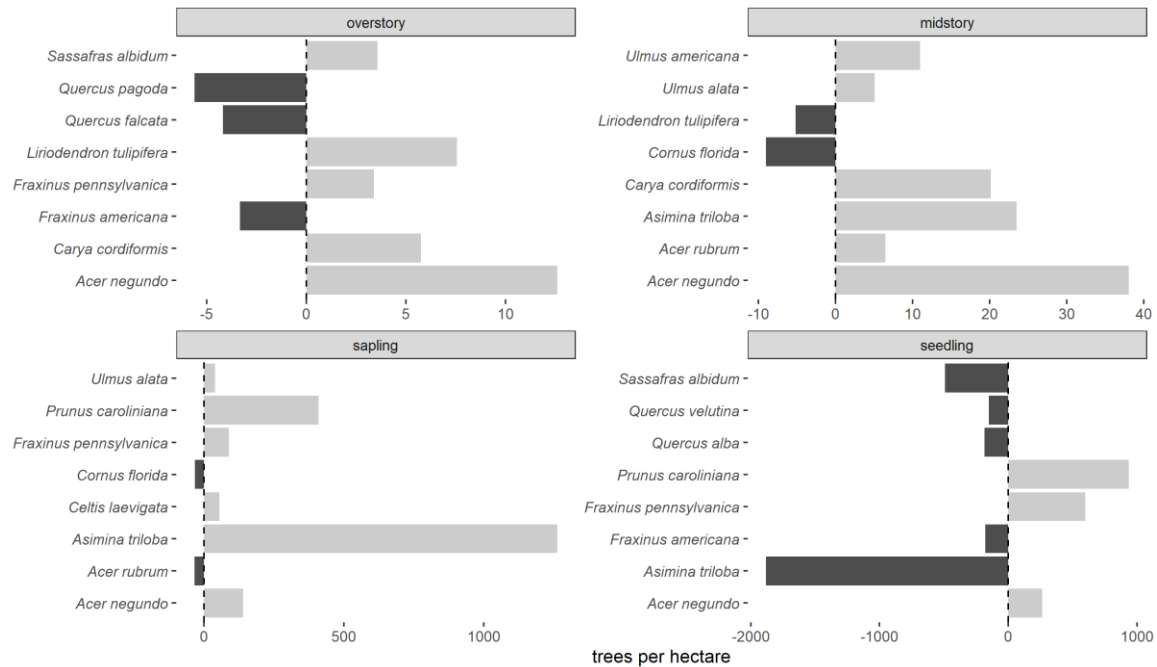


Figure 2.2 Change in density (trees per hectare) of species with the highest positive or negative capscale scores by size category in the Old Forest State Natural Area, Memphis, TN, USA between 1987 and 2019.

Bars to the right of zero indicate increased density. Bars to the left of zero indicate decreased density.

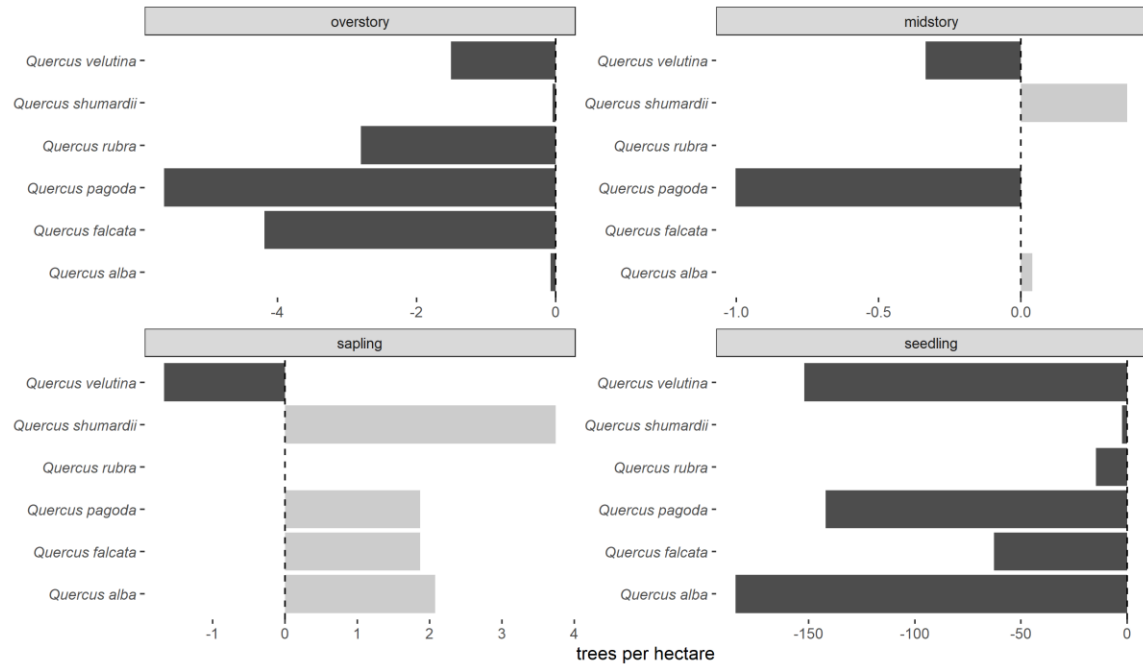


Figure 2.3 Change in oak (*Quercus* spp.) density (trees per hectare) by size category in the Old Forest State Natural Area between 1987 and 2019.

Bars to the right of zero indicate increased density. Bars to the left of zero indicate decreased density.



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CHAPTER III  
SEED PREDATION AND SEEDLING EMERGENCE OF NORTHERN RED OAK AND  
TULIP POLAR IN RESPONSE TO LEAF LITTER, UNDERSTORY VEGETATION,  
AND CANOPY COVER IN AN URBAN FORESTED NATURAL AREA

**Abstract**

Urban forested natural areas (UFNAs) provide valuable ecosystem services within the urban environment. Continuity of these ecosystem services depends on the regeneration of desired tree species, yet the dynamics of early tree establishment processes in these forests remain poorly understood. Understanding how site factors affect seed predation, germination and seedling emergence provides a foundation for informed management strategies in UFNAs. This study, conducted in the Old Forest State Natural Area in Memphis, Tennessee from December 2021 to November 2022, investigated the impacts of leaf litter removal, understory vegetation removal, and canopy condition (gap versus closed canopy forest) on seed predation of northern red oak (*Quercus rubra*, L.) and seedling emergence of northern red oak and tulip poplar (*Liriodendron tulipifera*, L.). We predicted lower predation rates in canopy gaps due to increased risk for small mammals such as eastern gray squirrel (*Sciurus carolinensis*) and in leaf litter retention treatments due to increased acorn hiding cover. We also predicted higher emergence rates in canopy gaps due to increased light levels and soil moisture, and in leaf litter removal treatments due to increased light levels and temperature fluctuations as well as a reduction in physical barriers to shoot emergence. Results revealed high predation rates (99.6%) on northern

red oak acorns, but with no significant variation among experimental treatments. Northern red oak seedling emergence (82%) was significantly higher in leaf litter removal treatments and under canopy gaps but was unaffected by understory vegetation removal. In contrast, tulip poplar seedling emergence (2.5%) was marginally higher in leaf litter removal and significantly higher in understory vegetation removal treatments. Our findings suggest that relying solely on natural regeneration in UFNAs without targeted interventions may lead to a decline in select tree abundance.

### **Introduction**

Urban forested natural areas (UFNAs) provide a host of ecosystem services to cities and towns that surround them such as air and water pollution reduction (Escobedo et al., 2011), carbon sequestration (Nowak et al., 2007), and biodiversity conservation (Ives et al., 2016; Lepczyk et al., 2017). Native trees, in particular, have co-evolved with, and provide habitat for, a diverse suite of native flora and fauna including insects, birds, and pollinators (Pickett et al., 2011) often serving as refugia for plants and animals in the urban environment (Lepczyk et al. 2017; Gentili et al., 2023). Although studies suggest the canopy of UFNAs is currently composed primarily of native tree species (Pregitzer et al., 2019), continued dominance of these species in the absence of management interventions is uncertain as studies have found native tree recruitment failures in urban forested natural areas (Burton et al., 2005; Labatore et al., 2016; Piana et al., 2021a; Nagele et al. 2024). Developing appropriate management strategies requires an understanding of factors affecting early establishment (i.e., seed germination, seedling emergence, survival, and growth), which are not well studied in urban forests (Piana et al., 2021c). During early establishment in rural forests, leaf litter, understory vegetation, and canopy gaps interact with species functional traits such as seed dispersal mechanisms and germination

strategies (hypogeal or epigeal) to create ecological filters and set the trajectory of future species composition (Clark et al., 2007; McConkey et al., 2012; Piana et al. 2019). For example, a covering of deep leaf litter can inhibit germination of small-seeded species more than large-seeded species (Sayer, 2006), dense understory vegetation reduces the regeneration of shade-intolerant species more than shade-tolerants (Lombaerde et al., 2020), and canopy gaps decrease seed predation from small mammal seed predators (Sharik & Buckley, 2002). However, it is not known if the urban environment modifies how these site factors affect seed predation, seed germination, and seedling emergence in UFNAs (Facelli & Pickett, 1991; Beckage et al., 2000; Beckage & Clark, 2005; Piana et al., 2019; Doroski et al., 2022). Thus, studies elucidating mechanisms by which leaf litter, understory vegetation, and canopy gaps affect seed predation and seedling emergence in UFNAs are necessary to ensure the continued recruitment of native trees.

Within the early establishment process, post-dispersal seed predation and removal through consumption or caching, is an important factor influencing early tree establishment in forests throughout the world (Hulme, 1998; Howe & Brown, 2001; Vander Wall et al., 2005; Larios et al. 2017). Seed predation can inhibit seed germination through consumption (Bogdziewicz et al., 2019) or enhance seed germination through gut passage scarification (Samuels & Levey, 2005), dispersal from the parent tree, and burial in soil (Vander Wall et al., 2005; Gu et al., 2017). Seed predation can also indirectly alter community composition by the preferential removal of seeds with different traits thereby changing the competitive dynamics within a forest (Hulme, 1998; Beckage & Clark, 2005), and limit the range of suitable sites (Brown & Vellend, 2014). While studies have demonstrated higher levels of seed predation in urban forests than in surrounding rural forests (Overdyck et al., 2013; Piana et al., 2019a), it

remains a little studied topic. Seed predation likely interacts with anthropogenic factors such as habitat fragmentation, heavy human use, and alternative food sources for predators (Piana et al., 2019a; Gelmi-Candusso & Hamalainen, 2019) leading to potentially species- and site-specific effects. Thus, a better understanding of the mechanisms affecting seed predation in UFNAs is important to evaluate the effectiveness of natural regeneration and potential impacts on native tree composition.

Similar to seed predation, leaf litter is widely known as an important factor impacting early establishment of tree species in forests throughout the world (Farris-Lopez et al., 2004; Dupuy & Chazdon, 2008). By changing the environment of the understory (Facelli & Pickett 1991) and by interacting with other environmental factors (George & Bazzaz 1999), leaf litter can have species-specific impacts on seed germination and seedling survival, thereby affecting tree species composition in forests (Sayer, 2006). For example, deep litter layers in forests have been shown to create physical impediments to seedling survival by limiting shoot emergence from below or preventing radicle penetration to the soil surface (Facelli & Pickett, 1991; George & Bazzaz, 1999; Brose, 2001; Sayer, 2006). Also, the presence of leaf litter layers has been shown to inhibit germination by reducing light quantity and quality at mineral soil level (Vazquez-Yanes et al., 1990), attenuating temperature fluctuations needed by some species for germination (Vazquez-Yanes & Orozco-Segovia, 1982; Molofsky & Augspurger 1992), and by hosting insect and fungal pathogens associated with damping off disease (Cram, 2003; Arthur et al., 2012; Greenler et al., 2020). In contrast, a covering of leaf litter can enhance germination by retaining moisture and preventing seed desiccation (Garcia et al., 2002; Dupuy & Chazdon, 2008).

