DISTURBED RELATIONSHIPS: PLANT-SOIL INTERACTIONS FOLLOWING TORNADO DAMAGE IN SOUTHERN APPALACHIAN FORESTS

by

UMA NAGENDRA

(Under the Direction of Chris Peterson)

ABSTRACT

In this dissertation, I describe the relationships between mature trees and seedlings in Northeast Georgia temperate forests, with a focus on how tornado disturbance can alter plant-soil relationships. I used field observations to assess spatial patterns between trees and same-genus seedlings in a Piedmont forest. A greenhouse experiment was used to examine differences in plant-soil feedbacks between tornado-damaged and intact Southern Appalachian forest areas. That experiment was followed by a two-part field transplant experiment which assessed the role of soil biotic feedbacks in a tornado-damaged landscape.

In the first study, I observe patterns of seedling mortality near mature trees of the same genus in a mapped Piedmont forest. I use a spatially-explicit approach to examine shifts in seedling spatial patterns and effects of neighboring trees on seedling performance. The findings are consistent with negative distance dependence patterns at the genus level. Shifts in seedling spatial distributions, seedling survival rates, and seedling survival probabilities supported the hypothesis that seedlings in this forest are negatively impacted by the influence of closely related trees.

In the second and third studies, I document changes to plant-soil feedbacks in a tornado track in the Southern Appalachian Mountains. The second chapter uses a greenhouse study to examine plant-soil relationships for common Southern Appalachian tree species and whether those relationships are neutral in tornado-damaged patches. The results suggest that plant-soil feedbacks are not neutral, but instead highly variable after severe wind disturbance. The nature of plant-soil feedback changes depended upon species identity.

The third chapter uses a two-part greenhouse and field experiment to compare effects of soil biotic conditioning and the abiotic environment on seedling growth and survival in tornado-damaged forest areas. Four years after a tornado, plant soil interactions for common southern Appalachian seedlings were to be the same in both intact- and tornado-damaged forest areas. While some plant-soil feedbacks are visible in the field, they were secondary determinants of seedling performance. Seedling survival was more affected by abiotic environmental characteristics regardless of soil inoculum origin. Overall, plant-soil feedbacks calculated from seedlings grown in the greenhouse did not match those calculated from field-transplanted seedlings.

INDEX WORDS: plant-soil feedbacks, tornado, natural disturbance, negative distance dependence, Appalachian mountains, mycorrhizae, soil pathogens, Janzen-Connell, seedling mortality, temperate forest, recruitment, relatedness

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# CHAPTER 1

# INTRODUCTION

## 1.1 Negative Distance Dependence in Temperate Forests

In forested ecosystems, dominant tree species are not passive inhabitants; after living in one location for decades, they shape the ecosystem itself through physical, chemical, and biotic interactions. Mature trees change their immediate surroundings through the resources they consume as they grow; through the resources they release as they senesce; through their physical form that shades, shelters, and structures ecosystems; and through the communities of other organisms that feed on, live in, and partner with them.

Mature forest trees share their environment with young seedlings—recruits of their own species, as well as other species that coexist in the same forest. As mature trees alter their surroundings, they in turn indirectly affect these seedlings. This indirect influence on seedlings can range from beneficial to detrimental, depending on whether it creates a comparatively favorable environment and protection from natural enemies, or if it limits the availability of key resources and increases the abundance of species-specific natural enemies. Individuals of the same species often inhabit the same ecological niche; they have similar resource requirements and vulnerabilities compared to distantly-related species. Because of this, mature trees often have an outsized effect on seedlings of the same species.

The relationships between mature trees and seedling recruits shape the spatial distributions of forest species and the structure of forest communities. In many ecosystems, seedlings are less likely to survive if they are close to mature trees of the same species—or in areas with a high density of that species. This pattern, called negative density and/or distance dependence (NDD), can result in over-dispersed spatial distributions (Connell *et al*. 1984). By limiting the over-dominance of competitive species, NDD provides a stabilizing force, and is thought to facilitate and maintain species-rich forest areas in both the tropics (Barry 2016; Carson *et al*. 2008; Comita *et al*. 2014; Hyatt *et al*. 2003) and temperate zones (Lambers and Clark 2003; Martínez *et al*. 2013; McCarthy-Neumann and Kobe 2010; Packer and Clay 2000; Reinhart *et al*. 2012a; Yamazaki *et al*. 2009). Species that exhibit the strongest NDD are often the least abundant (Mangan *et al*. 2010), and areas with high species diversity often contain more species that exhibit strong NDD (Johnson *et al*. 2012).

Negative Distance/Density Dependence describes a pattern with many potential mechanisms, ranging from niche-specific resource competition to pathogen accumulation. While the aboveground influence of mature trees on the surrounding ecosystem is relatively easy to see and study, the belowground influence is hidden. These 'buried' interactions, however, are key to understanding ecosystem processes. Belowground species-specific pests and pathogens in particular are more likely mechanisms for NDD patterns than the initial theory of aboveground herbivores (Mangan *et al*. 2010; Terborgh 2012).

### 1.2 Plant-Soil Feedbacks as a Mechanism for NDD

In many cases, the mechanism of NDD occurs belowground, via the accumulation of species-associated microbial communities (mutualists, pathogens, and decomposers) as well as through biogeochemical pathways. When species-specific soil alterations in turn influence the performance of others in that same species, the relationship is called a plant-soil feedback (Connell 1978a; Ehrenfeld et al. 2005; Klironomos 2002; Mangan et al. 2010). If a plant species harbors a soil environment that hinders conspecific recruits compared to recruits from other species, it can be a mechanism for soil-specific NDD. These negative (stabilizing) plant-soil feedbacks lead to over-dispersed distributions and may help maintain diverse plant communities (Mills and Bever 1998). Positive (self-promoting) plant-soil feedbacks, in which a plant species harbors a soil environment that benefits its own offspring compared to other species, favors instead clumped spatial distributions, single-species stands, and monodominance (Callaway *et al*. 2008; Corrales *et al*. 2016). Both negative (stabilizing) and positive (self-promoting) feedbacks are known to influence species composition, diversity, and productivity in many terrestrial ecosystems (Klironomos 2002; Mills and Bever 1998; Van Der Heijden *et al*. 2008). Some invasive species are able to persist in new areas due to positive feedbacks—whether through release of natural enemies in the soil, or active inhibition of competitors' mutualists. Diverse tropical forests are thought to be maintained by negative feedbacks mediated by pathogens, while monodominant tropical forests are through to be maintained by positive feedbacks mediated by biogeochemical cycles. Negative feedbacks mediated by pathogens can influence plant community spatial patterns in grasslands, shrublands, and forests in temperate and tropical areas.

### 1.3 Natural Disturbances and Plant-Soil Feedbacks

Interactions between plants and the soil occur throughout ecosystem development and succession. While most studies focus on plant-soil feedbacks at one point in time, or one static environmental condition, ecosystems themselves are subject to constant changes. The interactions between organisms in turn respond to those changing conditions. Shifts in nutrient status (De Deyn *et al*. 2004; Manning *et al*. 2008), light availability (McCarthy-Neumann and Ibáñez 2013), soil moisture (Kennedy and Peay 2007), and other ecosystem characteristics all influence the strength and direction of plant-soil feedbacks. Ecosystem characteristics such as these change year-to-year and through successional time, leading to long-term successional shifts in plant-soil feedbacks (Kardol *et al*. 2013). In some instances, plant-soil feedbacks may even be partial drivers of that successional change (Kardol *et al*. 2006; Van der Putten *et al*. 1993) by facilitating the establishment of new plant populations. Despite a growing body of literature on the population- and community-level importance of plant-soil feedbacks, we lack a working knowledge of how plant-soil feedbacks change during secondary succession (Kardol *et al*. 2013; Putten *et al*. 2013; Reynolds *et al*. 2003).

In particular, we need an understanding of how plant-soil feedbacks change after natural disturbances in order to make predictions about the consequences of plant-soil feedbacks in real ecosystems. Periodic disturbances shape the development of every ecosystem type (White and Jentsch 2001; Xi and Peet 2011). The same environmental characteristics that regulate plant-soil interactions are highly dynamic immediately after disturbances. The abrupt mortality and resource release caused by tornadoes, hurricanes, fires, and floods drastically change both abiotic and biotic ecosystem parameters, all of which influence plant-soil feedbacks.

The majority of studies on soil changes after disturbances focus on fire-prone systems (Peay *et al*. 2009; Rincón and Pueyo 2010) and anthropogenic disturbances in agricultural systems (Kulmatiski and Kardol 2008; Peay *et al*. 2009; Rincón and Pueyo 2010). Soil alterations in temperate forests due to wind disturbance is still very much a black box, despite the ubiquity of temperate forests across the globe and the commonality of wind disturbances as a major component of global disturbance regimes (Millar and Stephenson 2015; Pan *et al*. 2011). As we build knowledge of how soil biotic communities change after wind disturbances (Cowden and Peterson 2013; Egli *et al*. 2002; Reinhart *et al*. 2010), few have taken the next step to examine the resulting changes to plant-soil interactions.

Wind disturbances alter abiotic soil characteristics and biotic soil communities both directly and indirectly. Each of these types of changes on their own can alter plant-soil feedbacks. In a post-disturbance landscape, the combined effects also interact in ways that shift plant-soil interactions. First, severe wind disturbances remove forest canopy cover. This changes soil abiotic characteristics by increasing solar radiation, wind flow, and soil evaporation rates (Ritter *et al.* 2005), leading to hotter and drier soils. As leaf litter, root litter, and fallen trunks decompose, nutrients and carbon previously locked away in organic matter are released into more labile forms (Vitousek and Melillo 1979). Although there is a sudden input of organic matter, many of these nutrient pools are ephemeral; water-soluble nitrates quickly leach out of the system. The amount, quality, and content of the sudden organic inputs in particular may vary by species. Plant-soil feedbacks driven primarily by biogeochemical cycles could be altered simply by these abiotic post-disturbance changes.

Plant-soil feedbacks driven primarily by biotic interactions would be affected by post-disturbance changes in the soil biotic community. The soil microbial composition, diversity, and structure may change along with abiotic drivers such as hotter and drier soil or reduced nutrient availability. In addition, biotic drivers such as stress to the host plant, fewer active host root tips, and a shifting plant community would all contribute to altered microbial diversity and composition. Wind-damaged forest soils contain compositionally different soil microbial communities than intact temperate forest soils. Pine seedling roots in wind-damaged and intact forest areas hosted different ectomycorrhizal communities. The mycorrhizal community in wind-damaged areas was not only compositionally different, but also less diverse (Cowden and

Peterson 2013). Treefall gap areas may also contain a greater abundance of pathogenic fungi (Reinhart *et al*. 2010). In general, wind damage may lead to a less diverse, more generalist soil microbial community (Cowden and Peterson 2013; Egli *et al*. 2002; Jones *et al*. 2003; Reinhart *et al.* 2010). The shift towards easily-dispersed, fast-colonizing generalist species could dampen the strength of plant-soil feedbacks, leading to a "blank slate" of PSF immediately following a strong disturbance (Kardol *et al*. 2013; Reynolds *et al.* 2003).

Thirdly, abiotic and biotic changes interact in the post-disturbance landscape. Both the plants and soil biotic communities may function differently after a wind disturbance. The same plant-fungal pairs function differently in conditions with altered nutrients (Corkidi *et al*. 2002; Manning *et al*. 2008), water (Kennedy and Peay 2007), and even light (Kummel and Lostroh 2011; McCarthy-Neumann and Ibáñez 2013). For instance, a sudden increase in nutrient availability may increase the likelihood that plants will either avoid associating with root mutualists altogether (Treseder 2004) or suffer from a "parasitic" carbon-nutrient trade in which fungi benefit more than plants (Corkidi *et al.* 2002; Johnson et al. 1997; Johnson *et al*. 2010). All of these conditions would be relevant to a post-disturbance environment.

### 1.4 Specific Research Aims

In this dissertation, I present three separate chapters that each explore the plant-soil relationships between mature trees and seedlings in Northeast Georgia temperate forests. The first data chapter focuses on the generality of negative distance dependence in temperate forests. In the study, I observe patterns of seedling mortality in a mapped Piedmont forest, with an aim to detect genus-level negative distance-dependence patterns. By using spatially-explicit data in a mapped plot, this study contributes to a growing body of knowledge of the full extent and importance of NDD in North American temperate forests.

The second and third data chapters focus on plant-soil feedbacks in the southern Appalachian Mountains. These two studies use different methods to ask similar questions about the impact of natural disturbances on plant-soil feedbacks. The second chapter uses a greenhouse experiment with field-collected soils. In this study, I examine the nature of plant-soil feedbacks in intact southern Appalachian forests and determine whether those relationships are altered after a tornado. The third chapter builds upon that knowledge by testing whether feedbacks found in the greenhouse are also evident in the field. By using a multi-stage greenhouse and field experiment, I compare the importance of specific soil biotic changes to the overall post-tornado environment. These two data final chapters combined provide a uniquely comprehensive look at the ways in which tornado damage alters the plant-soil relationships of common southern Appalachian tree species. Taken as a whole, this dissertation provides multiple lenses to examine Northeast Georgia temperate forest plant-soil interactions.

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# CHAPTER 2

# NEGATIVE DISTANCE DEPENDENCE BY TREE GENUS IN A MAPPED PIEDMONT FOREST[1]

[1] Nagendra, U.J., McCoy, M.C., Seesanthar, V., and Peterson, C.J. Submitted to *Plant Ecology*, 1/19/17.

## 2.1 ABSTRACT

Long-lived sedentary organisms such as trees and corals can have profound effects on ecosystem structure and diversity. In many cases, adults inhibit the growth and survival of nearby young relatives. This negative distance-dependent mortality appears to be relatively common, more so than its potential mechanism would suggest. In addition, the importance of this pattern is widely understudied in biomes other than tropical forests. In this study, I examine whether these patterns can be seen in more distantly-related individuals, in a mature temperate forest. I use a spatially-explicit, seedling-centered approach. We established seedling plots in 1 ha of a mapped Piedmont forest dynamics plot to examine (a) shifts in seedling spatial patterns, and (b) effects of neighboring tree influence on seedling performance. Seedlings shifted away from same-genus trees in the second year. This spatial shift was most evident in the smallest seedlings (year 1= 7.75 +/- 6.73 m, year 2 sim = 7.77 +/- 6.50 m, year 2 obs= 12.96 +/- 8.58 m).

Middle-sized seedlings survived at lower rates when influenced by same-genus trees. Conversely, they survived at higher rates when influenced by different-genus trees. Our findings provide evidence for the existence of Negative Distance Dependence (NDD) patterns at the genus level in this mature piedmont temperate forest. Shifts in seedling spatial distributions, seedling survival rates, and seedling survival probabilities were all roughly consistent with the hypothesis that seedlings in this forest are negatively impacted by the influence of closely related trees.

## 2.2 INTRODUCTION

Due to their sessile nature, mature trees can have profound effects on nearby growing seedlings and saplings. When these tree-recruit interactions differentially affect same-species and different-species recruits, they shape the spatial distributions of forest species and the structure of forest communities. Trees may facilitate same-species recruits if they provide a comparatively favorable soil environment (abiotic and/or biotic), provide protection from natural enemies, or even indirectly share vital nutrients through roots. Alternatively, trees may negatively impact recruits through niche-specific resource consumption such as shading or water use, or by hosting species-specific natural enemies.

Plant species in many ecosystems exhibit negative density and/or distance dependence (NDD), in which individual performance is negatively correlated with the influence of the same-species. Ideas such as the Janzen-Connell hypothesis propose how specific types of NDD could result in over-dispersed spatial distributions (Connell *et al*. 1984). If these interactions are strong enough, they could theoretically facilitate and maintain high biodiversity, such as in the tropics. Janzen-Connell proposes that over-dispersion of species could result from a combination of clumped seed dispersal and greater seed and seedling mortality in areas with more influence by mature same-species trees. In the original hypotheses, greater seedling mortality was expected in areas closer to mature trees, or in areas with high density of same-species individuals. These areas would have higher populations of species-specific enemies. Although the original hypothesis focused on herbivores and above-ground pests, research has found that belowground species-specific pests and pathogens are more likely mechanisms for this pattern (Mangan *et al*. 2010; Terborgh 2012).

Since the introduction of the Janzen-Connell hypothesis, many studies have documented existence of the predicted spatial patterns and distance-dependent seedling mortality. In some studies, the amount of NDD was related to species diversity. On a community scale, species that exhibited the strongest negative density dependence were also the least abundant (Mangan *et al*.

2010). On a regional scale, areas with greater species diversity contained more species that exhibited negative density dependence (Johnson *et al*. 2012).

Although original Janzen-Connell writings suggested that the predicted NDD patterns would mainly exist in tropical forests because of higher host-specificity in the topics, many studies have demonstrated evidence of NDD patterns in temperate forests as well (Lambers and Clark 2003; Martínez *et al*. 2013; McCarthy-Neumann and Kobe 2010; Packer and Clay 2000; Reinhart *et al*. 2012b; Yamazaki *et al*. 2009). The literature for temperate NDD is growing, but meta-analyses and reviews continue to show bias towards studying NDD in tropical forests. A high number of studies take place in Panama, often at the same mapped plot (Barry 2016; Carson *et al*. 2008; Comita *et al*. 2014; Hyatt *et al.* 2003). A growing body of literature, conducted in a diverse array of locations and study systems, furthers our understanding of how widespread these phenomena may be, and if they contribute to community-wide patterns in species diversity or overall regional patterns of diversity.

Several meta-analyses and reviews offer different takes on the universality of NDD patterns. While many agree that there is evidence for distance-dependent mortality in many biomes, one meta-analysis suggests that the patterns only exist for a few species within each region, making it more of a special case than a universal pattern (Hyatt *et al*. 2003). Others note that the commonality of NDD across latitudes contradicts the hypothesis that distance-dependent mortality is responsible for the high biodiversity in the tropics (Comita *et al*. 2014; Lambers *et al.* 2002).

The commonality of NDD in many biome types suggests that the requirements for its occurrence may not be as stringent as originally proposed. Since Janzen-Connell theoretically requires highly species-specific natural enemies or microsite preferences, and pathogens and herbivores are rarely exclusive, NDD should be a rare occurrence. The growing body of literature suggests that NDD patterns are more common than should be possible given the rarity of highly specialized natural enemies worldwide. Instead, some have suggested that complex interactions of less-specialized or perhaps even generalist natural enemies could create very similar spatial patterns (Benítez *et al.* 2013; Hersh *et al*. 2012). NDD patterns have been found between more distantly related trees and seedlings (Lebrija-Trejos *et al*. 2014; Liu *et al*. 2012; Zhu *et al*. 2015) than would be expected by Janzen-Connell. The initial premise states that distance or density-dependent mortality would occur when seedlings and mature trees of a given species are relatively more vulnerable to certain natural enemies (pathogens, herbivores, pests). In many ecosystem types, closely related species coexist. Closely related individuals are often more likely to share vulnerabilities to the same natural enemies (Gilbert and Webb 2007). Individuals within a genus may exhibit density or distance-dependent mortality patterns, resulting in a genus-level phylogenetic NDD pattern.

The ability to state generalities about worldwide NDD patterns is limited by the sensitivity of study methods and modes of observation (Lambers *et al.* 2002). Some studies may miss observations by focusing on older age classes such as saplings and mature trees. Others may overestimate patterns by neglecting to compare for the overall effect of neighboring trees, regardless of relatedness.

Much of the initial evidence for distance-dependent mortality relies on seedling transects extending from a focal tree (Augspurger 1983; Packer and Clay 2000; Sugiyama 2015). This type of method assumes that the focal tree alone constitutes the total same-species influence on a given seedling. Studies using this method, then, must either violate this assumption and discount the additional effects of surrounding trees and saplings, or restrict their analyses to select communities in which mature adults are widely dispersed. This requirement effectively self-selects the types of species and communities included in NDD studies; common dominant tree species for many ecosystem types would inherently violate this assumption.

The use of mapped research plots has enabled more flexible and comprehensive study designs (Liu *et al*. 2012; Martínez *et al*. 2013; Peters 2003). When the locations, identities, and sizes of all individuals in an area are known, all neighboring individuals can be included in a seedling mortality model. This more flexible approach allows researchers to evaluate NDD patterns in many more ecosystem types, and for species of many abundances (common and rare). Only recently have studies gone the extra step to include mapped seedling locations, enabling individual-based analyses instead of quadrat-based analyses (Martínez *et al*. 2013; Peters 2003).

This study builds from the extensive body of work on Negative Distance Dependence to investigate the presence of genus-level negative distance-dependence in a mapped temperate Piedmont forest. Based on documented NDD in other North American temperate forests, I expect this forest to exhibit significant negative distance dependence in seedlings. I used the mapped locations of both trees and seedlings of common genera in a long-term forest dynamics plot to examine negative density dependence in terms of both (a) shifts in seedling spatial patterns and (b) effects of neighboring same-genus trees on seedling performance.

## 2.3 Methods

### <u>Overview</u>

This study takes a seedling-centered approach to assess genus-level Negative Distance Dependence patterns in a Piedmont forest. Instead of examining what the effect of one tree may be on a subset of seedlings around it, this approach examines the biotic environment consisting of all neighboring trees influencing an individual seedling. The utility of a mapped forest dynamics plot enables this spatially-explicit approach. Distance data was used to examine NDD patterns in two ways: (a) shifts in seedling spatial patterns, and (b) effects of neighboring tree influence on seedling performance. Seedling performance was measured with (b1) seedling survival rates, (b2) seedlings survival probabilities, and (b3) seedling growth.

Site description and plot setup

The study took place within a 12 hectare (300m x 400 m) long-term forest dynamics plot at the State Botanical Garden of Georgia (33.903029, -83.380543). The forest is a typical Piedmont mature secondary forest, dominated by White Oak (*Quercus alba*), American Beech (*Fagus grandifolia*), various Hickories (*Carya spp*.), and Tulip Poplar (*Liriodendron tulipifera*) with a sub-canopy of other oak species (*Q. nigra* and *Q. rubra*), Hophornbeam (*Ostrya virginiana*), and Sourwood (*Oxydendrum arboreum*). Soils consist of Louisburg and Madison sandy loams (NRCS). The area receives an average of 116 cm of precipitation annually, and daily temperature ranges from 21.0 – 33.0 ˚C in July to 0.61 – 12.2 ˚C in January (NCDC 2011). The long-term forest dynamics plot is subdivided into 1200 10m x 10m grid cells. Within each grid cell, all woody stems greater than 5 cm have been identified, mapped, and measured. For this study, a set of seedling demography plots were established within a 1 hectare area of this long-term forest dynamics plot. A 3m x 3m seedling plot was established in the northwest corner of each of 36 grid cells.

Seedling monitoring

In June 2014, all tree or shrub stems less than 1 m height within each 3m x 3m seedling plot were tagged and mapped, and their height and diameter measured. Although an unknown fraction of these were likely of vegetative origin, I refer to them herein as seedlings, for convenience. Seedlings were identified to genus. Seedling age was estimated as less than or greater than 1 year. Seedlings that appeared to be resprouts from older root mass were marked as greater than 1 year old.

In June 2015, seedling plots were revisited. Surviving seedlings were measured again for height and diameter, and genus classification was confirmed. Negative Distance Dependence predicts temporal shifts in seedling spatial patterns. Since young seedlings close to same-species trees are more likely to die, the overall distribution of seedlings should shift away from conspecific trees in subsequent years. This pattern should be particularly evident in the youngest age class of seedlings.

To examine this pattern, I calculated the distance to the nearest same-genus and different-genus neighbors for all seedlings present in 2014. These Year 1 nearest-neighbor distances were then compared to those of both observed seedlings survivors (Year 2 obs) and randomly simulated seedling survivors (Year 2 sim). If NDD patterns are evident in this forest, I would expect for surviving seedlings (Year 2 obs) to be farther from same-genus trees than the original cohort (Year 1) or due to chance (Year 2 sim). Seedling distance from different-genus trees, however, should remain equal across years.

First, seedlings were grouped into three roughly equal size classes using the ‘Hmisc’ package (Harrell and Dupont 2016) in the statistical program R (R Core Team 2015). Within each size class (Size Class 1: < 6.9 cm; Size Class 2: 7.0 cm – 11.6 cm; Size Class 3: > 11.6 cm), I generated a set of random seedling survivors (Year 2 sim) equal to the number of observed seedling survivors, using the ‘sample’ function in R. Randomizations were iterated 50 times to create a representative dataset. Nearest Neighbor distances were compared between Year (Year 1, Year 2 obs, Year 2 sim), relatedness (same- or different-genus) and size classes, using analysis of variance (ANOVA). Genus and grid cell were included as covariates. Tukey's HSD was used for post-hoc means separation. An increase in nearest same-genus neighbor distance in year 2 as compared with different-genus neighbor distance would be consistent with Janzen-Connell patterns.

I calculated the euclidean distance between each individual seedling and each tree within scales of a) 2 meters, b) 5 meters, and c) 10 meters. Each seedling-tree pairing was categorized as either same-genus or different-genus.

For each seedling, same-genus tree influence ($I_c$) and different-genus tree influence ($I_h$) were calculated using the formula: [ $I = \text{sum}(\text{basal area}_i/\text{distance}_i)$ ] in which *i* signifies tree. Both same-genus and different-genus tree influence was calculated for each individual seedling at all three scales (2 m, 5 m, and 10 m). Janzen-Connell predicts that seedling mortality should decline with increasing distance from conspecific adults.

Seedlings were grouped into seven Influence Classes based on the neighboring same-genus tree influence they experienced within 10 m. Seedling survival rate was calculated for each Influence Class and Size Class combination. I used linear regression to correlate seedling survival rate with Influence Class independently for each seedling Size Class. This process was repeated using neighboring different-genus tree influence.

I used a logistic regression and likelihood ratio to compare the probability of seedling survival given the surrounding influence of neighboring trees (both same-genus and different-genus). Seedling X,Y coordinates were included as covariates to control for spatial variability. This analysis was done independently for each seedling genus, and for each of the three spatial scales (2m, 5m, and 10m).

Linear regression was used to compare the effect of neighboring trees on the growth of surviving seedlings, using both height change and volumetric relative growth rate.

All analyses were done with the statistical program R (R Core Team 2015). Logistic and linear regressions were completed using the R package ‘car’ (Fox and Weisberg 2011), and seedling spatial patterns were analyzed through the R package ‘spatstat’(Baddeley et al. 2015).

## 2.4 Results

Seedlings were consistently farther from same-genus neighbor trees than different-genus neighbor trees in both years ($F(1, 58945) = 18316.29$, $p<0.001$; Table 2.1, Figure 2.1). Distance to same-genus neighbor increased with both seedling size class ($F(2, 58945) = 27.40$, $p < 0.001$) and with survey year ($F(1, 58945) = 4.05$, $p < 0.05$). Tukey’s post-hoc comparisons showed that the greatest increase in same-genus neighbor distance between the two years was for the smallest seedling size class (year 1= 7.75 +/- 6.73 m, year 2 sim = 7.77 +/- 6.50 m, year 2 obs= 12.96 +/- 8.58 m, Figure 2.1). Distance to nearest same-genus neighbor also increased for size classes 2 and 3, but these differences were not significant. Distance to nearest different-genus neighbor did not differ between the two survey years, regardless of seedling size class or genus.

When all genera are included in the model, seedling survival rate correlates negatively with same-genus tree influence. This effect was only significant for the middle seedling size class (Table 2.2, Figure 2.2). Conversely, seedling survival rate increased with greater different-genus tree influence. This effect was similarly also only significant for the middle seedling size class (Table 2.2, Figure 2.3).

*Ostrya*, *Quercus*, and *Ulmus* seedling survival probabilities were affected by neighboring tree influence of either same-genus or different-genus trees (Table 2.3). For other genera, seedling survival probability did not change with greater influence of either type.

At all three scales (2m, 5m, and 10m), Ostrya seedling survival increased with influence by different-genus trees. At 10m scale, *Quercus* seedling survival decreased with influence by same-genus trees. At the 2m scale, *Ulmus* seedling survival increased with influence by diff-genus trees. At 5 M scale, however, *Ulmus* seedling survival decreased with influence by same-genus (Table 2.3).

Neither same-genus nor different-genus tree influence was correlated with surviving seedling growth rate. No correlation was found for relative growth rate, or change in height, at any of the three scales. This pattern was the same for all three seedling size classes.

## 2.5 Discussion

Our findings provide evidence for the existence of Negative Distance Dependence (NDD) patterns at the genus level in this mature piedmont temperate forest. Shifts in seedling spatial distributions, seedling survival rates, and seedling survival probabilities were all roughly consistent with the hypothesis that seedlings in this forest are negatively impacted by the influence of closely related trees. These patterns could arise from several possible mechanisms, including a higher prevalence of natural enemies surrounding same-genus trees, as described in Janzen-Connell. Negative distance dependence could also be created by other mechanisms, however, such as intraspecific competition, shading, or nutrient dynamics.

The seedling distribution patterns showed the clearest evidence for NDD in this study. As predicted, the overall distribution of seedlings shifted away from same-genus trees in the second year of the study, more so than could be explained by chance seedling survival. This pattern was particularly evident in the smallest size class of seedlings, which is presumed also to include the youngest seedlings (Barot *et al*. 1999; Jansen *et al*. 2008; Wada and Ribbens 1997). Young seedlings that have yet to establish a strong root system are thought to be particularly vulnerable to environmental conditions, including the inhibiting effects of nearby same-genus trees. It is not surprising that this smallest seedling class exhibited both the highest mortality rates and the most evident spatial shift away from same-genus trees.