The impact of leaf litter on seed predation and seedling emergence in UFNAs is not well known (Piana et al., 2021a). Studies have documented shallower leaf litter depths in urban forests compared to surrounding rural forests (Kostel-Hughes et al., 1998; Piana, 2019) along with associated higher recruitment rates for small-seeded species as litter depth decreases (Kostel-Hughes et al., 2005). However, due to the exclusion of fire, many UFNAs may not experience periodic, albeit temporary, reductions in leaf litter that can create germination opportunities for certain groups of species such as oaks (*Quercus* spp., Royse et al., 2010). Thus, developing a better understanding of leaf litter dynamics in UFNAs is important for the development of management strategies designed to enhance native tree recruitment.

Along with leaf litter, understory vegetation is well known to influence early establishment in forests worldwide (Royo & Carson, 2006; Lombaerde et al., 2021) primarily through light attenuation (Lombaerde et al., 2020). For example, understory vegetation can reduce light quantity to < 1% of full sunlight and light quality (e.g., red: far-red ratios) by over 90% (Horsley, 1993; Messier et al., 1998), thereby reducing site suitability to species capable of germination, survival, and growth in low light environments (Oliver & Larson, 1996; Royo & Carson, 2006). In addition, understory vegetation interacts with other conditions that affect early establishment such as seed predator activity and leaf litter depths (Oliver & Larson, 1996; Royo & Carson 2008). For example, dense understory vegetation layers have been shown to provide hiding cover for granivorous small mammals leading to an increase in seed predation rates (George & Bazzaz, 1999; Royo & Carson, 2008; Greenler et al., 2019) along with increasing humidity levels favoring the growth of fungal pathogens of seedlings (Horsley, 1993). Some studies have found an association between dense understory vegetation and limited tree establishment in urban forests (Bornkamm, 2007; Piana et al., 2021a), others have found the

indirect effects of understory vegetation, such as increased seed predation, to be more important in explaining native tree recruitment failure (Labatore et al., 2016). Others found dense understory vegetation was not a factor in urban tree recruitment failure (Ettinger et al., 2017) and was associated with reduced seed predator activity in urban forests (Bowers & Breland, 1996). Given the potential impact to seed predation and seedling emergence, and the lack of clear trends, studies examining the impact of understory vegetation on early establishment in urban forests are important to inform management strategies in UFNAs.

Canopy gaps create regeneration opportunities by increasing light exposure, soil moisture, and temperature fluctuations (Collins et al., 1985; Canham et al., 1990) and are a critical component of the early establishment process globally (Runkle, 1981; Yamamoto, 2000; Muscolo et al., 2014). Gaps can also enhance species diversity due to their varied sizes and shapes (Denslow, 1987; Kneeshaw & Prevost, 2007; Muscolo et al., 2014; Kern et al., 2017). For example, shade tolerant species have been shown to dominate the regeneration in small gaps with opportunistic species dominating larger openings (Runkle, 1982). Canopy gaps can also interact with factors such as leaf litter, understory vegetation, and seed predator behavior leading to the inhibition of native tree regeneration (Schnitzer et al., 2000; Dupuy & Chazdon, 2008). For example, canopy gaps can lead to a dense understory vegetation thereby limiting the early establishment of native tree seedlings (Beckage et al. 2000; Royo & Carson, 2006). Also, leaf litter moisture levels may be lower in canopy gaps due to higher evaporation rates in open light environments (Kreye et al., 2018; Alexander et al., 2021) thereby reducing the prevalence of fungal and insect pathogens on seedlings (Cram, 2003; Reinhart et al., 2010). The role of canopy gaps in UFNAs is less studied but are often reported as invaded by vines and non-native species (Labatore et al., 2016; Johnson & Handel, 2016; Massad et al., 2019) and their ecological



degradation is a common concern of urban forest managers (Piana et al., 2021b). Thus, developing a better understanding of the impact of canopy gaps on regeneration dynamics in UFNAs is essential to ensure the recruitment of a diverse suite of native tree species and prevent the disruption of gap-dynamics (sensu Royo & Carson 2006).

The current study was conducted in a UFNA (Old Forest) of Memphis, Tennessee where a 1987 inventory (Guldin et al., 1990) documented a lack of native overstory tree recruitment, despite an abundant seed source and the presence of canopy gaps. The authors hypothesized that the presence of a dense understory vegetation layer had severely restricted tree regeneration. A second inventory in 2019 (see Chapter Two), revealed a substantial increase in non-native tree species abundance and a decrease in native tree regeneration compared to 1987. Thus, the current study sought to better understand early establishment processes, specifically the interacting effects of leaf litter, understory vegetation, and canopy gaps on seed predation and emergence rates of native overstory species in the Old Forest. We hypothesized that seed predation rates on northern red oak (*Quercus rubra* L.) would be lower in canopy gaps and in leaf litter retention treatments. These hypotheses are based on the assumptions that canopy gaps would increase risk to granivorous mammals from avian predators while leaf litter would provide acorn hiding cover. We also hypothesized that emergence rates of northern red oak and tulip poplar (*Liriodendron tulipifera*, L.) would be higher in canopy gaps and leaf litter removal treatments. These hypotheses are based on the assumptions that light levels and soil moisture would increase in canopy gaps while light levels and soil temperature fluctuations would increase while physical impediments would decrease in leaf litter removal treatments. Study results may contribute to the development of management strategies to ensure native tree recruitment in the Old Forest and other urban forested natural areas.

## Methods

### Study area

The current study was conducted in the Old Forest State Natural Area (Old Forest) in Memphis, Tennessee, USA. The Old Forest is a 58 ha mixed mesophytic forest located within the boundaries of the 138 ha Overton Park and situated within Braun's (1950) Western Mesophytic Region, Mississippi Embayment Section. More broadly, it is situated within the Humid Temperate Domain, Subtropical Division, Southeastern Mixed Forest Province (Bailey, 1995). Soils are well-drained Alfisols with a depth to restrictive layer of more than 200 centimeters (cm) with slopes of 2-5% (Sease et al., 1989). The climate is characterized by mild winters and hot summers. Average annual temperatures are 16.8 °C with the highest mean temperature of 27.9 °C occurring in July and lowest mean temperature of 4.8 °C occurring in January (USDC, 2020). Precipitation averages 134.5 cm per year with the highest monthly average of 14.6 cm occurring in December and lowest monthly average of 7.3 cm occurring in August (USDC, 2020). The site is known to contain over 330 species of vascular plants and 58 species of trees (Heineke, 2009). The overstory is composed primarily of oaks and tulip poplar with scattered specimens of cottonwood (*Populus deltoides* W. Bartram ex Marshall), sycamore (*Platanus occidentalis* L.), sweetgum (*Liquidambar styraciflua* L.), white and green ash (*Fraxinus americana* L., *Fraxinus pennsylvanica* Marshall; Guldin et al., 1990). The largest specimen recorded was a tulip poplar with a diameter at 1.3 meters (m) above mean ground level (dbh) of 180 cm, height of 50 m, and age of 189 years (Bridges 2019). The site contains at least 16 species of woody invasive plants including tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle), princess tree (*Paulownia tomentosa* Thunb.), paper mulberry (*Broussonetia papyrifera*

(L.) L'Her ex Vent.), and mimosa (*Albizia julibrissin* Durazz); Miller et al., 2010; Heineke, 2009).

### **Experimental design**

In 2022, canopy gaps  $> 150 \text{ m}^2$  and  $> 5 \text{ m}$  from a trail, roadway, or other gaps were identified through analysis of LiDAR data (Goddard, 2014) and aerial imagery. Twelve gaps meeting these criteria were randomly selected for sampling. Blocks were established to include each sample gap and the adjacent closed canopy forest. Each block consisted of two  $1 \times 2\text{-m}$  plots, one placed within the canopy gap and another place within the adjacent closed canopy forest at least  $5 \text{ m}$  from the edge of the expanded gap and no further than  $20 \text{ m}$  apart.

The experiment was designed as a  $3 \times 2$  split-split-plot factorial layout with treatments assigned to split plots using a randomized complete block design (Potvin, 2001; Dupuy & Chazdon, 2008; Gotelli & Ellison, 2018). Three treatments – canopy condition, understory vegetation treatment, and leaf litter treatment – each with two levels, were applied at the whole plot, split-plot, and split-split-plot levels, respectively. Canopy condition – gap or forest – was considered the whole plot treatment. Each plot was then split into two  $1 \times 1\text{-m}$  sub-plots. Within one randomly assigned sub-plot, all understory vegetation including trees  $< 10 \text{ cm}$  dbh were manually removed while the remaining sub-plot had understory vegetation retained. Resprouted stems were periodically clipped from the vegetation removal sub-plots throughout the experiment. Each  $1 \times 1\text{-m}$  sub-plot was divided into two  $0.5 \times 1\text{-m}$  sub-sub-plots. Within one randomly assigned sub-sub-plot, all leaf litter was carefully removed by hand while the remaining sub-sub-plot had leaf litter gathered from the surrounding forest floor placed at a uniform depth of  $4 \text{ cm}$ . Leaf removal treatments were reapplied to the leaf litter removal sub-sub-plots as needed throughout the experiment. Each sub-sub-plot was split into two  $0.5 \times 0.5\text{-m}$

quadrats. Within one randomly assigned quadrat, a seed predator enclosure was placed while in the remaining quadrat an open wire cage of the same size was placed (Figure 3.1). Seed predator enclosures consisted of 1.2-cm galvanized wire construction hardware cloth cut into a 1,000 cm<sup>2</sup> circle, mounted to a 15-cm sheet metal skirt, and trenched 15 cm into the soil. Quadrats without predator enclosures included galvanized hardware cloth cut to the same diameter of the predator enclosures but without a wire top and attached to the soil with garden staples. Plots were installed in December 2021.

Effects of all treatments and their interactions were tested on the emergence of tulip poplar and northern red oak. These test species were chosen to represent differing life strategies, growth rates, shade tolerances, and seed sizes from overstory species known to occur in this forest. Tulip poplar represented a shade-intolerant, fast growing, small-seeded species. Northern red oak represented a moderately shade tolerant, medium-fast growing, large-seeded species (Burns & Honkala, 1990). Within each predator enclosure, 20 tulip poplar seeds and 12 northern red oak acorns were placed systematically at a spacing based on supplier (F.W. Schumacher, Sandwich, MA) recommended sowing density. Tulip poplar seeds were placed on the soil surface while acorns were placed approximately 1 cm into the soil. In litter removal sub-sub-plots, seeds were placed on or in the soil before the litter layer was carefully replaced. Effects of all treatments and their interactions were tested on predation rates of northern red oak only as the seeds were large enough to observe removals and were unlikely to wash away during rain events, whereas tulip poplar seeds were considered too small to accurately relocate. Twelve northern red oak acorns were placed systematically throughout each predator accessible quadrat. The final sample sizes were 1,152 acorns and 1,920 tulip poplar seeds in predator enclosures and 1,152 acorns in predator accessible quadrats. All seeds were sourced from Kentucky and Tennessee,

purchased from F.W. Schumacher, Sandwich, MA, USA, and kept in cold storage until field placement in December 2021. Acorns were float tested before field placement (Bonner, 2008).