Shifts in seedling spatial distributions are expected when seedlings most influenced by same-genus trees experience lower survival rates than those least influenced by same-genus trees (Augspurger 1983; Clark and Clark 1984; Packer and Clay 2000). In this study, the survival rates of middle-sized seedlings were lowest when seedlings were most affected by same-genus trees. Conversely, greater influence of different-genus trees was associated with higher seedling survival, demonstrating that the NDD pattern was limited to same-genus trees. It is not surprising that the effect was not significant in the largest seedling size class, since the effect has been shown to be most significant in the smallest and/or youngest seedlings (Connell *et al*. 1984; Luo *et al*. 2012; Peters 2003).

Although it may seem puzzling that seedling growth rates were only significantly correlated to tree influence in middle-sized seedlings, and not in the smallest seedlings, there are some alternative explanations. One possible reason could be that small seedlings gain just as much benefit from nearby related trees (favorable soil chemistry, hosting mycorrhizal mutualists, etc.), as they experience inhibition (pathogens, shading, etc.). The interplay between benefit and inhibition is rarely simple or static; a given seedling's interactions with soil chemistry, structure, and biota will change as the seedling itself grows, and as the environment develops. It is possible that inhibitions outweigh benefits for middle-sized seedlings, but not for small seedlings.

The lack of demonstrable correlation in small seedlings may simply be due to small sample size in influence bins, since small seedlings were barely present in the largest same-genus tree influence bins. It should also be noted that our "middle" size class is still comparable to the "small" size class used in many other studies (Lambers and Clark 2003; Sugiyama 2015). Our focus on young and small seedlings weighted our analysis towards seedlings under 10 cm tall, which is the lower size limit of many seedlings studies.

Regardless of size class, it is interesting to note that our tree influence metric, which includes a measure of tree size in addition to distance, had a slightly different effect on seedling survival than distance alone. This suggests that seedlings experience the influences of larger, but farther trees more so than small nearby trees. Simply being a seedling's nearest neighbor, then, does not necessarily mean that tree has the largest effect on a seedling. That these two metrics also impacted two different size classes (small vs. medium seedlings) does indicate that the initial seedling distributions may have skewed the data—middle-sized seedlings happened to be closer to larger trees than small seedlings. Since this study is observational, seedling initial locations were as occurred in nature, and not experimentally standardized or randomized. With this data, we cannot tell whether initial distributions are due to seed rain, or external factors driving seed predation, mortality, or failure to germinate.

When divided out into separate genera, seedling survival probabilities were still consistent with NDD patterns. For three of the genera, seedlings were relatively positively affected by diff-genus tree influence and negatively affected by same-genus trees. For two genera, this manifested as positive diff-genus. For one, this manifested as negative same-genus. For each of these genera, effects were visible at different scales within 10 m, indicating that genera-specific mechanisms may be at work. It is possible that, for other genera, low sample size clouded the response.

Although tree influence affected seedling survival, it did not affect the growth of surviving seedlings. Though at first this result may appear contradictory, it fits within theoretical expectations. Seedlings with the lowest growth rates would also be the most likely to perish; surviving seedlings are already among the best performing individuals. Subtle differences in growth rate due to tree influence would likely not be visible in only these high-performing surviving seedlings. The result also implies that once seedlings have become established enough to survive after 1 year, they are not susceptible to tree influence. This idea is supported by high survival rates in larger/older seedlings in this study and others (Peters 2003; Sugiyama 2015; Zhu *et al*. 2015).

This work contributes to a growing body of literature demonstrating that Negative Distance Dependence patterns are not limited to tropical forests (Lambers and Clark 2003; Martínez *et al*. 2013; McCarthy-Neumann and Kobe 2010; Packer and Clay 2000; Reinhart *et al.* 2012b; Yamazaki *et al*. 2009). Spatial dynamics created by seedling responses to mature trees occur in many ecosystem types (Comita *et al*. 2014). The broader consequences of these spatial patterns on ecosystem structure, composition, and diversity, however, are yet to be clarified. If they are not unique to tropical forests, and indeed occur at multiple latitudes (Comita *et al*. 2014) and levels of biodiversity (Lambers *et al*. 2002), the hypothesis that they are a major factor in the creation of high biodiversity in the tropics may not be supported. Instead, this distance-dependent seedling mortality may be more universal across biomes as a mechanism of maintaining community structure.

In addition, this work demonstrates that Janzen-Connell type processes can affect individuals at a broader phylogenetic scale than species (Lebrija-Trejos *et al*. 2014; Liu *et al*. 2012; Zhu *et al*. 2015). Individuals related at the genus scale can also influence each other's survival and contribute to genus-level spatial distributions. By extending the concept of Negative Distance Dependence beyond species level, this work questions the need for strong species-specific natural enemies for NDD patterns to emerge.

## 2.6 Tables and Figures

Table 2.1. Summary of ANOVA examining tree relatedness (same- or different-genus) effects on seedling distance from neighboring trees in two survey years.

| Source | F | P |
|---|---|---|
| Tree Influence Type | 18316.29 | **< 0.001** |
| Survey Year | 4.053 | < **0.05** |
| Seedling Size Class | 27.40 | < **0.05** |
| Influence Type x Year Interaction | 2.84 | 0.058 |
| Influence Type x Year x Size Interaction | 39.36 | **< 0.001** |
| Seedling Genus | 2269.96 | **< 0.001** |
| Grid Cell | 258.31 | **< 0.001** |

Table 2.2. Summary of regressions examining neighboring tree influence (same-genus or different-genus) effect on seedling survival rates

| | Same-Genus Tree Influence | | | Different-Genus Tree Influence | | |
|---|---|---|---|---|---|---|
| Size Class | F | P | $R^2$ | F | P | $R^2$ |
| 1 | 2.43 | 0.14 | 0.12 | 0.12 | 0.73 | 0.01 |
| 2 | 6.08 | 0.02 | 0.25 | 5.83 | 0.03 | 0.23 |
| 3 | 0.21 | 0.65 | 0.01 | 1.49 | 0.24 | 0.06 |

Table 2.3. Summary of logistic regression likelihood ratios of seedling survival probabilities

| | *Acer* | | *Carya* | | *Fagus* | | *Nyssa* | | *Ostrya* | | *Quercus* | | *Ulmus* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P |
| (a) 2 meters | | | | | | | | | | | | | | |
| Diff-Genus Tree Influence | 0.02 | 0.88 | 0.00 | 0.95 | 1.58 | 0.21 | 0.64 | 0.42 | 4.83 | **< 0.05** | 0.12 | 0.73 | 4.25 | **< 0.05** |
| Same-Genus Tree Influence | 1.45 | 0.23 | 0.44 | 0.51 | 0.21 | 0.65 | 2.99 | 0.08 | 0.39 | 0.53 | 0.93 | 0.34 | 0.86 | 0.35 |
| X coordinates | 2.04 | 0.15 | 8.39 | < **0.01** | 4.72 | < **0.05** | 0.12 | 0.73 | 15.72 | < **0.001** | 0.47 | 0.49 | 0.23 | 0.63 |
| Y coordinates | 1.53 | 0.22 | 0.24 | 0.62 | 0.38 | 0.54 | 0.45 | 0.50 | 15.25 | < **0.001** | 0.10 | 0.75 | 0.88 | 0.35 |
| (b) 5 meters | | | | | | | | | | | | | | |
| Diff-Genus Tree Influence | 0.08 | 0.77 | 0.46 | 0.5 | 2.22 | 0.14 | 0.20 | 0.65 | 11.68 | < **0.001** | 0.04 | 0.84 | 3.08 | 0.08 |
| Same-Genus Tree Influence | 1.84 | 0.18 | 0.01 | 0.91 | 0.14 | 0.71 | 2.18 | 0.14 | 0.05 | 0.82 | 0.33 | 0.57 | 7.83 | < **0.01** |
| X coordinates | 2.46 | 0.12 | 7.15 | < **0.01** | 4.61 | < **0.05** | 0.18 | 0.67 | 7.16 | < **0.01** | 0.33 | 0.56 | 0.28 | 0.6 |
| Y coordinates | 1.64 | 0.2 | 0.13 | 0.72 | 0.19 | 0.66 | 0.28 | 0.59 | 15.65 | < **0.001** | 0.01 | 0.91 | 0.13 | 0.72 |
| (c) 10 meters | | | | | | | | | | | | | | |
| Diff-Genus Tree Influence | 0.30 | 0.58 | 0.36 | 0.55 | 1.11 | 0.29 | 0.35 | 0.56 | 5.45 | < **0.05** | 0.06 | 0.81 | 3.07 | 0.08 |
| Same-Genus Tree Influence | 2.01 | 0.16 | 0.00 | 0.98 | 0.55 | 0.46 | 2.24 | 0.13 | 0.96 | 0.33 | 6.21 | < **0.01** | 3.39 | 0.07 |
| X coordinates | 2.93 | 0.09 | 6.69 | < **0.01** | 3.42 | 0.06 | 0.71 | 0.40 | 12.77 | <**0.001** | 0.04 | 0.85 | 0.66 | 0.42 |
| Y coordinates | 1.85 | 0.17 | 0.10 | 0.75 | 0.09 | 0.77 | 0.32 | 0.57 | 6.98 | < **0.01** | 0.28 | 0.60 | 2.70 | 0.1 |

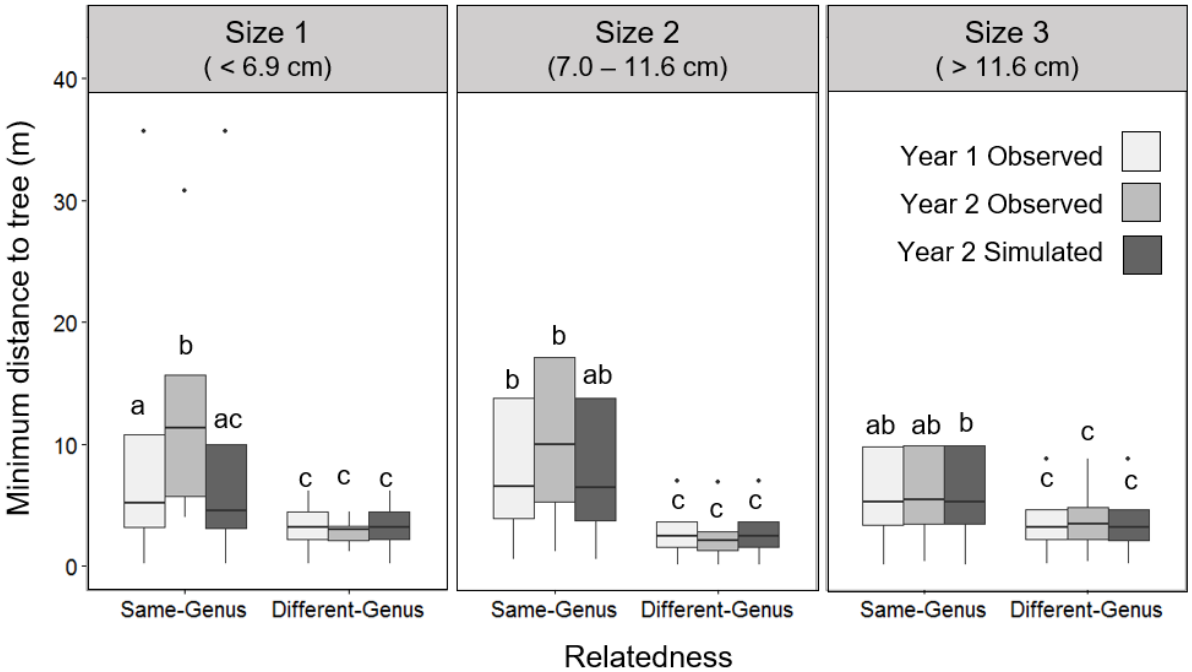


Figure 2.1. Distance to nearest same-genus and different-genus neighboring tree, for seedlings present in year 1 (light colors), those surviving in year 2 (dark colors), and those randomly simulated for survival in year 2 (middle shades). Boxes with different letters are significantly different according to a Tukey's Honest Significant Difference means separation test ($p < 0.05$)

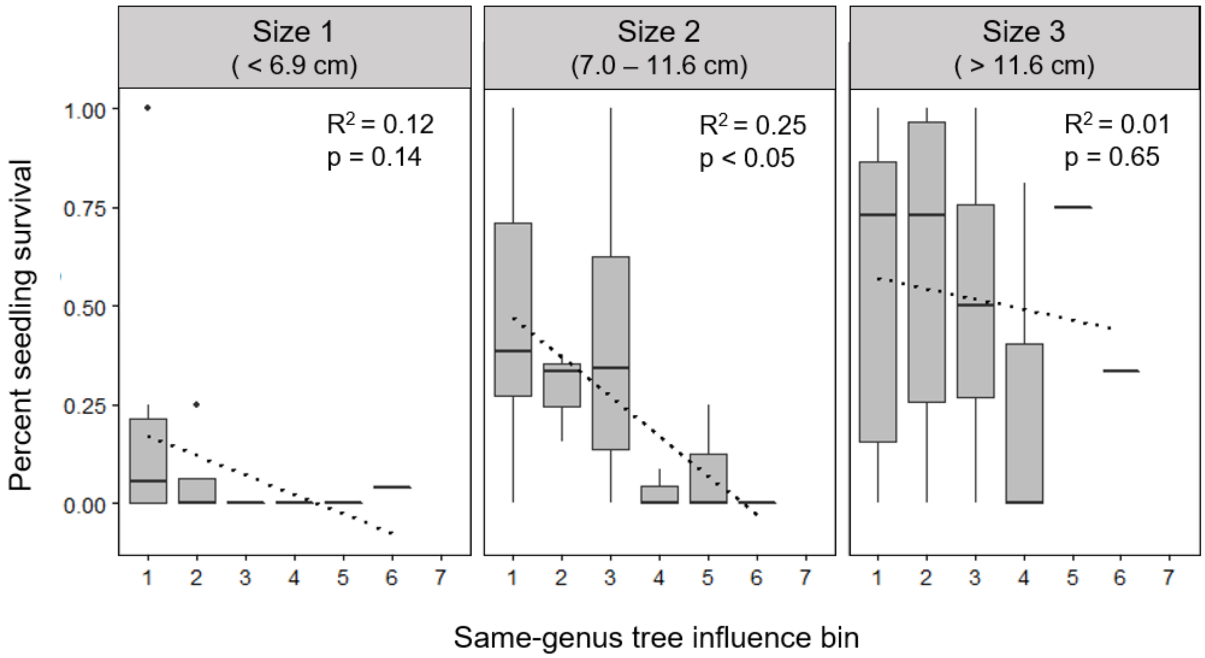


Figure 2.2. Observed seedling survival rate as a function of same-genus tree influence within 10 m. Lines represent modeled linear regressions. All genera are pooled.

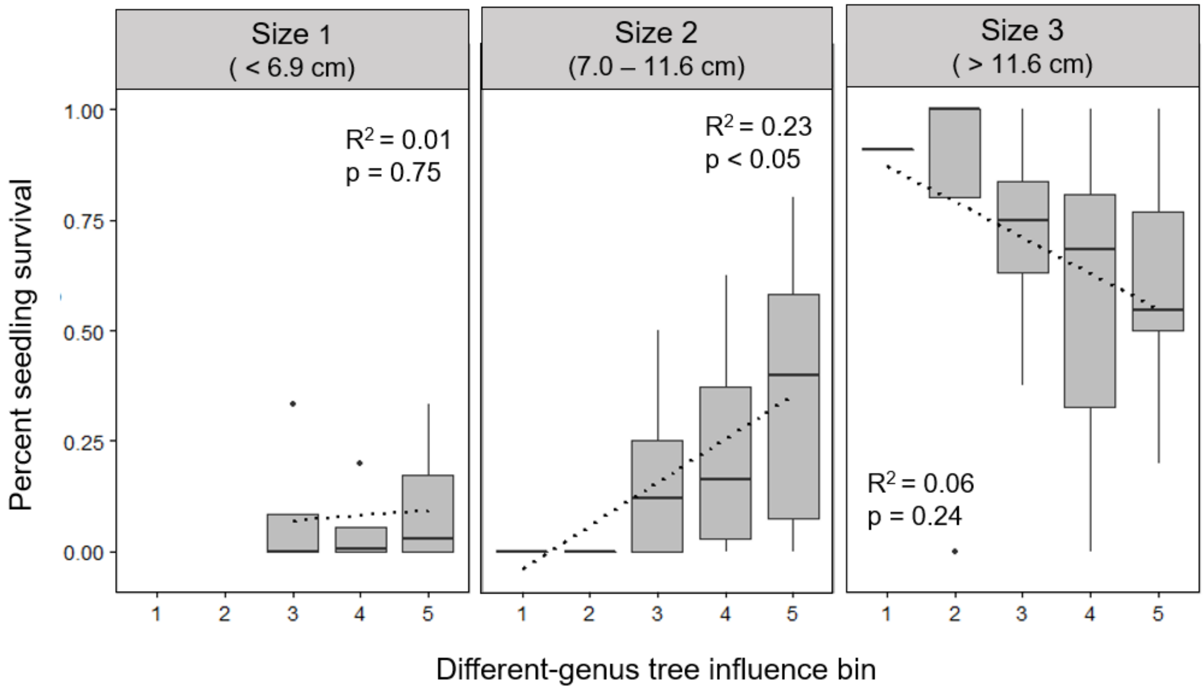


Figure 2.3. Observed seedling survival rate as a function of different-genus tree influence within 10 m. Lines represent modeled linear regressions. All genera are pooled.

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CHAPTER 3

# PLANT-SOIL FEEDBACKS DIFFER IN INTACT AND TORNADO-DAMAGED AREAS OF THE SOUTHERN APPALACHIAN MOUNTAINS, USA[2]

[2] Nagendra, U.J. and Peterson, C.J. 2016. *Plant and Soil*. *402*(1-2), 103-116. doi: 10.1007/s11104-015-2766-0. Reprinted here with permission of the publisher.

## 3.1 Abstract

Aims

Plant-soil feedbacks (PSF) greatly influence forest community structure and diversity. However, it remains unknown how feedbacks change after disturbances. Biotic and abiotic changes reduce soil microbial diversity after a severe disturbance. These post-disturbance changes may create neutral PSF. We examine a) differences in performance of three seedlings of southern Appalachian tree species in same-species and different-species soil and b) whether the relationship differs between intact forest and wind-damaged patches, as well as c) test mycorrhizal colonization rate as a potential mechanism.

Methods

In April 2011, a severe (EF-3) tornado damaged several thousand hectares of mature secondary mixed pine-oak forest in northeast Georgia, USA. In 2012, we collected soil from the base of mature trees in intact forest and in tornado-damaged patches. Three tree species seedlings were grown in same-species and different-species soil for three months. Height, biomass, and mycorrhizal colonization were compared.

Results

Results suggest that PSF are neutral to negative in intact forest. For *Nyssa sylvatica* Marsh., PSF were less negative in wind-damaged soils. *Quercus alba* L. exhibited the opposite response.  *Pinus strobus* L. PSF did not differ with wind damage.

Conclusions

We found that PSF may be changed by severe wind disturbance, but the nature of the changes depends upon species identity. Multiple soil mechanisms aside from mycorrhizal colonization likely drive disturbance-related PSF changes.

## 3.2 INTRODUCTION

Reciprocal interactions between plants and soil components –biotic and abiotic plant-soil feedbacks—are influential in many ecological processes (Ehrenfeld *et al.* 2005; Putten *et al.* 2013). Long-lived plants or patches of vegetation alter the local soil characteristics through the accumulation of species-associated microbial communities (mutualists, pathogens, and decomposers) as well as through biogeochemical pathways. Both negative (stabilizing) and positive (self-promoting) feedbacks are known to influence ecosystem composition, diversity, and productivity in many terrestrial ecosystems (Klironomos 2002; Mills and Bever 1998; Van Der Heijden *et al*. 2008), including both tropical forests (Comita et al. 2010; Connell 1978b; Mangan et al. 2010; Terborgh 2012) and temperate forests (Johnson *et al*. 2012; Reinhart *et al.* 2012b).

Although many studies of plant-soil feedbacks assume static conditions, ecosystems are inherently dynamic. Periodic disturbances are a major force in shaping ecosystem development (White and Jentsch 2001; Xi and Peet 2011). Tornadoes, hurricanes, fires, and floods cause abrupt and severe changes to both abiotic and biotic ecosystem parameters, all of which may influence plant-soil feedbacks.  Plant-soil feedback strength and direction are known to vary greatly in response to basic environmental conditions such as nutrient status (De Deyn *et al*. 2004; Manning *et al*. 2008), light availability (McCarthy-Neumann and Ibáñez 2013), and soil moisture (Kennedy and Peay 2007). These same environmental characteristics can be highly dynamic throughout ecosystem development, particularly after disturbances. As plant communities and ecosystem characteristics change through time, plant-soil feedbacks also change (Kardol *et al.* 2013). In some instances, plant-soil feedbacks may even be partial drivers of that change (Kardol *et al*. 2006; Van der Putten *et al*. 1993). Despite a growing body of literature on the population- and community-level importance of plant-soil feedbacks, we lack a working knowledge of how plant-soil feedbacks change after disturbances (Kardol *et al*. 2013; Putten *et al.* 2013; Reynolds *et al*. 2003).

In order to make conclusions and/or predictions about the consequences of plant-soil feedbacks in real ecosystems, we need an understanding of how plant-soil feedbacks change after natural disturbances. Although disturbance ecologists and soil microbial ecologists have examined soil changes after some disturbances, these studies focus on fire-prone systems (Peay et al. 2009; Rincón and Pueyo 2010) and anthropogenic disturbances in agricultural systems (Kulmatiski and Kardol 2008; Peay *et al*. 2009; Rincón and Pueyo 2010). Little attention has been given to temperate forests and wind disturbances, despite their global importance (Millar and Stephenson 2015; Pan *et al.* 2011). To date, few studies have specifically examined biotic soil changes post-wind disturbance (Cowden and Peterson 2013; Egli *et al*. 2002; Reinhart *et al.* 2010), and no studies have directly examined plant-soil feedbacks following natural disturbances.

Severe wind damage suddenly changes both abiotic and biotic components of the ecosystem. After canopy trees are removed or damaged, the soil experiences greater solar radiation, increased wind flow, and higher evaporation rates, leading to higher temperatures and lower moisture in upper soil (Ritter *et al.* 2005). The sudden flush of decomposing plant material releases stored nutrients into more labile forms, which are both more available to seedlings and at risk of leaching from the system (Vitousek and Melillo 1979). These abiotic changes may alter plant-soil feedbacks directly, through biogeochemical pathways, or indirectly, through microbial associations.

Both abiotic and biotic components of the post-disturbance environment could influence plant-soil interactions. Plants associate with root mutualists at lower rates when labile nutrients are available (Treseder 2004). For those mycorrhizal associations that are formed, carbon-nutrient "trades" may be altered, encouraging "cheating" relationships where fungi benefit more than plants (Corkidi *et al*. 2002; Johnson *et al*. 1997; Johnson *et al.* 2010). The death of mature trees and subsequent turnover of fine roots leads to a loss of host plants for obligate root mutualists. Cowden (Cowden and Peterson 2013) found that *Pinus spp.* seedlings in wind-damaged patches harbored ectomycorrhizal communities that were much less diverse and compositionally different from those in intact temperate forest patches. Wind damage has also been shown to influence the abundance of other soil microbial taxa, such as pathogenic fungi (Reinhart *et al*. 2010). Overall, the combination of abiotic and biotic changes in gaps created by wind damage leads to a less diverse, more generalist soil microbial community (Cowden and Peterson 2013; Egli *et al*. 2002; Jones *et al*. 2003; Reinhart *et al*. 2010).

This study uses a greenhouse experiment with field-collected soils to examine whether a severe wind disturbance can alter plant soil feedbacks in the Southern Appalachian Mountains, USA. In addition, we use a simple mycorrhizal colonization assay to investigate one of the possible mechanisms. We examine (Q1) differences in performance of three common southern Appalachian seedlings in same-species and different-species soil in intact forest patches, (Q2) whether those intact-forest plant-soil relationships are weakened in wind-damaged patches, and (Q3) whether mycorrhizal colonization rates correlate with plant-soil feedbacks.

Although few temperate forest species' plant-soil relationships have been measured directly, eastern temperate forests are thought to exhibit negative density dependence, particularly in diverse forests such as the Southern Appalachians (Johnson *et al.* 2012; Packer and Clay 2003; Reinhart *et al*. 2012b). Due to this overall trend, (H1) Southern Appalachian tree seedlings are expected to exhibit negative feedbacks in intact forests. Since strong biotic plant-soil feedbacks require species-specific relationships (Bever *et al.* 1997), a post-disturbance shift towards a generalist soil community should lead to weaker feedbacks (Kardol *et al*. 2013). Due to abiotic and biotic post-disturbance changes, we predict that (H2) plant-soil feedbacks in wind-damaged patches will be weaker (more neutral) than those in intact forest patches. Many of the potential plant-soil feedback mechanisms are altered after wind damage. Mycorrhizal associations in particular may potentially be affected by both biotic (loss of host species roots) and abiotic (shifts in nutrient availability) changes, leading us to predict (H3a) lower rates of mycorrhizal colonization in wind-damaged soils. If plant-soil feedbacks in these species are related to their mycorrhizal relationships, we would expect to see (H3b) greater mycorrhizal colonization in soil treatments that promote plant growth.

## 3.3 Methods

### Overview

This study examined the difference in plant-soil feedbacks between intact and wind-damaged forest areas using a greenhouse approach. A common tactic to measure plant-soil feedbacks is to measure seedling growth in soil that has been pre-conditioned by various species. In this study, we used field soil that had been naturally "conditioned" by mature trees within, or adjacent to, a recently tornado-damaged field site. Each soil treatment consisted of 2 factors, wind damage and soil origin. The wind damage factor contained 2 levels: intact forest and wind-damaged area. The soil origin factor contained 5 levels: one for each mature tree species under which soil was collected. Altogether, there were 10 soil treatment types, each of which also consisted of 4 individual focal trees from which soil was collected.

For every seedling species, “home” soil is soil collected from beneath a mature tree of the same species, while “away” soil was collected from beneath another species. The difference in plant growth between home and away soil shows whether that plant species’ soil has a positive or negative effect on future progeny, compared to other soils. This ‘home vs away’ contrast measures the indirect plant-soil feedback for an individual tree species—that is, comparing two types of live soil in lieu of comparing ‘home’ live soil to sterile soil as in a direct plant-soil feedback study (Putten *et al*. 2013).

Site Description

Boggs Creek Recreation Area (BCRA) is located within the Chattahoochee National Forest in northeast Georgia (34.67932°, -83.89561°). BCRA is a southern Appalachian forest dominated by oaks (*Quercus alba*, *Q. rubra*, *Q. prinus*) and pines (*Pinus strobus* and *P. virginiana*). Red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), black gum (*Nyssa sylvatica*), and sourwood (*Oxydendrum arboreum*) are also common. Elevations of our study sites ranged from 588 to 672 meters, with steep slopes leading down to the creek. Soils ranged with topography: Wickham fine sandy loam along the creek banks, Tallapoosa soils and Ashe and Edneyille stony loams along slopes, and Tallapoosa cobbly fine sandy loam along ridges (NRCS). Average temperature ranges from 3.94°C in winter to 22.77°C in summer, and average yearly precipitation is 157.7 cm (NCDC 2011).

In April 27-29, 2011 a total of 359 tornadoes formed across 14 states in the southern US. The outbreak on April 27 alone contained 208 tornadoes, including an EF-3 tornado with maximum winds of 120 mph that damaged BCRA and surrounding forest between 10:30 and 10:50 AM (NOAA 2011).

### Focal Tree Selection

We collected soil from beneath 4 mature individuals of 5 common species (*Quercus alba* L., *Nyssa sylvatica* Marsh., *Pinus strobus* L., *Liriodendron tulipifera* L., and *Oxydendrum arboreum* L.) in both intact and disturbed forest areas at BCRA (Figure 3.1, Appendix Table 1). Disturbed areas had been assessed for disturbance severity in summer 2011. Disturbance severity was calculated in a series of 20 x 20 m plots, as percentage of total tree basal area that was damaged after the tornado. Species identity and individual damage class (i.e. uprooted, snapped, canopy broken) was noted at that time for all trees within the plots. In winter 2011-2012, focal trees for this study were chosen from within 5 m of the previously assessed 20 x 20 m plots in order to ensure samples were from areas with moderate or severe plot-level damage. Plot-level disturbance severity of focal tree sites ranged from 64% to 93% tree basal area damaged. In addition, focal trees ranged in individual damage class from uprooted to broken canopy (Appendix Table 1). Some differences among focal tree species' average size and damage types reflect inherent differences between species. *P. strobus*, for instance, are generally larger and more prone to uprooting than *N. sylvatica*. No uprooted *N. sylvatica* trees were found in this study area (Appendix Table 1). Abiotic characteristics (canopy openness, soil moisture, and soil temperature) around focal trees was measured at three separate times during the 2012 growing season (Appendix Table 1).