Plots were checked for seed predation and seedling emergence approximately every three weeks from December 2021 through November 2022. Seedling emergence was defined as the appearance of a true leaf (Forcella et al., 2000). The number of removed acorns and emerged seedlings were tallied by species, plot, and treatment at the conclusion of the experiment.

### **Data analysis**

All analyses were performed using R Statistical Software (v4.3.2; R Core Team, 2023) and an  $\alpha$  of 0.05. Due to high levels of seed removal (99.6%), a model was not developed to test the significance of treatment on levels of seed predation. To test the relative importance of canopy condition, understory vegetation, and leaf litter, a generalized linear mixed model was fitted to the proportion of shoots that emerged as a function of the number of seeds added to each quadrat. Separate species-specific models were generated with treatments and their interactions included as fixed factors with block, block x canopy condition, and block x canopy condition x vegetation treatment included as random effects. Models were fitted with a beta binomial error distribution and a logit-link function using the glmmTMB package (Brooks et al., 2017). Significance of fixed effects was tested using Type II Wald chi-square tests. Treatment means were back transformed from the log odds ratio and reported as percentage emergence for each treatment combination. One quadrat was removed from the analysis due to vandalism (trampling) in the field.

## **Results**

### **Seed predation**

Of the 1,152 northern red oak acorns placed in the predator accessible quadrats, 1,147 (99.6%) were removed by seed predators during the experiment. Due to the high number of removals, statistical analysis of removals by treatment was not conducted.

### **Oak seedling emergence**

Of the 1,140 northern red oak acorns evaluated in the predator exclosures, 931 emerged (81.7%) with a range of 33.3% to 100% per quadrat. Emergence was significantly higher in the litter removal treatment (85.9%) than in the litter retention treatment (77.3%, Table 3.1, Table 3.2). Vegetation treatment did not have a significant effect on emergence rates. Emergence was also significantly higher in canopy gaps (84.6%) than in the closed canopy forest (78.8%). None of the interaction terms in the model were significant.

### **Tulip poplar seedling emergence**

Of the 1,900 tulip poplar seeds evaluated, 47 emerged (2.5%) with a range of 0% to 25% per quadrat. While there was a general trend toward higher emergence in the litter removal treatment (3.2%) compared to the litter retention treatment (1.7%), these results were not significant (Table 3.1, Table 3.2). Emergence was significantly higher in the vegetation removal treatment (4.3%) than in the vegetation retention treatment (0.6%). Canopy treatment did not have a significant effect on emergence rates and none of the interaction terms in the model were significant.

## Discussion

Results of the current study suggest that oak and tulip poplar face recruitment challenges in urban forested natural areas similar to those found in rural forests, albeit from potentially different sources and at potentially different magnitudes. For example, despite fewer taxa of seed predators, acorn removal rates for northern red oak in the current study were 99.6% and did not vary by experimental treatment. While the study duration was limited to one year, these predation rates may indicate that urban oak have fewer safe germination sites than rural oaks. Also, while oak germination responded favorably to leaf litter removal and canopy gap treatments, the background germination rates (77 to 86 %) suggest that barriers to oak recruitment in the Old Forest are not due to a lack of suitable sites. In contrast, tulip poplar emergence was low (0.6 to 4.3 %) but responded favorably to understory vegetation removal suggesting the barriers to its recruitment are related to a lack of suitable sites. However, the urban environment may create recruitment barriers for both test species (northern red oak and tulip poplar). Specifically, elevated levels of eastern gray squirrels in urban areas may increase seed predation rates on oak while the high densities of non-native tree species may create dense understory vegetation layers inhibiting successful emergence of tulip poplar. Therefore, without management intervention, the prevalence of oak and tulip poplar are likely to continue declining in the Old Forest.

### Seed predation

Seed predation levels on northern red oak acorns in the current study (99.6%) were consistent with Myster and Pickett (1993) who found 96% northern red oak acorn predation in the Hutcheson Memorial Forest, a UFNA of New Jersey. Also, northern red oak experience high rates of seed predation in rural forests, with 80% or more of the acorn crop being predated during

normal seed years and up to 100% predation in poor seed years (Shaw, 1968; Sander, 1990; Plucinski & Hunter, 2001). Thus, while studies have shown generally higher rates of seed predation across many species in UFNAs (Bowers & Breland, 1996; Aronson & Handel, 2011; Piana et al., 2019; Piana, 2019), our results may fall within a normal range for northern red oak during average to poor seed production years. Future studies should compare acorn predation levels to background rates of seed production in the Old Forest to determine if seed predation is limiting recruitment during mast years.

Seed predator taxa were not explicitly identified in the current study, though field camera footage revealed that eastern gray squirrel (*Sciurus carolinensis*) was the most common plot visitor. Also, no white-tailed deer (*Odocoileus virginianus*), eastern wild turkey (*Meleagris gallopavo*), or white-footed mice (*Peromyscus leucopus*) were known to inhabit the Old Forest (Roznik, 2020) nor was there evidence on field camera footage. Urban squirrel populations are theorized to be higher in urban forests due to the presence of supplemental anthropogenic food sources (Bower & Breland, 1996; Parker & Nilon, 2008). Thus, our results may be the result of an elevated squirrel population in the Old Forest and surrounding park as has been noted by others in other locations (Parker & Nilon, 2008; Overdyck et al., 2013). However, future studies should compare squirrel populations and seed predation rates between the current study site and the surrounding rural forests.

In the current study, seed predation rates were unaffected by leaf litter, understory vegetation, and canopy treatments. Others have found leaf litter provides hiding cover for acorns and reduces overall seed predation rates on oaks (Shaw, 1968; Sharik & Buckley 2002). However, it is possible that shallow leaf litter depths found in urban forests (Kostel-Hughes et al., 1998), or the 4-cm leaf litter depth used in the current study, do not provide sufficient cover



to prevent predation (Myster & Pickett, 1993). In addition, dense understory vegetation has been shown to provide hiding cover for small mammal seed predators resulting in increased seed predation rates (George & Bazzaz, 1999). However, squirrel populations in urban areas have been found to be higher than in rural areas (Overdyck et al., 2013) and exhibit more aggressive behavior (Parker & Nilon, 2008) possibly negating the effect of understory vegetation on seed predation rates. Finally, while seed predators are known to spend less time in open habitats that expose them to predators, such as canopy gaps (Hulme, 1998; Schreiner et al., 2000), this often does not reduce overall seed predation rates, but rather alters seed predator behavior such that seeds are removed from the open area before being consumed or cached (Plucinski & Hunter, 2001). However, studies documenting the effect of urban forest canopy gaps on seed predator behavior and predation rates are lacking.

Seed predation in UFNAs has been proposed as one of the reasons oaks face higher regeneration barriers in urban compared to rural forests (Bowers & Breland, 1996; Aronson & Handel, 2011; Piana et al., 2019). While current study results do not conclusively prove this, the high level of seed predation observed combined with the lack of oak seedlings in the forest (see Chapter Two) suggest that seed predation may play an important role in oak recruitment at this site. However, it should be noted that the current study measured seed removal, which is not synonymous with seed consumption (Vander Wall et al., 2005), for a single oak species over one year. Seed predator behavior may vary by oak species and over time. For instance, squirrels are known to preferentially cache red oak acorns and consume white oak acorns (Steele et al., 2001) and the percentage of seeds removed is typically lower during mast years (Plucinski & Hunter, 2001). Future research should examine acorn predation rates over multiple species and multiple

years to capture varying rates of seed production and better determine the role seed predation plays in oak regeneration in this UFNA.

### **Oak seedling emergence**

The emergence of the northern red oak in the current study generally followed patterns found in rural forests and provided insight into potential recruitment limitations in urban forested natural areas. Overall emergence rates for northern red oak were relatively high (82%) but consistent with the expected rates for the species (Hopper et al., 1985; Bonner, 2008). Also, the observed increase in northern red oak emergence within the **leaf litter** removal treatment was consistent with observations that leaf litter removal accentuates soil temperature fluctuations thereby enhancing the germination of many species (Facelli & Pickett, 1991; Baskin & Baskin, 2014). Litter removal should also reduce the likelihood of seed exposure to fungal pathogens known to inhabit damp leaf litter (Greenler et al., 2020) thereby reducing seed mortality. Further, these results were aligned with rural forest studies wherein oak germination is enhanced when leaf litter is removed via fire (Royse et al., 2010; Nation et al., 2021) or manually (Garcia et al., 2002). Conversely, Piana (2019) found no relationship between leaf litter removal and germination of northern red oak in UFNAs of New York. However, their background litter depths were shallower than in the surrounding rural forests. Thus, the effect of leaf litter on oak emergence may be site specific in urban forests.

Also, in the current study oaks emerged at higher rates in **canopy gaps** than in closed canopy forests but were unaffected by understory vegetation removal treatments. Canopy gaps and understory vegetation removal should have increased light quantity and quality in the understory (Horsley, 1993; Muscolo et al., 2014; Lombaerde et al. 2020). Therefore, our results suggested that oak emergence was more influenced by changes in soil temperature and moisture,

as has been documented in canopy gaps (Collins et al., 1990; Canham et al., 1990), rather than by increased light in the understory. Thus, current study findings were compatible both with the understanding that large-seeded species such as northern red oak are less dependent on the understory light environment than small-seeded species (Burns & Honkala, 1990; Baskin & Baskin, 2014) and also with the findings of other studies that found increased oak emergence in rural forest canopy gaps (Clinton et al., 1994; Rentch et al., 2003; Izbicki et al., 2020). However, to our knowledge, this was the first study examining oak seedling emergence in urban forest canopy gaps.

While our results suggest that oaks could be successfully germinated in urban forest canopy gaps, several studies have documented that urban forest gaps were heavily invaded by dense stands of non-native woody vegetation with little or no observable tree recruitment (Piana et al. 2021c; Massad et al. 2019; Doroski et al. 2018; Labatore et al. 2016). Further, studies have found dense understory vegetation inhibits oak seedling survival and growth (Lorimer et al. 1994). Thus, understory vegetation removal may be more important to seedling survival and growth in urban forest canopy gaps than to germination. Future studies should examine oak seedling survival and growth in response to understory vegetation removal treatments, particularly in urban forest canopy gaps.

### **Tulip poplar seedling emergence**

The emergence of tulip poplar seedlings in the current study was inconsistent with patterns found in rural forests and provides unique insight into the recruitment dynamics of the species in UFNAs. Overall emergence rates for tulip poplar were low (2.5%) but consistent with the expected rates for the species (Boyce & Kaiser, 1961; Kota et al., 2007). Also, increased tulip poplar emergence observed in understory vegetation removal treatments and marginally higher

emergence in leaf litter removal treatments was consistent with the ecology of small-seeded species that depend on understory light for germination (Clark & Boyce, 1964; Baskin & Baskin 2014). Both understory vegetation and leaf litter removal have been shown to improve light availability in the understory (Vazquez-Yanes et al., 1990; Facelli & Pickett 1991). Marginally higher emergence in leaf litter removal treatments was understandable, considering leaf litter can present a physical barrier to the germination of small-seeded species preventing the root radicle from reaching the soil or the epicotyl from reaching above the leaf litter layer (Farris-Lopez et al. 2004; Royo & Carson 2006). Thus, tulip poplar in the Old Forest appeared to respond strongly to increased light availability as predicted.

The lack of improved tulip poplar emergence in canopy gaps, however, was contrary to these findings as well as those of others that have noted tulip poplar seedling emergence improved in rural forest canopy gaps (Poulson & Platt, 1989; Lhotka, 2012). While no studies have examined urban forest canopy gap effects on tulip poplar seedling emergence, we anecdotally observed tulip poplar seedlings emerging in canopy gaps with understory vegetation removed outside the boundaries of the experimental quadrats suggesting these seedlings emerged from the seed bank. Also, a previous one-year duration study in the Old Forest found that tulip poplar seedlings only occurred in canopy gaps and not in the surrounding closed canopy forest (Massad et al., 2019). Thus, the lack of tulip poplar seedling emergence in our canopy gaps was likely not due to low light. Rather, given that tulip poplar seeds may require multiple seasons of stratification to obtain successful germination (Clark & Boyce, 1964; Olson, 1969), the lack of seedling emergence in canopy gaps was likely due to an insufficient stratification period (i.e., one season) used in this study.

To our knowledge, this is the first study examining how leaf litter, understory vegetation, and canopy gaps affect tulip poplar seedling emergence in UFNAs. Our results suggest the factors important to tulip poplar regeneration in rural forests are also important in UFNAs. Given the highly competitive nature of the species (Kota et al., 2007), abundant seed production, ability to store seeds for up to seven years (Burns & Honkala, 1990), and anecdotal observations of tulip poplar seedling emergence in canopy gaps just outside the study area, future studies should evaluate the viability of using understory vegetation removal treatments within existing canopy gaps to regenerate tulip poplar from seed rain in the Old Forest. However, care should be taken to ensure a tulip poplar seed bank exists before undertaking vegetation clearing as canopy gaps in UFNAs are often degraded by vines and invasive species (Massad et al., 2019; Piana et al., 2021b). Thus, developing a better understanding of regeneration dynamics in urban forest canopy gaps may be essential to ensuring successful recruitment of tulip poplar as well as other native tree species.

### **Limitations**

While the current study included two species with contrasting life history strategies (i.e., shade intolerant, wind dispersed versus shade intermediate, mammals dispersed), to develop a more comprehensive understanding of recruitment dynamics in UFNAs, future studies should include a wider variety of species including shade tolerant, understory, and non-native trees. Also, the current study examined seedling emergence over the course of one year within the boundaries of one site, limiting generalizability of these results to other areas and to longer term processes such as seedling survival. Future studies should include comparison sites and examine processes such as seedling survival and growth for longer periods of time.

## **Management implications**

In forests across the globe, seed predation, leaf litter, understory vegetation, and canopy gaps are critical components of early seedling establishment combining site conditions with species life history traits to determine future species composition. While the same factors important to rural forest regeneration appear to be important in urban forests, conditions of the urban environment, (e.g., elevated seed predator populations, invasive species, fragmentation, trampling) may create unique outcomes and require different management strategies. For instance, urban forest managers should consider direct planting of oak seedlings or incorporating seed protection into direct seeding strategies to overcome seed predation. Also, while the short-term reduction in leaf litter from prescribed fire would likely benefit oak germination and seedling emergence at the current study site, high seed predation rates may offset any gains in recruitment. The current study results suggest that tulip poplar regeneration could be achieved using natural seed rain combined with vegetation removal in existing canopy gaps. However, it is likely that continual interventions would be needed to control the high levels of non-native species known to invade these gaps. An improved knowledge of how site factors affect seed germination and seedling emergence in UFNAs will allow urban foresters to develop urban-specific silvicultural strategies to promote a diverse suite of native tree species and to develop management budgets to support those strategies. Regardless, without management interventions, relying on natural regeneration for native species recruitment through a policy of benign neglect bearing the “misplaced imprimatur of conservation” (Guldin et al., 1990, p.13) will inevitably lead to ecological degradation and a shift in species composition, likely away from desirable species like oak.

## Tables

Table 3.1 Emergence rates (percent) for northern red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*) by treatment in the Old Forest State Natural Area, Overton Park, Memphis, TN in 2022.

	gap	forest	vegetation removal	vegetation retention	litter removal	litter retention
<i>Quercus rubra</i>	84.6	78.8	79.7	83.7	85.9	77.3
<i>Liriodendron tulipifera</i>	2.3	2.6	4.3	0.6	3.2	1.7

Table 3.2 Analysis of Deviance results for northern red oak (*Quercus rubra*) and tulip poplar (*Liriodendron tulipifera*) emergence in the Old Forest State Natural Area, Overton Park, Memphis, TN in 2022.

treatment	northern red oak			tulip poplar		
	df	Chi-square	p-value	df	Chi-square	p-value
canopy (can)	1	5.020	0.025	1	0.047	0.829
vegetation (veg)	1	1.514	0.219	1	9.899	0.002
litter (lit)	1	7.294	0.007	1	2.239	0.135
can*veg	1	0.118	0.731	1	0.012	0.912
can*lit	1	0.116	0.733	1	1.646	0.200
veg*lit	1	2.057	0.151	1	0.049	0.825
can*veg*lit	1	0.313	0.576	1	0.000	0.997

Treatments: canopy = canopy gap or closed canopy forest; vegetation = understory vegetation removal or retention; litter = leaf litter removal or retention.

## Figures

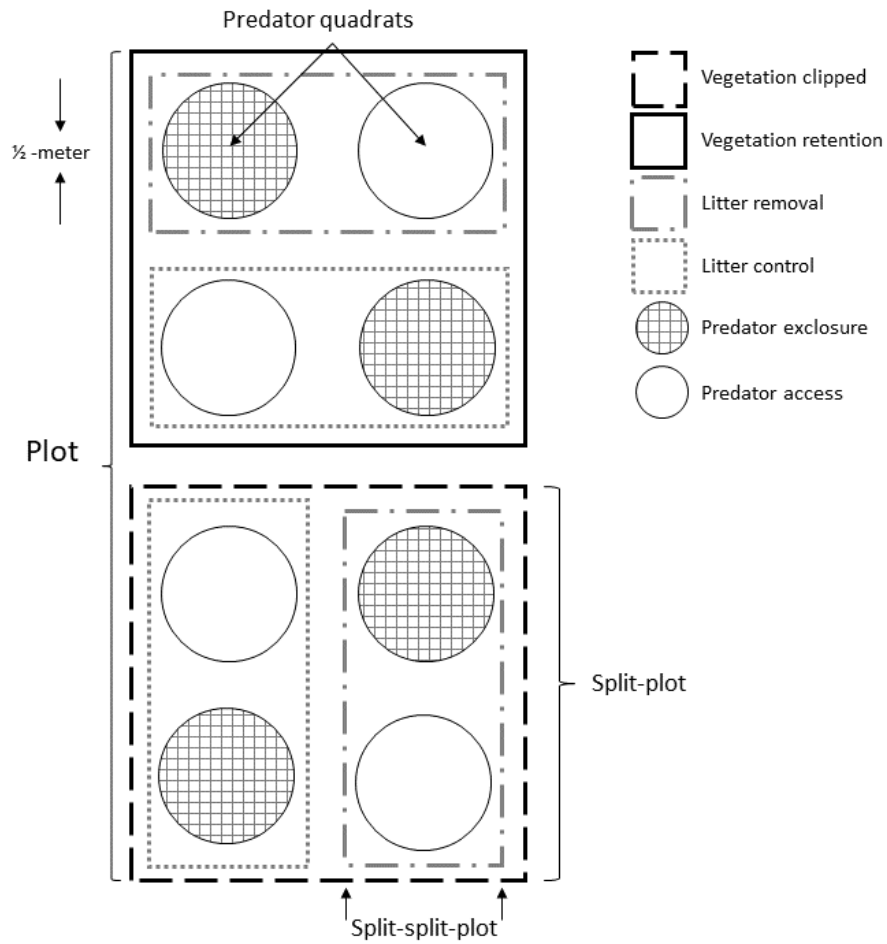


Figure 3.1 Split-split plot randomized complete block design with eight unique treatment combinations and predator exclusions.

Eight unique treatment combinations consist of canopy treatment (not shown), vegetation treatment, and litter treatment. Plots were placed in the Old Forest State Natural Area, Overton Park, Memphis, TN in 2022.



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CHAPTER IV  
ALLELOPATHIC POTENTIAL OF *PRUNUS CAROLINIANA* IN AN  
URBAN FORESTED NATURAL AREA

**Abstract**

Urban forested natural areas (UFNAs) play a vital role in providing ecological and social benefits in urban landscapes, yet they face numerous threats, including the potentially negative impacts of regionally relocated tree species on germination and growth of native vegetation. This study focused on the allelopathic potential of cherry laurel (*Prunus caroliniana* Aiton), a horticultural species increasingly abundant and well outside its historic range in the Old Forest State Natural Area in Memphis, Tennessee. Through vegetation surveys, germination bioassays, and pot culture experiments, we investigated the relationship between cherry laurel and the abundance, composition, and diversity of understory vegetation, the impact of its leaf leachates and root exudates on native tree germination, and the effect of its leaf residue on early growth of native trees. Results indicate a 16% decrease in native understory vegetation abundance within one meter of cherry laurel trees with no difference in species composition or diversity. Bioassays revealed exposure to cherry laurel leaf extracts was associated with decreased germination rates for all recipient species (-14% in sugar maple (*Acer saccharum* Marshall), -46% in cherry laurel, -10% in northern red oak (*Quercus rubra* L.)), while exposure to cherry laurel root extracts was associated with increased germination rates for sugar maple (49%) and cherry laurel (20%) but decreased germination rates for northern red oak (-28%). Pot culture experiments were

inconclusive due to confounding effects of activated carbon (included in soil to adsorb and control for allelochemicals) on the growth of recipient species. While further research is needed to disentangle the effects of cherry laurel from experimental artifacts, this study underscores the importance of considering allelopathic potential and impacts to plant abundance, composition, and diversity from regionally transplanted species in UFNAs.

## **Introduction**

Within an increasingly urban society, urban forested natural areas (UFNAs), fragmented remnants of natural forests, provide a variety of biological and social benefits such as air and water quality improvements, wildlife habitat, improved property values, and improved human health (Kuo & Sullivan, 2001; Nowak et al., 2009; Donovan & Butry, 2010). However, delivery of these benefits is threatened by human-induced ecological stressors such as fragmentation; disrupted disturbance regimes; poor air, soil, and water quality; and biological invasion by non-native plants, insects, and pathogens (Nowak et al., 2009; Motard et al., 2011; Loeb, 2012; Doroski et al., 2018). Invasive plants, those not native to the ecosystem being considered and causing ecological or economic harm (USDA, 2024), are of particular interest in UFNAs because of their proximity to urban landscapes where non-native plants are commonly used (Gavier-Pizarro et al., 2010). Many invasive plants originate as ornamental landscape species (Duinker et al., 2017), which have been relocated and planted across major geographical or continental boundaries, while others have been moved within a regional context (Nackley et al., 2017). Plants relocated within a regional context are sometimes discounted as causing less ecological harm, are often not included on state invasive plant lists, and thus receive less attention from urban forest managers (Simberloff et al., 2012; Berthon et al., 2021). However, these regional transplants are often novel to the ecosystem in which they have been transplanted and can

therefore have similar impacts as plants relocated across continents (Nackley et al., 2017). Invasive plants, regardless of origin, may become so abundant in their new habitat that they displace native species (Aronson & Handel, 2011; Johnson et al., 2015), reduce native biodiversity (Vila et al., 2011), and necessitate costly control efforts (Johnson & Handel, 2016). While urban forests are particularly vulnerable to invasive plants due to frequent introduction of non-native plant material (Gavier-Pizarro et al., 2010; Duinker et al., 2017), the impact of, and mechanisms behind, invasion by regionally transplanted species, sometimes termed “native invasion,” is a little studied topic in urban forestry (Lehvavirta, 2007). Therefore, research into impacts and mechanisms of native plant invasion is relevant to urban forest managers when determining appropriate management strategies.