### Soil Collection

Soil samples were gathered in February and March 2012, before the spring growing season. It is important to note that soils were collected nearly 1 year after the tornado. Since environmental characteristics can change rapidly with time since disturbance, this experiment may not describe dynamics at other time points. At each mature tree, a total of 3 liters of soil from up to 10 cm in depth was taken 1.5 m from the base of the tree (Figure 3.2). The 3-liter sample was composed of 3 separate 1-liter samples taken from equidistant locations around the tree base that were then bulked together, in order to capture the average soil surrounding a single tree. If a damaged tree was uprooted, soil was taken from the undisturbed ground (i.e. not from the root pit or tipup mound). After collection, soil was stored in a greenhouse cold room at 40˚F (4.4˚C) until all samples were collected.

Greenhouse methods

Seeds of all five species were purchased (Sheffield's Seed Company), overwintered, and sown in greenhouse potting mixture. Only three species germinated successfully. These were *Nyssa sylvatica* and *Quercus alba*, classified as shade tolerant with response to canopy opening; and *Pinus strobus*, classified as intermediate in shade tolerance (Burns and Honkala 1990). Seedlings of these three species were transplanted after 1 week of growth into pots with 1:1 mixture of live field soil and a steam-pasteurized sand-turface greenhouse mix. Samples of field soil from each focal tree were kept separate in the greenhouse and experimental design. To prevent cross-contamination, basins and instruments were soaked in a bleach solution between handling different focal tree soil samples.

Each pot contained one seedling in one focal tree soil. Seedlings in same-species ('home') soil were replicated 8 times (2 pots per focal tree soil), while seedlings in different-species ('away') soil were replicated 4 times per origin x wind combination (1 pot per focal tree soil), for a total of 144 seedlings (Figure 3.2). This unbalanced replication design maximized statistical power for the crucial statistical test (home-vs-away contrasts) while maintaining small overall pot numbers. Pots were arranged on the bench in a randomized complete block design. Since splashing water can cause spores to cross-contaminate soils, we left a lip of at least 1.5” on each pot, and spaced pots 4-5” apart on the greenhouse bench to minimize splashing during watering. Seedlings grew in the greenhouse for three months. Differences in plant response were measured via changes in height over three months, and final total plant biomass. Plant biomass was divided at the root collar to calculate root:shoot ratio.

<u>Mycorrhizal colonization methods</u>

After three months of growth, aboveground and belowground portions of all plants were harvested and dried in an oven at 60°C for biomass calculations. Before drying, small portions of Q. alba and P. strobus roots were set aside for ectomycorrhizal colonization scoring. Although mycorrhizae are only one of many potential mechanisms that could drive plant-soil feedback patterns, we chose to use a simple mycorrhizal colonization assay for this first study. Future studies should include assays for multiple potential mechanisms. For each plant, approximately 20 cm total length of fine roots was set aside and scored under a dissecting scope for percent of root tips that were visibly ectomycorrhizal. Root samples of at least 15 cm could not be obtained from 16 of the 96 *Q. alba* and *P. strobus* seedlings, leading to slightly lower replication in the mycorrhizal analysis. After scoring, these roots were dried, weighed, and added to the biomass calculations for *Q. alba* and *P. strobus* seedlings. After drying and weighing, fine roots from N. sylvatica were cleared with KOH and stained with Direct Blue. Approximately 50 root sections per plant were scored for presence/absence of hyphae, arbuscules, and vesicles using a compound light microscope. Arbuscular mycorrhizal colonization was calculated as percent of root sections containing hyphae, arbuscules, and/or vesicles. *N. sylvatica* roots from *L. tulipifera* and *P. strobus* soil were not able to be retained for mycorrhizal analysis.

Statistical analysis

Final height and biomass of seedlings in each soil treatment were compared via ANCOVA using the package 'car' in the statistical software R (Fox and Weisberg 2011; R 2014). Initial seedling height was included as a covariate in the height analysis, and bench location was included as a block (Table 3.1). Within this main model, the soil origin treatment effect informs our first hypothesis (H1), and the soil origin x wind interaction informs our second hypothesis (H2). Without means separation, however, the main model alone cannot fully address the hypotheses. Within each forest damage type, separation of means between soil origins was done in JMP first with a priori orthogonal 'home v away' contrasts and second with Dunnett's test using 'home' soil as control (Table 3.2). 'Home v away' contrasts are a specific measure of a species' indirect plant-soil feedbacks, a test of our first hypothesis (H1), in which we expect seedlings to perform significantly better in 'away' soil than in 'home' soil. Comparing the 'home v away' contrasts between forest damage types tested our second hypothesis (H2), in which we expected significant 'home v away' contrasts in intact forest soil, but not in damaged-forest soil. Dunnett's tests were then used to clarify patterns that emerged from the main model and a priori 'home v away' contrasts. Because they were used to support existing patterns, not to provide independent analyses, statistical alpha-level was not adjusted.

Because this experimental design includes full reciprocal transplants for three of the species, a standard pairwise feedback index can also be calculated (Bever *et al*. 1997). A pairwise feedback index allows us to examine how population-level feedbacks (i.e. 'does species A promote or inhibit its own progeny?') may scale up into community-level patterns (i.e. 'will plant-soil feedbacks encourage species A and B to coexist or not?'). For a given species pair A and B, a feedback value (I) was calculated from the growth of each seedling species (either A or

B) in soil conditioned by each species (represented by subscripts a or b), as explained in Bever *et al*. 1997: [I = ( Aa-Ab ) - ( Bb-Ba ) ].

Pairwise feedback values were calculated for the change in height of *Nyssa sylvatica*, *Quercus alba*, and *Pinus strobus* seedlings in both intact forest soil and wind-damaged area soil. Each feedback value (I) was calculated from mean treatment responses (change in height). In order to determine if mean pairwise feedback values were different from zero, we constructed 95% confidence intervals by sampling with replacement for 1000 iterations. A resulting negative pairwise feedback value (I) indicates that species A and B may coexist, whereas a positive value indicates that the two species may self-segregate. If confidence intervals overlap with zero, plant-soil feedbacks may have little to no effect on species distribution patterns.

In order to test our third hypothesis (H3), ecto- and arbuscular mycorrhizal colonization for each species was arcsin transformed and compared with ANOVA using the package 'car' in the statistical software R (Fox and Weisberg 2011; R 2014). Colonization for each species was compared across wind damage treatments and soil origin. Separation of means between soil origins was done in JMP first with a priori orthogonal 'home v away' contrasts and second with Dunnett's test using 'home' soil as control (Appendix Table 2, Appendix Table 3).

## 3.4 RESULTS

### *Nyssa sylvatica* seedlings

**Change in height:** *N. sylvatica* seedlings grew taller overall in wind-damaged soils (36.3 +/- 13.02 cm) compared to intact forest soils (33 +/- 14.15 cm, ($F(1,34)=4.72$, $p<0.05$), regardless of soil origin ($F(4,34)=2.39$, $p=0.07$; Figure 3.3A, Table 3.1). In addition, there was a significant wind x soil origin interaction ($F(4,34)=3.66$, $p<0.05$). In particular, home vs away contrasts showed that *N. sylvatica* seedlings responded to same-species soil in intact forest soils ($F(41,34)=3.66$, $p=0.06$), but not in wind-damaged soils ($F(1,34)=0.83$, $p=0.37$, Table 3.2). Specifically, *N. sylvatica* seedlings grew taller in other species' intact soils (35.51+/- 15.66 cm) than in their own species' intact soils (28.86 +/- 10.74 cm, $F(41,34)=3.66$, $p=0.06$; Table 3.2), indicating a negative feedback. This trend was driven by *N. sylvatica* seedlings growing taller in intact-forest *Q. alba* soil (47.12 +/- 10.61 cm, $F(1,34)=8.98$, $p<0.01$; Figure 3.3A, Table 3.2). In contrast, the opposite pattern was evident but not significant in wind-damaged soil. *N. sylvatica* seedlings grew taller in their own wind-damaged soil (39.34 +/- 12.60 cm) than in *Q. alba* soil (29.38, +/- 6.47 cm, $F(1,34)=2.90$, $p=0.097$; Table 3.2).

**Biomass:** *N. sylvatica* seedling total biomass did not change with either soil origin ($F(4,34)=0.63$, $p=0.64$) or wind ($F(1,34)=0.0045$, $p=0.95$ ; Table 3.1, Appendix Figure 1A). The root:shoot ratio of *N. sylvatica* seedlings did not differ with wind ($F(1,34)=2.26$, $p=0.14$) or soil origin treatments ($F(4,37)=0.75$, $p=0.56$; Table 3.1, Appendix Figure 2A).

**Mycorrhizal colonization:** The percent of *N. sylvatica* root sections containing AMF structures (hyphae, arbuscules, and vesicles) did not vary with either wind damage ($F(1,23)=0.73$, $p=0.40$) or soil origin ($F(2,23)=1.53$, $p=0.24$, Figure 3.4A). For a detailed breakdown of arbuscular mycorrhizal structures in *N. sylvatica* roots, see supplemental material (Appendix Figure 3, Appendix Table 2).

*Quercus alba* seedlings

**Change in height:** Orthoganal contrasts showed that, although *Q. alba* seedling height did not differ between 'home' and 'away' soils in intact forest soils ($F(1,34)=0.07$, $p=0.79$), they did differ between 'home' and 'away' in wind damaged soils ($F(1,34)= 5.28$, $p< 0.05$; Figure 3.3B, Table 2). *Q. alba* seedlings grew taller in other species' wind-damaged soils (9.09 +/- 3.79

cm) than in their own species' wind-damaged soils (5.28 +/- 6.74 cm(, F(1,34)= 5.28, p< 0.05; Table 3.2), indicating a negative feedback. This relationship was driven primarily by *Q. alba* seedlings growing taller in wind-damaged *N. sylvatica* soils (11.68 +/- 2.31 cm, F(1,34)=4.48, p<0.05) as well as in wind-damaged *O. arboreum* soils (9.08 +/- 2.58 cm, F(1,34)=3.23, p=0.081; Figure 3.3B, Table 3.2). In the main model, *Q. alba* seedling height did not differ with wind (F(1,34)=0.34, p=0.56) or overall soil origin treatments (F(4,34)=0.56, p=0.69; Table 3.1), nor was there a wind x soil origin interaction (F(4,34)=0.10, p=0.42).

**Biomass:** *Q. alba* seedling biomass did not change with either soil origin (F(4,34)=1.09, p=0.38) or wind (F(1,34)=2.06, p=0.16; Table 3.1, Appendix Figure 1B). *Q. alba* seedlings, did exhibit a greater root:shoot ratio when grown in soil from intact forest areas (3.69 +/- 0.27) compared to wind damaged areas (2.74 +/- 0.20; F(1,37)=10.34, p<0.01; Table 3.1, Appendix Figure 2B). Root:shoot ratio of *Q. alba* seedlings did not differ with soil origin treatment (F(4,37)=1.20, p=0.33).

**Mycorrhizal colonization:** Percent of *Q. alba* root tips with visible ectomycorrhizae differed with soil origin (F(4,26)=2.97, p<0.05) but not wind damage (F(1,26)=0.08, p=0.78). Specifically, the home vs away contrast showed that *Q. alba* seedlings had the lowest percentage of ectomycorrhizal root tips when grown in their own intact soil (22.65 +/- 12.40 %) than in other species' intact soils (60.01 +/- 20.61%, p<0.01), particularly compared to *P. strobus* soil (63.34 +/- 36.83%, p<0.01), *L. tulipifera* soil (59.47 +/- 11.26%, p<0.05), and *N. sylvatica* soil (68.94 +/- 18.58%, p<0.05; Appendix Table 3; Figure 3.4B).

<u>*Pinus strobus* seedlings</u>

**Change in height:** *P. strobus* seedling height did not differ with wind (F(1,34)=0.0026, p=0.95) or soil origin treatments (F(4,34)=0.20, p=0.93; Table 3.1), nor was there a wind x soil origin interaction ($F(4,34)= 0.35$, $p=0.84$; Figure 3.3C, Table 3.1). In addition, home vs. away contrasts were not significant in either intact ($F(1,36)=0.20$, $p=0.66$) or wind-damaged ($F(1,36)=0.16$ $p=0.69$) soil for *P. strobus* seedlings (Table 3.2).

**Biomass:** *P. strobus* seedling biomass did not differ in either wind ($F(1,34)=0.78$, $p=0.38$) or soil origin treatments ($F(4,34)=0.13$, $p=0.97$; Appendix Figure 1, Table 3.1). The root:shoot ratio of *P. strobus* seedlings did not differ with wind ($F(1,37)=0.72$, $p=0.40$) or soil origin treatments ($F(4,37)=0.12$, $p=0.97$; Table 3.1, Appendix Figure 2).

**Mycorrhizal colonization:** Percent of *P. strobus* seedling root tips with visible ectomycorrhizae did not differ with either wind damage ($F(1,28)=1.1$, $p=0.30$ ) or soil origin ($F(4,28)=2.09$, $p=0.11$; Appendix Table 3; Figure 3.4C ).

Pairwise feedback indices

Pairwise feedback values for *Nyssa sylvatica* – *Quercus alba* were strongly negative in intact forest soils (-19.1 +/- 6.4), but neutral in wind-damaged soils (3.5 +/- 5.5; Fig 3.5). Pairwise values for the other two possible pairings (*Nyssa sylvatica* – *Pinus strobus* and *Pinus strobus* – *Quercus alba*) did not differ between intact forest and wind-damaged soils. Pairwise feedback values were consistently neutral for *Nyssa sylvatica* – *Pinus strobus* (Intact: -0.3 +/- 5.4; Wind: -1.0 +/- 6.0; Fig 5), and consistently negative for *Pinus strobus* – *Quercus alba* (Intact: -5.4 +/- 2.4; Wind: -4.7 +/- 3.6; Fig 3.5).

## 3.5 Discussion

Overview

Although no empirical tests of post-disturbance plant-soil feedbacks have been previously published, theories suggest that any strong plant-soil feedbacks in intact forests would be weakened after a disturbance—leading to a soil 'blank slate' (Kardol *et al*. 2013). Contrary to this theory and to the second hypothesis (H2), our results suggest wind disturbance does not appear to have a consistent neutralizing effect on plant-soil feedbacks. In this experiment, plant-soil feedbacks in wind-damaged soil were more neutral, more positive, or unchanged from intact-soil, depending on the seedling species involved. No clear post-disturbance plant-soil feedback pattern emerged, suggesting that post-disturbance plant-soil dynamics are more complicated and variable than current theory explains. Plant-soil feedbacks in both intact and wind-damaged forest soil were largely driven by particular species pairings, as well. Although intact forest plant-soil feedbacks were largely negative when significant, as expected in the first hypothesis (H1), these feedbacks were driven by specific species pairs. In addition, mycorrhizal colonization was not a significant driver of plant-soil feedback differences, contrary to the third hypothesis (H3). The variability in wind-damaged soil plant-soil feedbacks suggests that numerous mechanisms may underlie post-disturbance plant-soil feedbacks depending on the plant species.