To be considered invasive, once a non-native plant is introduced within a UFNA, it must successfully establish and spread to disrupt or otherwise cause harm to the native ecosystem (Richardson et al., 2000, Blackburn et al., 2011). To explain the process of successful plant invasion, several potential mechanisms have been proposed: release from herbivores or pathogens that were present in its native range but are absent in its new habitat (Keane & Crawley, 2002; Blumenthal, 2005), rapid evolution of competitive traits such as faster growth rates (Blossey & Notzold, 1995), and life history characteristics such as rapid growth and prolific seed production (Muller-Scharer et al., 2004). Allelopathy, the chemical interaction between plants (Molisch, 1937), has also been proposed as a mechanism for non-native plant invasion, especially when the introduced plant produces and releases secondary metabolites (i.e., allelochemicals) that are novel to the invaded ecosystem (Callaway & Aschehoug, 2000). In the introduced plant's native range, allelochemicals often have little effect on the coevolved vegetation; however, they can strongly impact growth and reproduction of plants that have not

evolved in their presence (Callaway & Ridenour, 2004), and thus, may aid in non-native plant establishment and spread into the new ecosystem. While allelopathy has been widely studied as a mechanism for understanding plant invasion in rural forests and grasslands (Zhang et al., 2021), little is known about allelopathic mechanisms within UFNAs (but see Warren et al., 2017).

Effects of allelochemicals on recipient (i.e., target) plants have the potential to either inhibit or promote germination and growth depending on plant structure and pathway from which the allelochemical was derived (i.e., leaf leachate, root exudate, or plant residue), species of the target plant, and interaction of the allelochemical with soil biota (Orr et al., 2005, Pisula & Meiners, 2010). For example, in their meta-analysis of allelopathy experiments, Zhang et al. (2021) found that plant residues, decomposing leaves and roots, had the most negative effect on “plant performance” compared to leaf leachates and root exudates. Also, in an urban natural area of Buffalo, NY, Warren et al. (2017) found that root exudates of common buckthorn (*Rhamnus cathartica* L.) had greater inhibitory effects on the germination of four native plants than leaf leachates and that cottonwood (*Populus deltoides* W. Bartram ex Marshall) germination was inhibited by root exudates while eastern white pine (*Pinus strobus* L.) germination was enhanced. The soil substrate in which the allelochemicals are delivered also impacts their effect with native soil microbes having the capacity to degrade allelochemicals and neutralize allelopathic effects in some cases (Li et al., 2017) while allelochemicals may inhibit the growth of mycorrhizal fungi thereby indirectly inhibiting plant growth in others (Callaway et al., 2008). Developing an understanding of, and appropriate management response to, allelopathy in UFNAs is complicated by the interplay between target species, allelopathic pathway, and soil conditions. Thus, research examining the effects of allelopathy in UFNAs that incorporates combinations of locally relevant target species, a variety of allelopathic pathways, and a variety

of soil conditions is important for the development of effective management strategies. Also, given the vulnerability of UFNAs to invasive plants, developing an understanding of the allelopathic potential of non-native plant species is important for developing appropriate management responses, particularly for those species that are regionally relocated and therefore less likely to receive scrutiny from UFNA managers, policy makers, and the public (Simberloff et al., 2012; Nackley et al., 2017; Berthon et al., 2021).

The current study took place in the Old Forest State Natural Area (Old Forest), a UFNA in Overton Park, Memphis, Tennessee where cherry laurel was noted to have increased in abundance by 1,349 trees per ha, 99% of which was in the seedling and sapling size category, over a 32-year period (see Chapter Two). Though it did not appear in a 1987 inventory of the Old Forest (Guldin et al., 1990), a 2019 inventory (see Chapter Two) identified it as the second most abundant tree species. Cherry laurel is an understory tree, commonly used in horticulture and landscaping and was listed as an ornamental within Overton Park as early as 1944 (Taylor, 1944). It is native to southeastern U.S. coastal plains (Gilman et al., 2018) and historically limited primarily to the islands off the Carolinas and further south (Michaux, 1819). However, cherry laurel is also known to escape from cultivation, readily germinate in forest understories, and is widely reported throughout the southeastern U.S. (Data Portal, 2020). In fact, Ruter (2019) points out that breeding cherry laurel for sterility or substantially lower fruit production is important to allow its continued use in landscaping without the negative effects of its spreading and naturalization. While no specific studies are known to have tested allelopathy within cherry laurel, there are several indications that it has the potential to use secondary metabolites to its advantage. For example, consistent with several other members of the *Prunus* genus (Fisher 1980), the fruits and seeds of cherry laurel contain cyanogenic glycosides, which are thought to

deter premature frugivory on unripe fruits (Heiling & Gilbert, 2016). However, the cleavage of cyanogenic glycosides in the environment produces hydrogen cyanide, which is suspected of having a role in plant allelopathy (Blenis et al., 2004, Cipolloni & Gruner, 2007, Kremer & Ben-Hammouda, 2009). While the presence of cyanogenic glycosides does not prove allelopathy, it suggests an allelopathic *potential* within the species. Thus, the dramatic increase of cherry laurel in the Old Forest, well outside its historic native range, provides an opportunity to study allelopathic potential in the context of native invasion in a UFNA and provide a basis for native invasive management decisions. Therefore, the current study aimed to examine the relationship between cherry laurel stems in the Old Forest and understory plant abundance and species composition; analyze the allelopathic potential of cherry laurel leaf leachates and root exudates on the germination of native overstory trees; and evaluate the allelopathic potential of cherry laurel leaf residue on early growth of native overstory trees.

## **Methods**

### **Study area**

The current study was conducted in the Old Forest State Natural Area (Old Forest) within Overton Park, Memphis, Tennessee, USA. The Old Forest is a 58-hectare (ha) hardwood forest within the Western Mesophytic Region of the Eastern Deciduous Forest (Braun, 1950). Soils are Memphis Silt Loam, a strongly acidic, well-drained Alfisol (Sease et al., 1989) with 2-5% slopes. The site is within the USDA plant hardiness zone 7B defined as having average annual extreme minimum temperatures of -12.2 °C (USDA, 2012). The climate is characterized by mild winters and hot summers with average annual temperatures of 16.8 °C and average rainfall of 134.5 centimeters (cm) per year (USDC, 2020). The vegetation is characterized by over 330 species of flowering plants including 58 species of trees (Heineke, 2009). The overstory is

primarily composed of tulip poplar (*Liriodendron tulipifera* L.) and 11 species of oak (*Quercus* spp., Guldin et al. 1990). There are approximately 80 non-native plant species including at least 17 listed as invasive (Heineke, 2009, Miller et al., 2010, TNIPC, 2024).

## **Experiments**

To evaluate the allelopathic potential of cherry laurel, three experiments were conducted including a vegetation survey, germination bioassay, and seedling pot culture. The vegetation survey examined the relationship between cherry laurel and understory plant abundance and species composition. The bioassay analyzed the effect of cherry laurel leaf leachates and root exudates on the germination of native overstory trees, and the pot culture experiment evaluated the effects of cherry laurel leaf residue on the growth of native overstory tree seedlings.

### *Vegetation survey*

#### *Experimental design*

In April 2021, eighty paired plots were established within the Old Forest with one pair centered on a 2.0 to 5.5 cm diameter at 1.3 meters (m) above ground (dbh) cherry laurel tree and the other pair centered on a nearby (< 20m) pawpaw (*Asimina triloba* (L.) Dunal) tree of similar dbh. Pawpaw was chosen to represent an understory tree similar in mature height to cherry laurel but well within its historic native range (Immel, 2001). Plots were visited three times during the 2021 growing season, once in May (spring), July (summer), and October (fall). During each visit, a 1-m radius circular plot was centered on each focal species (cherry laurel and pawpaw) within which all vascular flora, including tree seedlings (less than 1.3 m tall), were recorded by species and abundance (stem count). Within a similarly placed 5-m radius circular plot, all tree saplings (> 1.3 m tall and < 2.5 cm dbh) were recorded by species and dbh.



### ***Data analysis***

All analyses were conducted using R (R Core Team, 2023) and an  $\alpha$  of 0.05. A two-tailed paired t-test was used to compare plot level differences in plant abundance between cherry laurel and pawpaw plots for each season (spring, summer, fall). One-way repeated measures ANOVA with season (spring, summer, fall) as predictor, difference in plant abundance between cherry laurel and pawpaw plots as response variable, and plot as a random effect was used to evaluate the effect of season on differences in plant abundance between the two focal species. Tree seedlings (< 1.3 m tall) were included in the overall vegetation abundance analysis and analyzed separately using a two-tailed paired t-test for each season. Tree sapling (1-10 cm dbh) abundance was collected once and therefore evaluated using a single two-tailed paired t-test. Differences in species composition for each season were analyzed using Permanova with a Bray-Curtis distance metric. Plot-level plant diversity was measured with the Shannon Diversity Index:

$$H_{Sh} = - \sum [(p_i) * \ln(p_i)] \quad (4.1)$$

where  $p_i$  equals the ratio of number of individuals of species  $i$  to the total number of all individuals (Gotelli & Chao, 2013; Gotelli & Ellison, 2018).

### ***Germination bioassay***

#### ***Experimental design***

The bioassay experiment was arranged as a two-way factorial treatment design using a randomized complete block layout and consistent with the recommended methodologies of Dorning and Cipollini (2006) and Caboun and John (2015). The two factors were cherry laurel parts (root and leaf) and extract concentrations (full strength, 50% strength, and distilled water).

Thirty randomly selected cherry laurel trees approximately 2-3 cm dbh were uprooted from the Old Forest using a leveraged pulling tool (Uprooter, Grants Pass, OR, USA) in the fall of 2021. Roots were separated from the stem at ground level, with the soil gently washed from the root surfaces, and leaves clipped from branches. Roots and leaves were air dried in a climate-controlled room until a constant weight was achieved. Dried roots were coarsely ground using an electric grinder (Mueller Hyper Grind Precision Electric Spice Grinder, Summit, NJ, USA) while dried leaves were coarsely ground by hand. Ground roots and leaves were soaked in distilled water at room temperature for 72 hours at a ratio of one gram (g) of dried plant material per five milliliters (ml) of distilled water (1:5 w/v) and vacuum-filtered through a Whatman No. 1 filter three times. Half of the filtered solutions were stored at full strength and half diluted with distilled water to 50% strength. These concentrations were consistent with the recommendations of John et al. (2006) to address the common criticism of bioassay experiments in allelopathy that laboratory conditions do not replicate field conditions particularly due to the use of unreasonably high concentrations of allelochemicals, which can cause inhibitory effects on germination unrelated to allelochemicals. All solutions were stored in 240 ml Boston Round amber bottles at 4 °C until use. Distilled water was used as the control solution. Three incubators (Percival Model I-30 BLL, Perry, IA, USA) were outfitted with four equally spaced shelves. Within each incubator, two shelves were randomly assigned a root extract treatment with the remaining two shelves receiving leaf extract treatment. Each shelf was divided into 24 sections and each section randomly assigned a species x treatment combination, including controls, for a total of two sets of species x treatment combinations on each shelf. Seeds of four recipient (target) species: northern red oak (*Quercus rubra* L.), tulip poplar, sugar maple (*Acer saccharum* Marshall), and cherry laurel were purchased from Louisiana Forest Seed Company (Lecompte, LA, USA) and

placed in cold storage (4 °C) until the start of the experiment. Northern red oak, tulip poplar, and sugar maple were chosen to represent native overstory species with different life history traits (shade tolerance, seed size, and dispersal) while cherry laurel was chosen to test for autotoxicity. Five seeds of tulip poplar, sugar maple, or cherry laurel were placed in separate 100 x 15 mm plastic Petri dishes filled with sand, while northern red oak seeds, due to their large seed size, were placed in 3.8 cm x 4.6 cm 5-cell germination trays filled with sand. Each petri dish was wetted with 5 ml of extract solution or distilled water, and each northern red oak tray was wetted with 1 ml of extract solution or distilled water once every two weeks for the duration of the experiment. Each petri dish and germination tray was lightly wetted with distilled water as needed to maintain damp sand throughout the experiment's duration. Each incubator was set to a 12-hour dark photoperiod and 12-hour light photoperiod. For 12 weeks, each incubator was set to 1 °C during the dark photoperiod and 5 °C during the light photoperiod. After 12 weeks, the incubators were set to 15 °C during the dark photoperiod and 25 °C during the light photoperiod. Each petri dish and germination tray was inspected weekly and new germinants were recorded by species, incubator, shelf, and treatment. The experiment continued until there were no new germinants for two consecutive weeks and lasted a total of 24 weeks. Germination was defined as the emergence of a root radicle at least 2 millimeters (mm) long.

### ***Data analysis***

All analyses were conducted using R Statistical Software (v4.3.2; R Core Team, 2023) and an  $\alpha$  of 0.05. To evaluate the relative importance of plant part and concentration, a generalized linear mixed model was fitted to the relative germination rate (number of seeds that germinated in a treatment relative to number of seeds germinated in the control, abbreviated RGR) as suggested by Rho (1986) for each species and treatment combination. Separate species-

specific models were generated with treatments and their interactions included as fixed factors while shelf and incubator were included as random effects. Models were fitted with a negative binomial error distribution and a logit-link function using the glmmTMB package (Brooks et al., 2017). Treatment means were back transformed and reported as RGR for each treatment by species combination. Post-hoc comparisons were performed for each significant treatment factor using the emmeans package (Lenth, 2024) with Tukey p-value adjustments for multiple tests.

### *Pot culture*

#### *Experimental design*

The pot culture experiment was designed as a 2 x 2 factorial design with soil (inoculated or sterile) and activated carbon (present or absent) as the two factors, each with two levels for a total of four distinct treatment combinations per species. Sterile soil mixture consisted of perlite and sand at a 2:1 (v/v) ratio and was designed to determine if cherry laurel allelochemicals were transferred directly to the recipient seedling. Inoculated soil mixture also contained perlite and sand at the same ratio but with the addition of 5% (v/v) native soil gathered from the Old Forest in areas free of cherry laurel and was designed to determine if allelochemicals impacted the recipient seedling through interactions with the soil microbial community. Half of the pots had activated carbon (Carolina Biological Supply, Burlington, NC, USA) added at 2% (v/v) to adsorb allelochemicals thereby acting as a control for each soil mixture (Callaway & Aschehoug, 2000, Inderjit & Callaway, 2003). Each pot had 5mg of dried and ground cherry laurel leaf litter, gathered from the Old Forest in fall 2021, mixed uniformly into the growing medium. The amount of leaf litter added to each pot was determined by averaging the dry weight of leaf litter collected from the base of 30 cherry laurel stems located throughout the Old Forest. Seeds of target species (northern red oak, tulip poplar, sugar maple, and cherry laurel) were purchased

from Louisiana Forest Seed Company (Lecompte, LA, USA) and stratified at 4 °C for 60-90 days before being transferred to germination trays in an indoor temperature and humidity-controlled greenhouse. Upon shoot emergence seedlings were transplanted individually into 10-cm diameter, 500-ml planting pots containing one of the four treatment combinations. Seedlings were grown on shelves under grow lights (FEIT – model GLP24FS/19W/LED, Rivera, CA, USA) in a 3.7 x 2.4 m indoor greenhouse (Eagle Peak, Downey, CA, USA) with thermostatically controlled heat (Palma Greenhouse Heater 2.0, Elk Grove Village, IL, USA) set at 26 °C from 6 am to 8 pm and 20 °C from 8 pm to 6 am and the humidifier (Inkbird IHC 200, Guangdong, CHN) set at a minimum of 60% relative humidity for eight weeks with each species x treatment combination represented on each shelf. Each seedling was watered with 30 ml of distilled water twice per week, 30 ml of liquid fertilizer (Hoagland's No 2 at 1/8 strength) once per week, and systematically rearranged on each greenhouse shelf once per week. Seedlings were grown for eight weeks, after which each seedling was harvested and separated at the root collar into above-ground and below-ground parts. Parts were dried at 60 °C for 72 hours and weighed. Each species by treatment combination was replicated 24 times for sugar maple, tulip poplar, and cherry laurel and 48 times for northern red oak. At the conclusion of the experiment, three soil samples from each pot mix were sent to a commercial soil laboratory (Waypoint Analytical, Memphis, TN, USA) and analyzed for pH, nutrient content, and cation exchange capacity. To evaluate the effects of activated carbon on plant growth in the absence of cherry laurel leaves, 20 seedlings of each species were grown in 9-cm diameter, 400-ml pots for approximately six weeks in one of two growth mediums (sand/perlite 2:1 v/v, or sand/perlite plus 2% activated carbon) after which plants were harvested, separated into above and below ground parts, dried, and weighed.

## ***Data analysis***

All analyses were conducted using R Statistical Software (v4.3.2; R Core Team, 2023) and an  $\alpha$  of 0.05. Biomass (mg) was analyzed using a linear mixed effects model with activated carbon treatment (present or absent) and soil treatment (sterile mix or native inoculation) included as fixed effects and greenhouse shelf as a random variable. Separate models were developed for each species and for each biomass component (total, below-ground, above-ground). Where necessary, biomass was log-transformed to achieve a normal distribution. Treatment means were back transformed and reported as biomass (mg) for each species by treatment combination. Post-hoc comparisons were performed using the emmeans package (Lenth, 2024) with Tukey p-value adjustments for multiple comparisons. Biomass in the carbon trial was analyzed using a linear mixed effects model with activated carbon treatment (present or absent) as the fixed effect and greenhouse shelf as a random effect for each species. Where necessary, biomass was log transformed to achieve a normal distribution.

## **Results**

### **Vegetation survey**

Plant abundance (stem count) was significantly higher in pawpaw plots than cherry laurel plots in spring and fall, and marginally higher in summer (Table 4.1). Mean differences in plant abundance between cherry laurel and pawpaw did not vary by season ( $F_{2,78}=0.118$ ,  $p = 0.889$ ). Mean tree seedling abundances were not significantly different between cherry laurel and pawpaw plots in spring ( $t = -0.55$ ,  $df = 39$ ,  $p = 0.585$ ), summer ( $t = -0.56$ ,  $df = 39$ ,  $p = 0.582$ ), or fall ( $t = -1.16$ ,  $df = 39$ ,  $p = 0.253$ ); however, sapling abundance was significantly higher ( $t = 3.51$ ,  $df = 39$ ,  $p = 0.001$ ) in pawpaw plots (8.9 saplings/plot) than in cherry laurel plots (6.6 saplings/plot). Species composition was not significantly different between the cherry laurel and

pawpaw plots in spring ( $F_{1,78} = 0.759$ ,  $p = 0.871$ ), summer ( $F_{1,78} = 0.862$ ,  $p = 0.639$ ), or fall ( $F_{1,78} = 1.0538$ ,  $p = 0.360$ ). Native plant diversity, as measured by the Shannon Diversity Index ( $H_{Sh}$ ), was slightly higher in the spring and fall in pawpaw plots ( $H_{Sh} = 2.6$ ) compared to cherry laurel plots ( $H_{Sh} = 2.5$ ) but was not different in the summer ( $H_{Sh} = 2.1$ ).

### **Germination bioassay**

Averaged over all species and both concentrations, leaf extracts had a negative impact as indicated by a relative germination rate less than one (RGR = 0.87) while root extracts had a positive impact on germination as indicated by a relative germination rate greater than one (RGR = 1.19, Figure 4.1). However, leaf and root RGR were only significantly different in cherry laurel where leaf extracts inhibited germination (RGR = 0.54) while root extracts stimulated germination (RGR = 1.21, Table 4.2). Averaged over all species and both plant parts (leaf and root), full strength extract concentration had a negative impact (RGR = 0.86) while 50% extract concentration had a positive impact (RGR = 1.2) on germination. However, the means were not significantly different in sugar maple or cherry laurel and approached significance ( $p = 0.078$ ) in northern red oak.

### **Pot culture**

In the carbon trial, average total biomass was 10% lower in carbon treatments than in non-carbon treatments, although these differences were not significant for any species. Average root biomass was 17% lower in carbon treatments than in non-carbon treatments with cherry laurel differences being significant ( $\chi^2 = 9.343$ ,  $df = 1$ ,  $p = 0.002$ ) and tulip poplar differences being marginally significant ( $\chi^2 = 3.761$ ,  $df = 1$ ,  $p = 0.053$ ). Average shoot biomass was 1% lower in carbon treatment than non-carbon treatment and did not differ significantly for any

species. In the experiment, mean total biomass was 14% lower in carbon treatment compared to non-carbon treatment and mean root biomass was 20% lower in carbon treatments compared to non-carbon treatments when averaged across all species and both soil treatments. Mean shoot biomass, however, was 22% higher for northern red oak and 13% lower for all other species in carbon treatments compared to non-carbon treatments (Table 4.3).

Total, root, and shoot biomass of sugar maple was significantly higher ( $t = -4.277$ ,  $df = 69$ ,  $p = 0.0003$ ;  $t = -3.835$ ,  $df = 69$ ,  $p = 0.002$ ;  $t = -3.498$ ,  $df = 69$ ,  $p = 0.005$ ; respectively) in sterile soil without activated carbon compared to sterile soil with activated carbon while no other sugar maple treatment means differed. Total biomass of cherry laurel was significantly higher in both the non-carbon treatments compared to the carbon treatments regardless of soil condition and in the inoculated soil treatments compared to sterile soil (Table 4.4). In contrast, cherry laurel root biomass did not vary by soil condition but was significantly higher in the non-carbon treatments compared to the carbon treatments (Table 4.4). Shoot biomass of cherry laurel in the inoculated soil mix without carbon was significantly higher than in the inoculated soil mix with carbon ( $t = -3.697$ ,  $df = 68.3$ ,  $p = 0.002$ ), the sterile soil mix with carbon ( $t = 4.009$ ,  $df = 68.3$ ,  $p = 0.009$ ), and the sterile soil mix without carbon ( $t = 4.887$ ,  $df = 68.3$ ,  $p < 0.001$ ). Similarly, total, root, and shoot biomass of tulip poplar was significantly higher in non-carbon treatment compared to carbon treatment regardless of soil condition and in inoculated soil compared to sterile soil regardless of carbon treatment (Table 4.4). Total biomass of northern red oak did not differ among treatments; however, root biomass was significantly higher in non-carbon treatments while shoot biomass was significantly lower in non-carbon treatments regardless of soil condition (Table 4.4).



## Discussion

The current study provides partial support for allelopathy in cherry laurel growing in a UFNA outside its native range. However, the degree to which allelopathy may be occurring, and its ecological significance remains unclear. The decreased abundance of understory vegetation and tree saplings in areas within 1 m of cherry laurel stems is consistent with allelopathic effects (Vila et al., 2011) but also with resource competition (Inderjit & del Moral, 1997). Further, the species-specific changes in relative germination rates (germination rate of treatment relative to germination rate of control) are also consistent with allelopathic effects (Zhang et al., 2021) but could be due to the osmolality or nutrient content of extract solutions (Inderjit & del Moral, 1997). However, the impact of leaf residue on early growth and survival could not be determined from the pot culture experiment due to confounding effects to plant biomass from the addition of activated carbon to the experimental pots.