Question 1— Do seedlings perform differently in same-species and different-species soil in intact forest patches?

Results suggest that plant-soil feedbacks in southern Appalachian forest soils are highly variable and depend on tree species identity. Feedbacks in intact forests, when significant, tended to be negative—consistent with other studies in temperate forests (Johnson *et al*. 2012; Putten *et al*. 2013; Reinhart *et al*. 2012b). These negative feedbacks were only present for a few specific heterospecific pairings, however. Overall feedbacks appeared to be largely driven by seedling responses to one or two heterospecific species. Because of this, we would expect feedbacks measured in the field to vary greatly depending on which neighbor species are present.

Namely, the growth of *N. sylvatica* and *Q. alba* seedlings were mainly affected by *N. sylvatica* and *Q. alba* soil. Wind-damaged *O. arboreum* soil also affected *Q. alba* seedlings, but on the whole, main effects seemed to be related to *N. sylvatica* and *Q. alba* specifically. The overall poor growth and survival of *P. strobus* seedlings may have masked any potential patterns for this species. In intact site soils, *N. sylvatica* seedlings grew better in *Q. alba* soil than in their own, whereas *Q. alba* seedlings grew equally well in all soils. This result is consistent with abundance patterns seen in the existing forest stands at BCRA, in which *Q. alba* is much more abundant than *N. sylvatica* (Peterson, unpublished data). If plant-soil feedbacks influence adult tree distributions in this ecosystem, we would expect to find *N. sylvatica* and *Q. alba* trees together, due to the preference of *N. sylvatica* seedlings for *Q. alba* soil. There is no clear relationship specifically between the spatial distribution of *N. sylvatica* adults and *Q. alba* adults in this site, however (Peterson, unpublished data), indicating that ecological processes other than plant-soil feedbacks (niche differences, competition, light requirements, etc.) may be driving adult tree distributions.

<u>Question 2—Are intact-forest plant-soil relationships weakened in wind-damaged patches?</u>

The response of feedbacks to wind damage also depended greatly on species identity. Both *N. sylvatica* and *Q. alba* seedlings exhibited a change in plant-soil feedback between intact and wind-damaged forest soils, although these changes were in opposite directions.

Contrary to predictions in the literature, severe disturbance did not appear to “neutralize” plant-soil feedbacks (Kardol *et al*. 2013). The results suggest instead that wind damage can either weaken or strengthen negative plant-soil feedbacks, depending on the species involved. The individual mechanisms that drive each species’ plant-soil feedback will react differently to disturbance, host species death, heat, and dry soils.  An individual tree species’ vulnerability to strong winds or ability to resprout would also differentially influence the post-disturbance soil biota. These species-specific differences may be exhibited in our data; no uprooted *N. sylvatica* individuals were found in our study area. Both pathogens and mutualists have the potential to be sensitive to disturbance-related changes (Cowden and Peterson 2013; Reinhart *et al.* 2010). The direction and magnitude of any feedback alterations will depend on which organisms are most affected, how specific or general their relationships are, and interactive effects with the changing abiotic and biotic conditions (Corkidi *et al*. 2002; McCarthy-Neumann and Ibáñez 2013).

*N. sylvatica* and *Q. alba* exhibited opposite but related feedback patterns in wind-damaged soils: *N. sylvatica*'s preference for *Q. alba* intact soils (negative feedback) switched to a somewhat positive feedback in wind damaged soils. *Q. alba*, on the other hand, had a neutral feedback in intact soils but a preference for *N. sylvatica* wind-damaged soils (negative feedback). In the field, we'd expect to see more *N. sylvatica* seedlings surviving to sapling stage in areas where *N. sylvatica* was common, compared to areas where *Q. alba* was common. In terms of a community-level response to wind disturbance, this may mean that *N. sylvatica* could be more likely to regenerate in patches after a severe disturbance, whereas *Q. alba* would survive to sapling stage in more species-diverse patches.

Overall, however, the feedback changes might not greatly influence species composition patterns. In intact forests, the pairwise plant-soil feedbacks suggested that we would observe *N. sylvatica* and *Q. alba* coexisting. In wind-damaged areas, the opposite but related feedback changes that the two species exhibited appear to effectively balance each other out, leading to a neutral paired feedback (Figure 3.5). Post-disturbance plant-soil feedbacks for these two species would promote neither coexistence nor mutual inhibition, and other ecological processes would instead drive distribution patterns. A major determinant of post-disturbance dynamics is species' ability to respond to light availability, which has also been shown to mediate some plant-soil interactions (McCarthy-Neumann and Ibáñez 2013). In order to predict how post-disturbance plant-soil feedbacks would influence seedling regeneration, future experiments will need to account for the influences of the high light environment.

Question 3—Do mycorrhizal colonization rates correlate with plant-soil feedbacks?

One component of the soil microbial community that could drive plant-soil feedback patterns is mycorrhizae. Ectomycorrhizal colonization was consistent with an overall negative feedback for *Q. alba* seedlings, since it exhibited the lowest colonization in its own intact soil. This pattern was only evident in intact soil, not wind-damaged soil, however. In wind-damaged patches, *Q. alba* soil lost its mycorrhizal 'disadvantage.' Interestingly, it is in wind-damaged *Q. alba* soil where *Q. alba* seedlings comparatively performed the worst. One possible explanation is that the ectomycorrhizal species inhabiting *Q. alba* roots are "cheaters," such that the plant does not benefit from the root-fungal association, and instead loses carbon relative to the nitrogen it gains from the fungus (Johnson *et al*. 1997). A combination of a more generalist ectomycorrhizal community (Cowden and Peterson 2013) and a sudden flush of nutrients may push the mycorrhizal symbiosis towards the parasitic end of the mutualism-parasitism spectrum (Treseder 2004). For *N. sylvatica* seedlings, however, arbuscular mycorrhizal colonization did not change with either soil origin or wind disturbance, suggesting that soil factors other than mycorrhizae are influencing its plant-soil feedback change.

Importantly, this measure of mycorrhizal colonization does not distinguish between different fungal species. With these methods, we cannot confirm that "cheater" mycorrhizae inhabit *Q. alba* roots, or that the same community of fungal associates inhabit both intact and wind-damaged soil. Furthermore, the overall amount of colonization may not matter as much as the identity of fungal associates colonizing the roots (Klironomos 2003). Even when overall colonization remains the same between intact and wind-damaged soils, seedling growth can still be affected if wind damaged soils contain different communities of fungal associates (Vogelsang *et al*. 2006). Future studies should consider not only colonization rates, but also community composition of fungal associates.

Even though ectomycorrhizal colonization for *Q. alba* seedlings showed treatment responses similar to seedling growth, we cannot pinpoint ectomycorrhizae as the cause of any feedback change. In this experiment, biotic and abiotic soil changes are interrelated. Soil nutrient availability in particular has the potential to change drastically post-disturbance. By using large relative quantities of field soil in the greenhouse pots, we cannot rule out nutrient effects. In fact, abiotic differences between intact and wind-damaged soils could help explain the observed decrease in root:shoot ratio in *Q*. *alb*a seedlings in wind-damaged soil. A release of labile nutrients could reduce *Q.alba*'s biomass allocation to root structures.

Conclusions and Future Directions

This study showed that plant-soil feedbacks in a mid-successional southern Appalachian forest are highly dependent on species identity, and interacting neighbors. Feedbacks in wind-damaged-site soils differed from those in intact-site soils, but not in a consistent manner. Although the literature suggests that plant-soil feedbacks would be weaker, or more neutral, after severe disturbance (Kardol *et al*. 2013), our study shows that disturbance can either strengthen or weaken negative plant-soil feedbacks.

The greenhouse environment and limited number of species does restrict the conclusions we can draw from this particular experiment, however. Soil manipulation (extraction, mixing, transporting) is itself a soil disturbance, meaning that even our intact-site soils have been somewhat disturbed by the time seedlings were transplanted. Greenhouse manipulations should be complemented by field-based experiments involving in-situ seedling transplants and targeted environmental manipulations. A combination of approaches including field studies will be essential in the future to examine the relationship between disturbance and plant-soil feedbacks.

Nearly all climate change models agree that North American ecosystems will experience a greater proportion of very strong tropical cyclones (typhoons and hurricanes) in the coming decades (Knutson *et al.* 2010; Knutson and Tuleya 2004; Solomon 2007). The number of tornado-prone weather events is also expected to increase for the eastern United States (Brooks 2013; Diffenbaugh *et al.* 2013), although it is still unclear whether or not that will effectively increase the frequency or strength of tornadoes (Brooks 2013). Instead of single-storm events, groups of tornadoes may be more likely (Elsner *et al.* 2014), such as the 200 tornadoes that affected Alabama and North Georgia in April 2011 (NOAA 2011). Although predictions vary about the strength and frequency of storms in general (Mitchell *et al*. 2006), the trend in these studies suggests a shift of overall disturbance regimes in the eastern US. Examining post-disturbance changes to major ecosystem processes, including plant-soil feedbacks, will help us understand how this shift in disturbance regimes will affect eastern forests. If, as this study suggests, plant-soil feedbacks do not "neutralize" after disturbances, post-disturbance soil changes may play a larger role in forest regeneration than previously assumed. Exploring how the context-dependent relationships between plants and soil respond to this dynamic environment will help further our understanding of natural regeneration processes as well as predict ecosystem responses to a changing climate.

## 3.6 Tables and Figures

Table 3.1. Analysis of three tree seedling species' change in height (a), total biomass (b), and root:shoot ratio (c) after three months greenhouse growth in soil treatments

| | *Nyssa sylvatica* | | *Quercus alba* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|
| Source | F | P | F | P | F | P |
| (a) change in height | | | | | | |
| Soil Origin | 2.39 | 0.07 | 0.56 | 0.69 | 0.20 | 0.94 |
| Wind | 4.42 | **0.04** | 0.34 | 0.56 | 0.00 | 0.96 |
| Soil Origin*Wind | 3.72 | **0.01** | 1.00 | 0.42 | 0.35 | 0.84 |
| Initial Height | 25.16 | < **0.0001** | 0.56 | 0.69 | 9.28 | <**0.01** |
| block | 1.82 | 0.16 | 2.20 | 0.11 | 0.70 | 0.56 |
| | | | | | | |
| (b) total biomass | | | | | | |
| Soil Origin | 0.63 | 0.64 | 1.09 | 0.38 | 0.13 | 0.97 |
| Wind | 0.0045 | 0.95 | 2.06 | 0.16 | 0.77 | 0.38 |
| Soil Origin*Wind | 0.74 | 0.57 | 1.14 | 0.36 | 0.52 | 0.72 |
| Initial Height | 26.97 | <**0.001** | 0.99 | 0.33 | 11.83 | <**0.01** |
| Block | 0.97 | 0.42 | 1.44 | 0.25 | 0.52 | 0.72 |
| | | | | | | |
| (c) root:shoot ratio | | | | | | |
| Soil Origin | 0.78 | 0.55 | 1.14 | 0.35 | 0.12 | 0.97 |
| Wind | 2.34 | 0.14 | 9.80 | <**0.01** | 0.72 | 0.40 |
| Soil Origin*Wind | 0.86 | 0.50 | 1.82 | 0.16 | 0.28 | 0.89 |
| Block | 5.98 | <**0.01** | 1.77 | 0.16 | 0.67 | 0.57 |

Table 3.2. Home vs Away and species-specific (Dunnett's test) contrasts performed on three tree seedling species after three months greenhouse growth in soil treatments.

| | *Intact* | | *Wind* | |
|---|---|---|---|---|
| Soil origins contrasted | F | P | F | P |
| (a) *Nyssa sylvatica* seedlings | | | | |
| Home vs Away | 3.66 | **0.06** | 0.83 | 0.37 |
| NYSY vs LIST | 0.70 | 0.41 | 1.10 | 0.30 |
| NYSY vs OXAR | 1.42 | 0.24 | 2.58 | 0.12 |
| NYSY vs PIST | 0.14 | 0.71 | 0.10 | 0.75 |
| NYSY vs QUAL | 8.98 | <**0.01** | 2.91 | 0.10 |
| (b) *Quercus alba* seedlings | | | | |
| Home vs Away | 0.07 | 0.79 | 5.28 | **0.03** |
| NYSY vs QUAL | 0.15 | 0.70 | 4.48 | **0.04** |
| QUAL vs LIST | 0.08 | 0.78 | 1.65 | 0.21 |
| QUAL vs OXAR | 1.03 | 0.32 | 3.23 | **0.08** |
| PIST vs QUAL | 0.73 | 0.40 | 1.42 | 0.24 |
| (c) *Pinus strobus* seedlings | | | | |
| Home vs Away | 0.20 | 0.66 | 0.16 | 0.69 |
| NYSY vs PIST | 0.00 | 1.00 | 0.36 | 0.55 |
| PIST vs QUAL | 0.03 | 0.87 | 0.17 | 0.68 |
| PIST vs LIST | 0.60 | 0.44 | 0.88 | 0.36 |
| PIST vs OXAR | 0.10 | 0.75 | 0.66 | 0.42 |