The species-specific effects of leaf and root extracts on relative germination suggest allelopathic compounds from cherry laurel could result in species composition change. For instance, exposure to cherry laurel root extracts stimulated sugar maple germination but inhibited northern red oak germination. Yet, vegetation surveys revealed no differences in species composition nor substantial differences in Shannon Diversity Index between cherry laurel and pawpaw plots. Successful completion of a pot culture study may have revealed changes in growth and survival of recipient species that would have clarified the allelopathic potential of cherry laurel and its ecological significance in an urban forested natural area. Regardless, the results of the current study do support the need for future research on cherry laurel and its potential impacts on species composition in UNFAs. To obtain the most comprehensive understanding possible, future studies should examine other pathways by which cherry laurel

allelochemicals may be released into the environment, such as leaf residue, as well as the effects of cherry laurel extracts and residues on a wider variety of recipient species.

### **Vegetation survey**

Consistent with allelopathic mechanisms of plant invasion and the findings of other researchers, the current study found decreased vegetation abundance near the putative allelopathic plant, cherry laurel (Parker et al., 1999, Warren et al., 2017). However, unlike others, there was not an accompanying substantive change in species composition, richness, or diversity. The decrease in vegetation abundance could be indicative of cherry laurel allelochemicals reducing competition and contributing to its successful establishment and spread (Orr et al., 2005; Hierro & Callaway, 2021). However, a negative correlation between invasive and native plant abundance does not necessarily imply a causal relationship due to the invasive plant. For instance, some researchers have found that native plants inhibit the establishment of invading plants through competition and allelopathy (Adomako et al., 2019), while others have found the negative correlation between invasive and native plant abundance explained primarily by environmental variables (e.g., soil texture) and human disturbance (e.g., cultivation and fire suppression; Surrrette & Brewer, 2008). Also, reduced native plant diversity and richness associated with the presence of invasive plants has been documented (Collier et al., 2002; Vila et al., 2011) along with changes in species composition (Warren et al. 2017). The seeming contradiction between decreased abundance but similar composition could indicate that cherry laurel allelochemicals have equivalently negative effects on most resident species or that it has established in the Old Forest by opportunistically occupying growing space where existing plant density was low. Given that cherry laurel is considered shade intolerant (Niinemets & Valladares, 2006), it is unlikely to have established in the low light levels under an intact forest

canopy. However, recent (2011) large-scale clearing of invasive Chinese privet (*Ligustrum sinense* Lour) by the land manager (OPC, pers comm) may have provided available growing space and light levels that cherry laurel subsequently utilized. Future studies should seek to clarify whether cherry laurel's establishment and spread in the Old Forest was a result of allelopathy, available growing space, or a combination of factors.

Along with documented reduction in vegetation abundance, the current study also found a reduction in sapling abundance near cherry laurel trees, but no difference in tree seedling abundance. Allelopathy has been proposed as one factor leading to native tree species recruitment failure either directly through allelochemical impacts on seedling emergence, growth, and survival (Pisula & Meiners, 2010; Warren et al., 2017) or indirectly through the development of a recalcitrant understory vegetation layer that subsequently inhibits seedling recruitment (Royo & Carson, 2006). However, allelopathic impacts also vary by plant life stage (Orr et al., 2005). Thus, the current study's conflicting findings of no difference in seedling abundance but a reduced sapling abundance in cherry laurel plots could indicate that allelochemicals have a more pronounced impact on early growth and survival of saplings than on germination and emergence of seedlings. It is also possible that cherry laurel and pawpaw both represent recalcitrant understory vegetation layers and therefore have similar impacts on seedling recruitment due to their density and subsequent reductions in available light in the understory (Baumer & Runkle, 2010). However, the differential survival of saplings between the two suggests that cherry laurel's impacts likely consist of more than competitive exclusion (Hierro & Callaway, 2021). While vegetation surveys cannot prove the presence of allelopathy, they do provide a rationale for continued study (Inderjit & Callaway, 2003) including a more detailed analysis of cherry laurel's impact on seedling germination, growth, and survival.

## **Germination bioassay**

Similar to others, the current germination bioassay found higher levels of germination inhibition from leaf extracts than root extracts (Zhang et al., 2021) and from higher extract concentrations compared to lower concentrations (Pisula & Meiners, 2010; Cipollini & Bohrer, 2016). Also, consistent with other findings (Orr et al., 2005; Pellisier, 2012) these effects ranged from inhibition to stimulation as a result of exposure to cherry laurel extracts depending on the extract concentration, extract origin (leaf or root), and recipient species (sugar maple, cherry laurel, northern red oak). For example, germination of cherry laurel was inhibited by exposure to leaf extracts while northern red oak and sugar maple germination were unimpacted. Similarly, germination of sugar maple was stimulated by exposure to root extracts, while northern red oak and cherry laurel germination were unaffected. Also, full strength extract, regardless of origin (leaf or root), reduced the germination rates of northern red oak but had no significant impact on sugar maple and cherry laurel. In contrast, the 50% extract, regardless of origin, stimulated germination of sugar maple but had no impact on northern red oak and cherry laurel. It is unclear what concentrations of potential allelochemicals would be present in the Old Forest under natural conditions (Inderjit & Nilsen, 2010), nor the pathway by which cherry laurel would release allelochemicals. However, the variety of reactions to germination demonstrated in the current study suggest cherry laurel could have an impact on species recruitment and ultimately species composition and underscore the importance of continued research into mechanisms, pathways, and consequences of cherry laurel allelochemicals on a wide variety of Old Forest plant species.

## **Pot culture**

Results of the current pot culture experiment were inconclusive due to confounding effects of activated carbon on the growth of recipient species. In previous studies, activated

carbon has been utilized to adsorb putatively allelopathic compounds thereby allowing a distinction between potential growth of the recipient plant and realized growth in the presence of potentially allelopathic compounds (Nilsson, 1994; Callaway & Aschehoug, 2000; Inderjit & Callaway, 2003). However, activated carbon has also been shown to produce experimental artifacts such as modified nutrient availability, soil pH, and the release of biologically active compounds (Lau et al., 2008; Kabouw et al., 2010; Biederman & Harpole, 2013). In the current carbon trial, the addition of activated carbon (2% v:v) was associated with reduced total biomass of all seedlings and with significant reductions in root biomass of cherry laurel and tulip poplar. Similarly, the experimental results showed reduced total biomass associated with the addition of activated carbon for all recipient species except northern red oak. While it is tempting to interpret these results as evidence that cherry laurel releases allelochemicals that stimulate growth of recipient plants, it is also possible that recipient plants responded to a modified growing environment due to the presence of activated carbon.

Several possible explanations for reduced growth of recipient plants receiving activated carbon treatments have been proposed with the most common being reduced nutrient availability (Blumenthal et al., 2003; Lau et al., 2008; Spokas et al., 2012), particularly in alkaline soils such as those found in the current study (Biederman & Harpole, 2013; Jeffrey et al., 2017). Activated carbon is often described as increasing nutrient availability primarily through increased nitrogen (N) mineralization and nutrient retention (Lau et al., 2008; Gao et al., 2020). However, it has also been associated with nutrient immobilization whereby activated carbon either provides a suitable substrate for the growth of bacteria, which sequester nutrients making them less available to roots (Blumenthal et al., 2003), or sorbs to N so strongly that it becomes less available to plants (Thomas & Gale, 2015). Other explanations for reduced plant growth in carbon addition studies

have included the release of soluble salts or phytotoxic compounds from the surface of activated carbon (Thomas & Gale, 2015) while others have shown that improvements to plant growth reverse after a threshold level of activated carbon is reached (Biederman & Harpole, 2013, Gao et al., 2020). However, the specific threshold varies by plant and appears unknown in temperate tree species (Biederman & Harpole, 2013; Joseph et al., 2021). In the current study, the observation of reduced below-ground biomass is not consistent with an expected optimal allocation response to nutrient poor conditions (Bloom et al., 1985; McConnaughay & Coleman, 1999). Thus, the observed results are likely due to combinations of confounding factors rather than nutrient immobilization alone.

Pot culture experiments offer an opportunity to examine the effects of potentially allelopathic leaf residues on the early growth and survival of recipient species in a controlled environment. Given that leaf residues are often reported as the most influential aspect of allelopathy on plant performance (Zhang et al., 2021), and that early growth and survival is an important and vulnerable stage of species establishment (Leck & Outred, 2008), pot culture studies are important for determining potential consequences of allelopathy on species composition. However, many pot culture studies utilizing woody species suffer from a lack of methodological information (Kawaletz et al., 2014). For example, activated carbon is often used as a “control” in pot culture studies, but many do not conduct or report results of a carbon trial (Lau et al., 2008). While the effects of allelopathy could not be disentangled from the effects of activated carbon in this study, results demonstrate the value of conducting and reporting carbon trials to inform the design of future experiments and interpretation of pot culture results.

The current study appears to be the first to evaluate the role of cherry laurel in an urban forested natural area and provides partial support for the allelopathic potential of the species.

Vegetation surveys suggest cherry laurel may alter community structure but not composition, while bioassays suggest cherry laurel has the potential to alter germination success through leaf leachates and root exudates. However, the role of cherry laurel leaf residue on early growth was inconclusive. These results combined with the documented increase in cherry laurel density from previous studies (see Chapter Two) suggest continued research is warranted into the mechanisms and impacts of cherry laurel spread in urban forests. Given that early growth is an important and vulnerable stage of species establishment (Leck & Outred, 2008), future studies should seek to better understand the effects of cherry laurel on a variety of species at this life stage. While the current study results suggest cherry laurel has the potential to affect species composition in urban forested natural areas, results have limited applicability due to the narrow range of conditions experienced in laboratory experiments compared to field conditions. Therefore, future research should also include field studies that can provide results and implications of practical relevance to urban forest managers. The promotion of native plants in horticulture and landscaping for their cultural and biodiversity values (Kermath, 2007; Tallamy, 2009) may result in the regional relocation of plant species considered “native” to a political jurisdiction (Nackley et al., 2017). As urban landscaping is often in close proximity to urban forests (Duinker et al., 2017), the current study demonstrates the need for urban forest managers and researchers to be vigilant in monitoring and studying both “native” and exotic plant introductions in UFNAs.

## Tables

Table 4.1 Mean (sd) and difference of plot level plant abundance (stems per plot) analyzed by two-tailed paired t-test in the Old Forest State Natural Area, Overton Park, Memphis, TN by 2021 season and focal species.

	<i>P. caroliniana</i>	<i>A. triloba</i>	difference	t-value	df	p-value
Spring	<b>75.2 (31.8)</b>	<b>85.6 (35.3)</b>	<b>10.5</b>	<b>2.06</b>	<b>39</b>	<b>0.046</b>
Summer	62.0 (25.5)	72.1 (35.4)	10.2	1.88	39	0.067
Fall	<b>46.7 (20.0)</b>	<b>59.2 (32.5)</b>	<b>12.5</b>	<b>2.196</b>	<b>39</b>	<b>0.017</b>

Bold text indicates significant results at  $p < 0.05$ .

Table 4.2 Analysis of Deviance (Type II Wald chi square tests) results for relative germination rate of seeds treated with cherry laurel (*Prunus caroliniana*) leaf and root extracts at two concentrations.