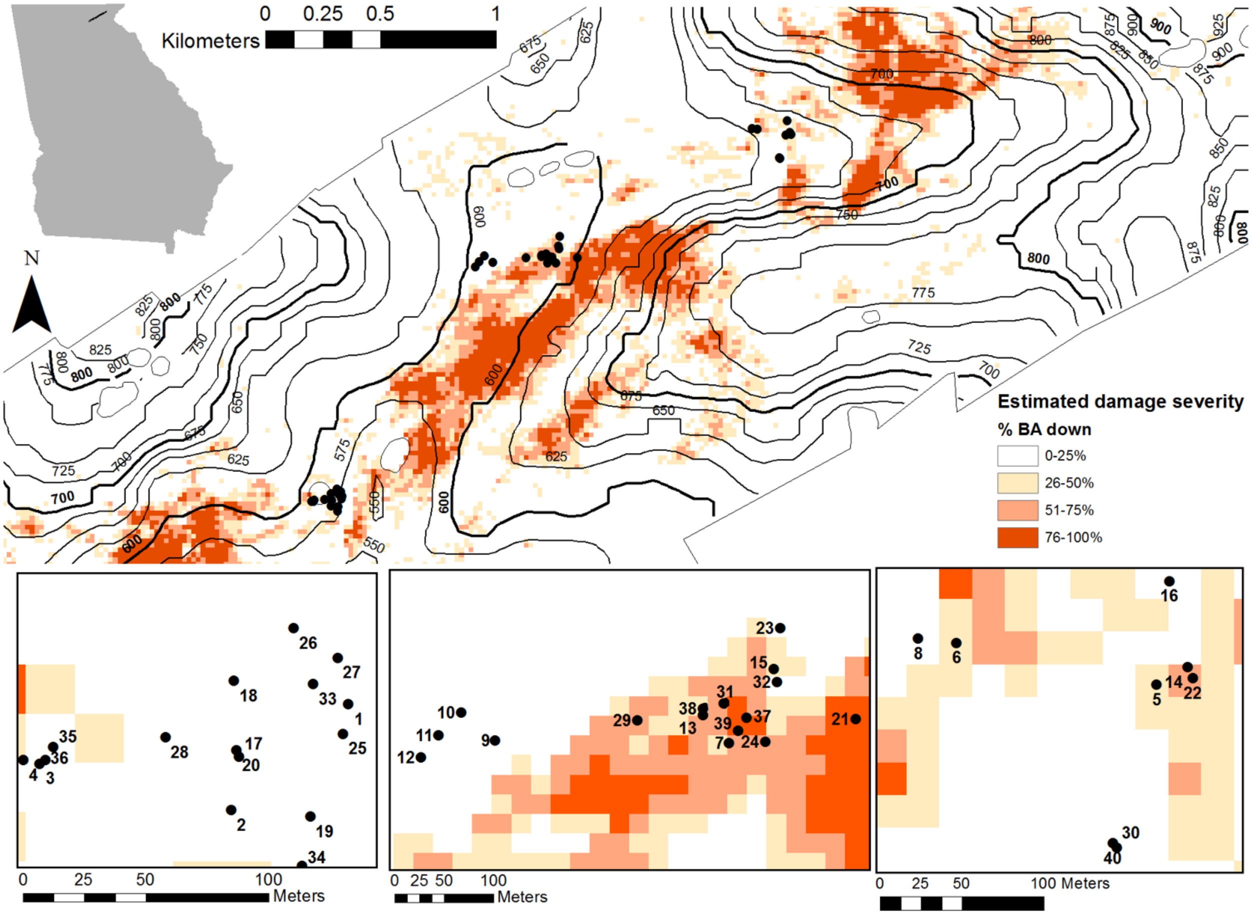
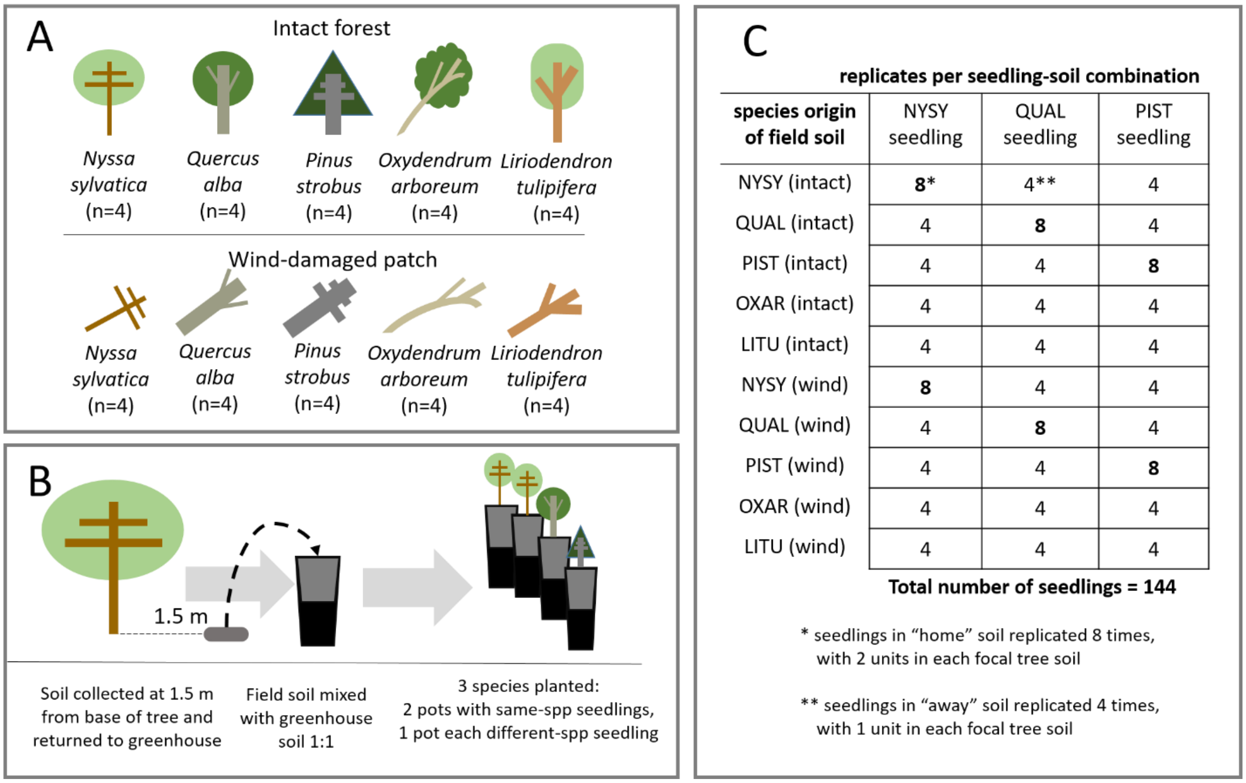



Figure 3.1. Locations of sampled trees. Soil samples were taken from beneath mature trees of five species in both intact and tornado-damaged areas of Boggs Creek Recreation Area, Chattahoochee National Forest. Tornado damage categories (% basal area down) are estimates based on aerial photography, used for visualization only (Peterson et al. 2016). For more detailed breakdown of individual tree characteristics, see Table 3.1.




| species origin of field soil | NYSY seedling | QUAL seedling | PIST seedling |
|---|---|---|---|
| NYSY (intact) | **8*** | 4** | 4 |
| QUAL (intact) | 4 | **8** | 4 |
| PIST (intact) | 4 | 4 | **8** |
| OXAR (intact) | 4 | 4 | 4 |
| LITU (intact) | 4 | 4 | 4 |
| NYSY (wind) | **8** | 4 | 4 |
| QUAL (wind) | 4 | **8** | 4 |
| PIST (wind) | 4 | 4 | **8** |
| OXAR (wind) | 4 | 4 | 4 |
| LITU (wind) | 4 | 4 | 4 |

**Total number of seedlings = 144**

* seedlings in "home" soil replicated 8 times, with 2 units in each focal tree soil

** seedlings in "away" soil replicated 4 times, with 1 unit in each focal tree soil

Figure 3.2. Experimental design. (A) Soil samples were taken from the base of mature trees of five species. Trees were located either in intact forest, or in areas substantially damaged by a recent tornado. (B) Seedlings of three species were grown in each soil type. (C) Replication was weighted towards conspecific seedling-soil combinations. Species were Nyssa sylvatica (NYSY), *Pinus strobus* (PIST), *Quercus alba* (QUAL), *Oxydendrum arboreum* (OXAR), and *Liriodendron tulipifera* (LITU).

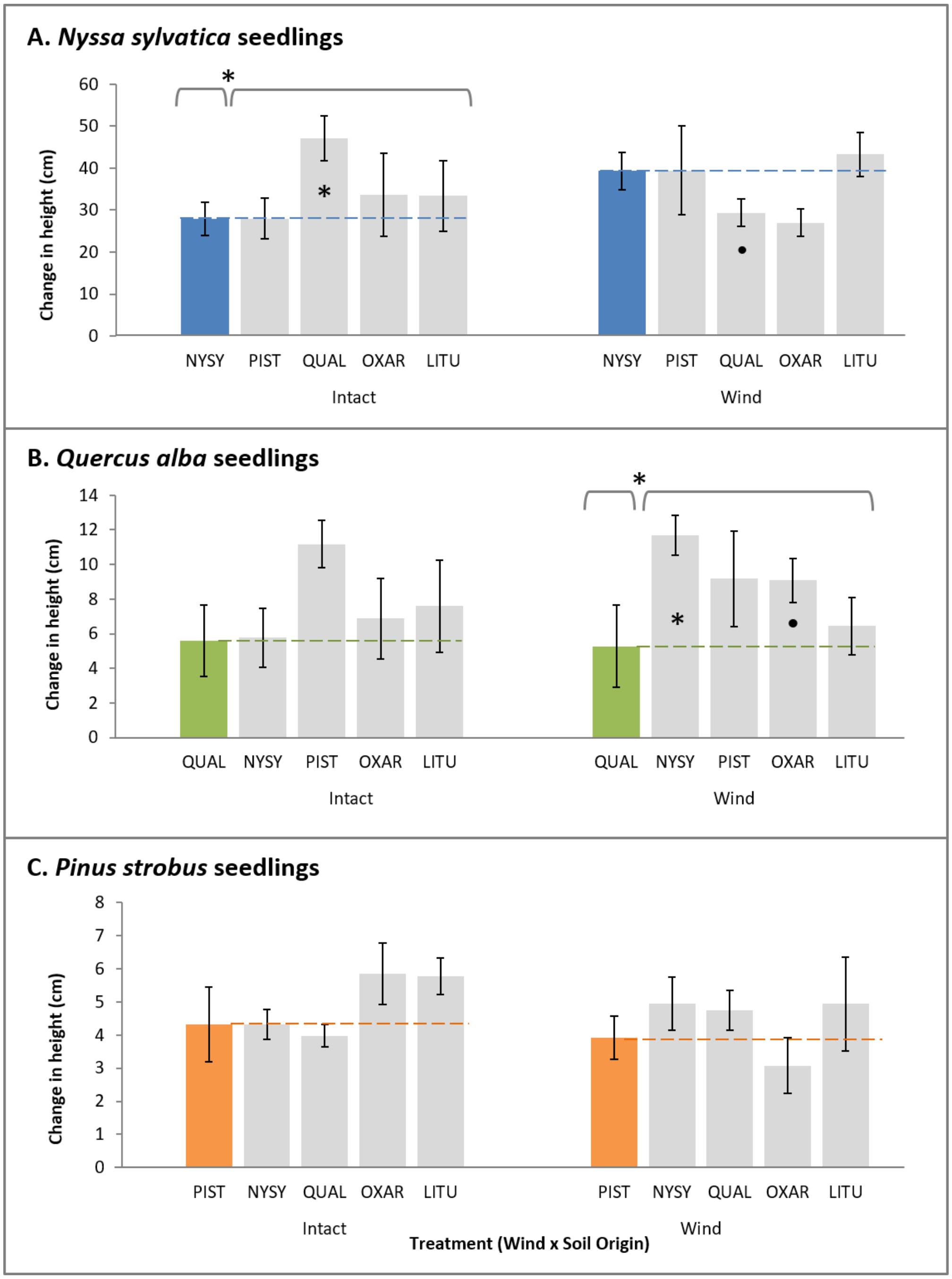



Figure 3.3. Change in height (cm) of *Nyssa sylvatica* (A), *Quercus alba* (B), and *Pinus strobus* (C) seedlings after three months in the greenhouse. Soil was collected from beneath mature trees of five spp ("Soil Origin" treatment), and from intact or wind-damaged forest plots ("Wind" treatment). Brackets represent home vs. away contrasts, with the seedling growth in "home" soil represented by the colored (dark) bar and dotted line. Symbols within bars represent contrasts between "home" soil and specific "away" soils. Species were Nyssa sylvatica (NYSY), *Pinus strobus* (PIST), *Quercus alba* (QUAL), *Oxydendrum arboreum* (OXAR), and *Liriodendron tulipifera* (LITU). (* = p <0.05 ; dots = p <0.1 )

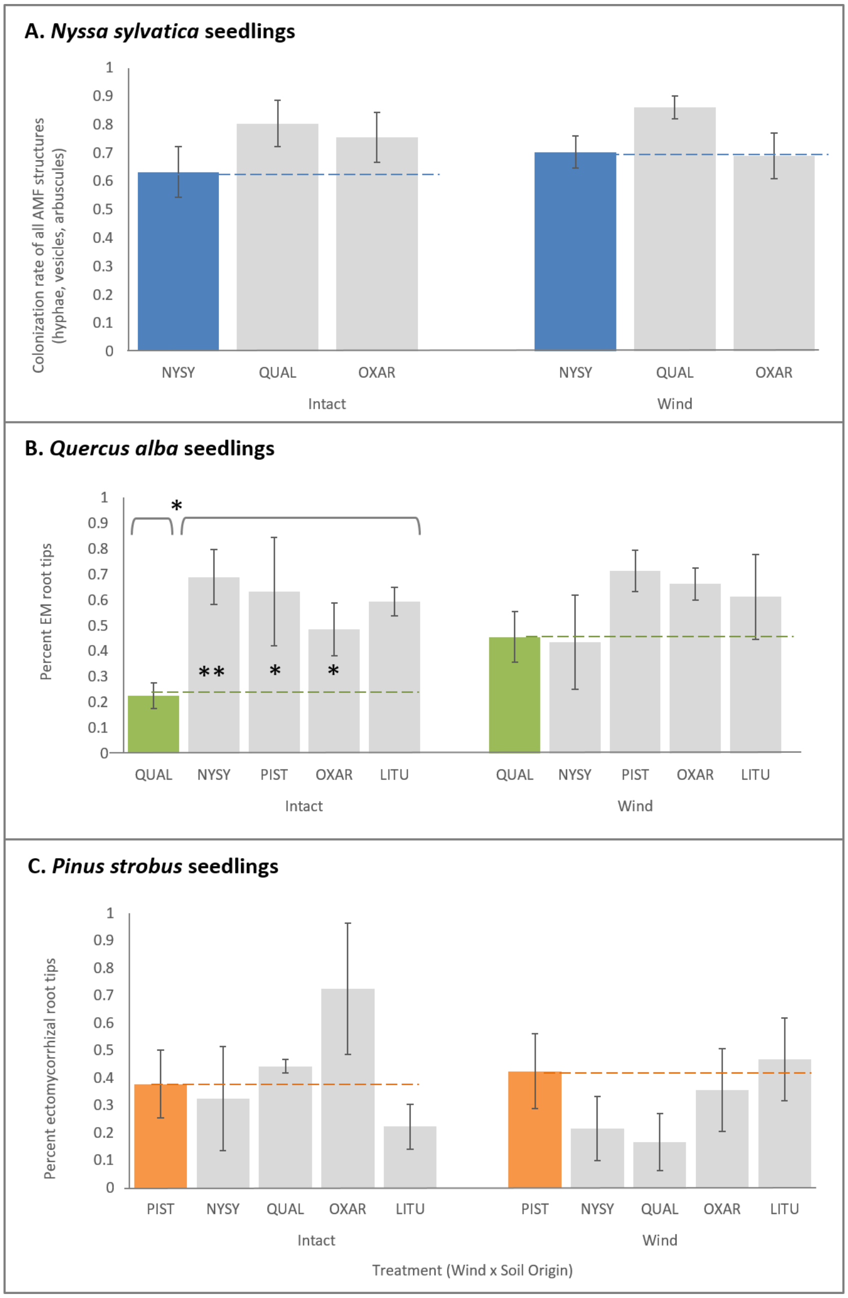



Figure 3.4 Arbuscular mycorrhizal colonization of (A) *Nyssa sylvatica* and ectomycorrhizal colonization of (B) *Quercus alba* , and (C) *Pinus strobus* seedling roots after three months growth in the greenhouse. Soil was collected from beneath mature trees of five spp ("Soil Origin" treatment), and from intact or wind-damaged forest plots ("Wind" treatment). Brackets represent home vs. away contrasts, with the seedling growth in "home" soil represented by the colored (dark) bar. Symbols within bars represent contrasts between "home" soil and specific "away" soils. Species were Nyssa sylvatica (NYSY), *Pinus strobus* (PIST), *Quercus alba* (QUAL), *Oxydendrum arboreum* (OXAR), and *Liriodendron tulipifera* (LITU). Roots from *Nyssa sylvatica* seedlings in LITU and PIST soils were not retained. (** = p< 0.01,* = p <0.05 ;)