	<i>Acer saccharum</i>			<i>Prunus caroliniana</i>			<i>Quercus rubra</i>		
	Chisq	df	p-value	Chisq	df	p-value	Chisq	df	p-value
concentration	4.492	2	0.106	3.682	2	0.159	5.099	2	0.078
plant part	1.690	1	0.194	<b>4.010</b>	<b>1</b>	<b>0.045</b>	0.924	1	0.336
part*concentration	3.967	2	0.138	2.449	2	0.294	1.270	2	0.530

Bold text indicates significant results at  $p < 0.05$ . Concentrations = 1g dried plant material per 5 ml distilled water or 0.5 g of dried plant material per 5 ml distilled water. Leaf and root extracts prepared from *P. caroliniana* collected within the Old Forest State Natural Area, Overton Park, Memphis, TN in fall 2021.



Table 4.3 Biomass (mg) by species and treatment in pot culture experiment.

biomass	soil	carbon	<i>Acer saccharum</i>	<i>Prunus caroliniana</i>	<i>Quercus rubra</i>	<i>Liriodendron tulipifera</i>
total	sterile	no	324 <sup>a</sup>	395	3050	226
	sterile	yes	237 <sup>b</sup>	369	3082	152
	inoculated	no	276 <sup>ab</sup>	467	3052	241
	inoculated	yes	279 <sup>ab</sup>	379	2945	187
root	sterile	no	196 <sup>a</sup>	159	2115	153
	sterile	yes	144 <sup>b</sup>	120	1890	99
	inoculated	no	166 <sup>ab</sup>	162	2069	160
	inoculated	yes	167 <sup>ab</sup>	126	1801	119
shoot	sterile	no	122 <sup>a</sup>	236 <sup>a</sup>	934	73
	sterile	yes	100 <sup>b</sup>	249 <sup>a</sup>	1191	54
	inoculated	no	108 <sup>ab</sup>	305 <sup>b</sup>	983	81
	inoculated	yes	108 <sup>ab</sup>	253 <sup>a</sup>	1144	68

Carbon treatments = activated carbon added to soil mix (2% v:v) and no carbon addition. Soil treatment = sand/perlite (2:1 v/v) mix and sand/perlite plus field soil (5% v:v) from Old Forest State Natural Area, Overton Park, Memphis, TN. Values with different letters represent significant differences for that portion of measured biomass based on Tukey post-hoc comparisons. Total, root, and shoot biomass for *Q. rubra* and *L. tulipifera* as well as total and root biomass of *P. caroliniana* was not evaluated with pairwise post-hoc comparisons as there were no significant interactions between treatments.

Table 4.4 Analysis of Deviance (Type II Wald chi square tests) results for biomass (mg) in pot culture experiment by recipient species and treatment.

species	treatment	total biomass			root biomass			shoot biomass		
		Chisq	df	p-value	Chisq	df	p-value	Chisq	df	p-value
<i>Acer saccharum</i>	carbon	<b>8.890</b>	<b>1</b>	<b>0.003</b>	<b>7.196</b>	<b>1</b>	<b>0.007</b>	<b>6.508</b>	<b>1</b>	<b>0.011</b>
	soil	0.001	1	0.977	0.163	1	0.687	0.474	1	0.491
	carbon*soil	<b>9.404</b>	<b>1</b>	<b>0.002</b>	<b>7.509</b>	<b>1</b>	<b>0.006</b>	<b>5.743</b>	<b>1</b>	<b>0.0166</b>
<i>Prunus caroliniana</i>	carbon	<b>11.964</b>	<b>1</b>	<b>0.001</b>	<b>17.485</b>	<b>1</b>	<b>2.9e-05</b>	<b>3.858</b>	<b>1</b>	<b>0.050</b>
	soil	<b>6.130</b>	<b>1</b>	<b>0.013</b>	0.595	1	0.441	<b>13.389</b>	<b>1</b>	<b>0.000</b>
	carbon*soil	3.584	1	0.058	0.491	1	0.484	<b>10.604</b>	<b>1</b>	<b>0.001</b>
<i>Liriodendron tulipifera</i>	carbon	<b>46.711</b>	<b>1</b>	<b>8.23e-12</b>	<b>48.758</b>	<b>1</b>	<b>2.90e-12</b>	<b>20.540</b>	<b>1</b>	<b>5.8e-06</b>
	soil	<b>6.359</b>	<b>1</b>	<b>0.012</b>	<b>4.084</b>	<b>1</b>	<b>0.043</b>	<b>8.909</b>	<b>1</b>	<b>0.003</b>
	carbon*soil	1.451	1	0.228	1.501	1	0.221	0.677	1	0.411
<i>Quercus rubra</i>	carbon	0.024	1	0.878	<b>4.860</b>	<b>1</b>	<b>0.028</b>	<b>8.762</b>	<b>1</b>	<b>0.003</b>
	soil	0.110	1	0.740	0.233	1	0.629	0.018	1	0.894
	carbon*soil	0.339	1	0.560	0.142	1	0.706	0.188	1	0.665

Carbon treatments = activated carbon added to soil mix (2% v:v) and no carbon addition. Soil treatment = sand/perlite (2:1 v/v) mix and sand/perlite plus field soil (5% v:v) from Old Forest State Natural Area, Overton Park, Memphis, TN. Bold text indicates significant at  $\alpha = 0.05$ .

## Figures

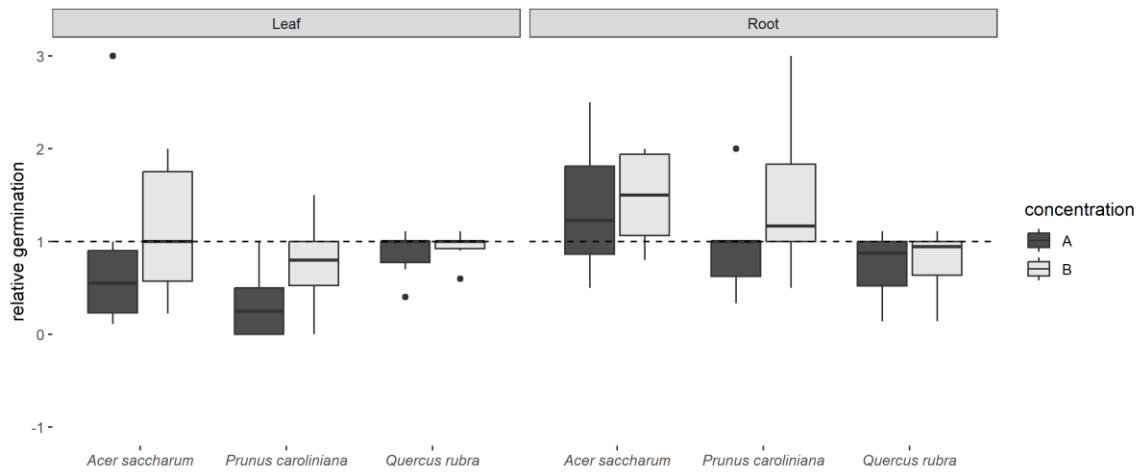


Figure 4.1 Relative germination rates (RGR) from 2021 bioassays by species and treatment with cherry laurel (*Prunus caroliniana*) leaf and root extract at 2 concentrations.

Concentration A = 1 g dried plant material/5 ml distilled water. Concentration B = 0.5 g dried plant material/5 ml distilled water. RGR above 1 indicates stimulatory effects, while RGR below 1 indicates inhibitory effects, RGR = 1 indicates no effect. Boxes indicate 25-75 percentile, whiskers indicate 75-100 percentile, dots represent outliers. Leaf and root extracts prepared from *P. caroliniana* collected from the Old Forest State Natural Area, Overton Park, Memphis, TN.

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## CHAPTER V

### CONCLUSION

Clearly, the ecosystem services provided by urban forested natural areas (UFNAs), are a vital component of quality of life in urban areas. Yet, little research has examined the nature of changing species composition and structure in UFNAs over time, the germination and emergence of native trees in these areas, nor the encroachment of non-native horticultural species on sensitive UFNAs. The three chapters presented above were designed to address limits in the extant research and provide beginning guidance specifically designed for managers of UFNAs.

The Old Forest State Natural Area (Old Forest), a UFNA in Memphis, TN, underwent significant changes in species composition and stand structure between 1987 and 2019. The introduction and proliferation of non-native tree species contributed to the changing composition and was a major factor behind the densification of the midstory and sapling layers along with slight decreases in tree species diversity (Shannon Diversity Index). Concurrently, oak (*Quercus* spp.) abundance declined sharply particularly in the overstory where oak density (trees per hectare) dropped by more than half. The densification of the midstory and sapling size categories with non-native, small-statured, and opportunistic tree species make it likely that, without management interventions, the Old Forest will continue to see reductions in species diversity over time and the continued decline of oak prevalence.

Contrary to expectations, clearing of the dense understory vegetation did not lead to enhanced northern red oak (*Quercus rubra* L.) germination and emergence in the Old Forest.

However, seed predation rates for unprotected acorns were 99.6% suggesting that seed predator populations, consisting mostly of eastern gray squirrels (*Sciurus carolinensis*), may be affecting oak recruitment success in the Old Forest. While germination and emergence rates improved within canopy gaps and leaf litter removal treatments, average rates were relatively high regardless of treatment (77-86%) suggesting that barriers to northern red oak recruitment are more likely due to seed predation and seedling survival than to site conditions inhibiting germination and emergence. In contrast to northern red oak, tulip poplar (*Liriodendron tulipifera* L.) germination and emergence was enhanced by understory vegetation removal. Further, abundant regeneration was anecdotally observed in canopy gaps outside the experimental plots suggesting the presence of a tulip poplar seed bank or abundant seed rain, although the experiment did not find higher germination and emergence rates of tulip poplar in canopy gaps. These findings highlight the need for management interventions targeted at the species of interest. For instance, these results suggest that managers should rely on direct planting rather than natural regeneration for northern red oak, while tulip poplar regeneration could be achieved through vegetation removal in existing canopy gaps utilizing the seed bank or abundant seed rain. However, given the abundance of non-native species observed in the previous study, regular monitoring, and control of competing invasive species should be included in management strategies.

The most abundant non-native tree species in the Old Forest, cherry laurel (*Prunus caroliniana* Aiton), exhibited allelopathic potential with vegetation abundance significantly reduced near cherry laurel trees when compared to vegetation near pawpaw (*Asimina triloba* (L.) Dunal) trees. While the reduction might have been due to competition, bioassays showed reduced germination rates of northern red oak with exposure to cherry laurel leaf and root

extracts. While impacts of cherry laurel leaf litter on early growth and survival of northern red oak, sugar maple (*Acer saccharum* Marshall), and tulip poplar could not be determined, the reduction in vegetation abundance and the impacts of cherry laurel extracts on germination of northern red oak suggest cherry laurel could have an impact (positive or negative) on native tree species recruitment and ultimately species composition. The results highlight the importance of carefully considering the ecological impacts of regionally relocated species when developing management strategies in UFNAs.

While further research is needed to clarify tree recruitment dynamics in UFNAs, particularly native tree recruitment limitations, the current studies add to the limited knowledge base for these systems and provide direction for future research priorities. In particular, studies examining a wider range of species over longer periods of time as well as studies comparing UFNA dynamics to surrounding rural forests will be helpful in developing and guiding management strategies. Despite these limitations, the results of the current studies demonstrate the necessity of management interventions in UFNAs to sustain native tree species populations. Ideally, site-specific management strategies can be developed alongside research projects to address the unique challenges of managing and studying UFNAs, especially when contrasted with rural forest management methods and knowledge.