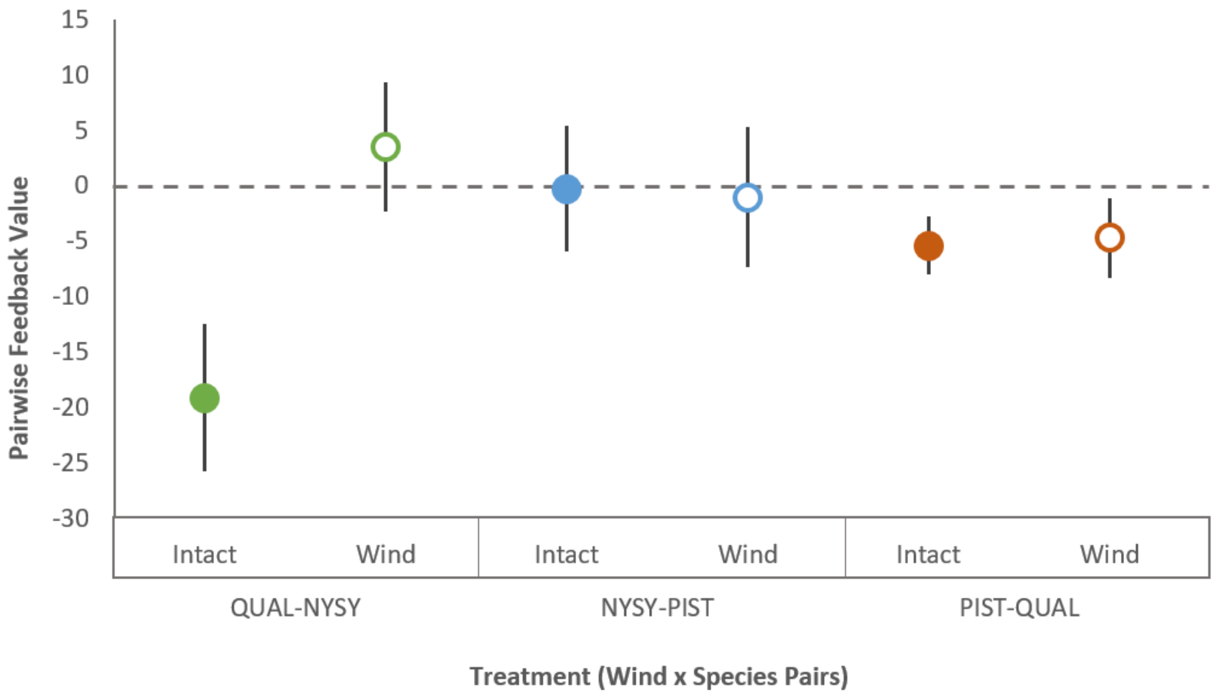



Figure 3.5. Pairwise feedback indices for three reciprocal pairs of tree seedlings: *Nyssa sylvatica* (NYSY), *Quercus alba* (QUAL), and *Pinus strobus* (PIST). Pairwise values were calculated from mean treatment response (change in height), using the formula $I = (A_a - A_b) - (B_b - B_a)$, in which *A* and *B* represent seedling change in height and subscripts *a* and *b* indicate the soil species type in which the seedling was grown. Filled circles show feedback values for seedlings grown in intact forest soil, and open circles show feedback values for seedlings grown in wind-damaged soil. Error bars represent 95% confidence intervals. Confidence intervals were constructed by sampling with replacement for 1000 iterations to determine if mean feedback values were different from zero (shown as dotted line).

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# CHAPTER 4

# FIELD TRANSPLANTS SHOW NO DISTURBANCE EFFECT ON SOUTHERN APPALACHIAN PLANT-SOIL FEEDBACKS[3]

[3] Nagendra, U.J., Alexander, L, Ginn, L., Statler, A, and Peterson, C.J. To be submitted to *Oecologia*

## 4.1 Abstract

Despite the ubiquity of natural disturbances, plant-soil feedback responses to natural disturbances are understudied. After a disturbance, both the soil biotic communities and the plants themselves may perform differently under the new abiotic conditions experienced in a post-disturbance forest. In this experiment, I look for field-based evidence of species-specific alterations to PSF in a tornado damaged area of the southern Appalachian Mountains. This field experiment investigates three components of post-disturbance plant-soil feedbacks: 1) feedbacks due to direct abiotic changes in soil, water, and light, 2) feedbacks due to alterations to the soil biotic community alone and 3) feedbacks due to interactions between soil biotic communities and direct abiotic changes. First, seedling roots were grown in soil inoculum gathered from beneath common mature trees in order to condition seedling roots to one particular soil origin type. Second, inoculated seedlings were then transplanted into field plots.

In both the greenhouse and the field, a history of wind damage had little to no effect on the function of soil inoculum. In addition, in this two-part experiment, plant-soil feedbacks calculated from seedlings grown in the greenhouse did not match those calculated from field-transplanted seedlings. Fast-growing species *Pinus strobus* and *Acer rubrum* were inhibited by conspecific soil microbial communities in the greenhouse, but not in the field. *Nyssa sylvatica*'s soil microbial community improved same-species seedling performance in the greenhouse but inhibited it in the field. Only one species (*Nyssa sylvatica*) exhibited negative plant-soil feedbacks in the field. When feedbacks were present in the field, they were secondary determinants of seedling performance, as seedling survival in the field was more closely related to larger-scale abiotic environmental characteristics.

## 4.2 INTRODUCTION

As long-lived sessile organisms, trees form complex relationships with the soil they grow in. Trees influence local soil characteristics through passively or actively attracting taxon-associated biotic communities, through the quality and content of their leaf and root litter contributions, and through exudations of chemical compounds. When taxon-specific soil alterations in turn influence survival and/or growth rates of the same taxon, the relationship is called a plant-soil feedback (Bever *et al*. 1997; Ehrenfeld *et al*. 2005; Putten *et al*. 2013). Reciprocal interactions between plants and soil characteristics, or plant-soil feedbacks (PSF) can have profound impacts on plant community diversity and composition (Connell 1978a; Ehrenfeld et al. 2005; Klironomos 2002; Mangan et al. 2010).

Positive (self-promoting) PSF, in which a plant species harbors a soil environment that benefits its own offspring compared to other species, favors clumped spatial distributions, dense growth, single-species stands, and monodominance (Callaway *et al*. 2008; Corrales *et al.* 2016). Negative (stabilizing) PSF, in which a plant species harbors a soil environment that hinders its own offspring compared to others, favors over-dispersed spatial distributions, sparse growth, multi-species stands, and the maintenance of diversity (Mills and Bever 1998). The most abundant plant species within a community tend to exhibit stronger positive feedbacks, whereas those species with strong negative feedbacks tend to be rare (Klironomos 2002; Mangan *et al*. 2010). Consequently, highly diverse plant communities in both tropical forests (Connell 1978a; Terborgh 2012) and temperate forests (Packer and Clay 2000; Reinhart *et al*. 2012b) often contain a greater percentage of species that exhibit negative plant-soil feedbacks.

As ecosystems change and develop, plant-soil feedbacks change as well (Kardol *et al.* 2013), sometimes contributing directly to ecosystem development and plant community succession (Kardol *et al*. 2006; Van der Putten *et al*. 1993). Within ecosystem types, plant-soil feedback strength and direction are known to change in response to environmental variation such as soil fertility (Manning *et al*. 2008), water status (Kennedy and Peay 2007), and light availability (Kummel and Lostroh 2011; McCarthy-Neumann and Ibáñez 2013; Record et al. 2016). These same environmental characteristics vary throughout ecosystem development, most notably immediately following natural disturbances (Canham *et al*. 1990; Canham and Marks 1985; Vitousek 1985; Vitousek and Melillo 1979). These periodic disturbances are major driving forces that shape ecosystem development throughout the world (White and Jentsch 2001; Xi and Peet 2011) and cause abrupt changes to numerous ecosystem characteristics that influence plant-soil feedbacks. But despite the ubiquity of natural disturbances, plant-soil feedback responses to natural disturbances are understudied (Kardol *et al.* 2013; Putten *et al*. 2013; Reynolds *et al.* 2003). Understanding the role of plant-soil feedbacks in shaping plant communities immediately following natural disturbances will contribute to our knowledge of ecosystem development as disturbance regimes continue to shift along with global climate (Brooks 2013; Elsner *et al*. 2014; Solomon 2007).

Current knowledge of plant-soil interactions following disturbances is limited to fire (Peay *et al.* 2009; Rincón and Pueyo 2010) and anthropogenic disturbances, usually in agricultural systems (Kulmatiski and Kardol 2008; Peay *et al.* 2009; Rincón and Pueyo 2010). Few studies focus on biotic soil changes after wind disturbances (Cowden and Peterson 2013; Egli *et al*. 2002; Reinhart *et al.* 2010). With increasing knowledge about direct changes to soil characteristics and biotic communities following disturbances, we are now more able to assess the interactions between plants and soil following disturbances.

A previous greenhouse experiment using soils collected from the southern Appalachian Mountains (Nagendra and Peterson 2016) demonstrated that plant-soil relationships were altered in soils from wind-damaged areas, compared to soils from intact areas. The experiment found generally negative PSF in intact area soils and one switch to positive PSF in wind-damaged area soils. That experiment alone, however, cannot confidently assess what alterations to PSF tree seedlings experience in a post-disturbance environment. The post-disturbance environment involves numerous other changes to important plant resources that a greenhouse experiment cannot capture.

After wind disturbance, the sudden changes to light availability, nutrient cycling, and soil moisture greatly influence seedling growth and survival (Canham and Marks 1985). Additional environmental variability created by site heterogeneity, woody debris, and even topographic variability could further complicate plant-soil interactions. All in all, the plant-soil feedbacks seen in the greenhouse study may only be a small component of realistic plant-soil interactions in a post-disturbance forest.

Disturbances affect plant-soil interactions via a combination of several interacting components. First, severe wind disturbances directly change soil abiotic characteristics. Namely, the removal of canopy cover increases solar radiation, wind flow, and soil evaporation rates (Ritter *et al*. 2005) while also releasing stored nutrients and carbon from organic material into more labile forms (Vitousek and Melillo 1979). This leads to hotter and drier soil with bursts of ephemeral nitrogen pools. If these abiotic changes vary by species, the disturbance would then directly alter *abiotic* plant-soil interactions.

Secondly, post-disturbance abiotic and biotic changes could alter plant-soil feedbacks by mediating shifts in the soil biotic community. Hotter and drier soil, reduced nutrient availability, stress to the host plant, fewer active host root tips, and a shifting plant community would all contribute to altered microbial diversity and composition. Compared to intact temperate forest areas, wind-damaged areas may contain a compositionally different, less diverse ectomycorrhizal fungal community (Cowden and Peterson 2013), with greater abundance of pathogenic fungi (Reinhart *et al.* 2010). This less diverse, more generalist soil microbial community (Cowden and Peterson 2013; Egli et al. 2002; Jones *et al*. 2003; Reinhart *et al.* 2010) could dampen the strength of plant-soil feedbacks, leading to a "blank slate" of PSF immediately following a strong disturbance (Kardol *et al.* 2013; Reynolds *et al.* 2003). Our previously mentioned greenhouse study (Nagendra and Peterson 2016) combined these first two pathways (abiotic and biotic) by focusing on post-disturbance changes to whole soil.

The third pathway is less well studied. After a disturbance, both the soil biotic communities and the plants themselves may perform differently under the new abiotic conditions experienced in a post-disturbance forest. An altered nutrient regime may increase the likelihood that plants will either avoid associating with root mutualists altogether (Treseder 2004) or suffer from a "parasitic" carbon-nutrient trade in which fungi benefit more than plants (Corkidi et al. 2002; Johnson *et al.* 1997; Johnson *et al.* 2010). The same plant-fungal pairs function differently in conditions with altered nutrients (Corkidi *et al.* 2002; Manning *et al.* 2008), water (Kennedy and Peay 2007), and even light (Kummel and Lostroh 2011; McCarthy-Neumann and Ibáñez 2013). All of these conditions would be relevant to a post-disturbance environment. In order to best examine the effects of disturbances on plant-soil feedbacks, therefore, we must consider the indirect changes to plant-microbial behavior in the field as well as direct changes to soil biotic composition and soil abiotic conditions.

Greenhouse experiments provide necessary controlled assessment of potential plant-soil feedbacks (Kulmatiski *et al*. 2008; Putten *et al*. 2013), but recent reviews of plant-soil literature have called for an increase in field studies (Kulmatiski *et al*. 2008; Putten *et al*. 2013). Although field studies are time-consuming and require a complex experimental design (Kulmatiski and Kardol 2008), they can both validate effects seen in the greenhouse and examine how existing environmental variation influences these effects.

In this experiment, I look for field-based evidence of species-specific alterations to PSF in a tornado damaged area of the southern Appalachian Mountains. In contrast to a greenhouse experiment, this field experiment investigates three components of post-disturbance PSF: 1) feedbacks due to direct abiotic changes in soil, water, and light, 2) feedbacks due to alterations to the soil biotic community alone and 3) feedbacks due to interactions between soil biotic communities and direct abiotic changes.  Specifically, I ask whether field-based plant-soil feedbacks after a wind disturbance are (Q1) the same in both intact and wind-damaged forest areas and (Q2) mediated by biotic, abiotic, or interactive soil changes.  This experiment also provides insight to (Q3) whether alterations to PSF after a tornado observed in a greenhouse are both retained in a field setting and strong enough to be visible in the context of other environmental changes.

Due to the many changes in overall environment, plant community, and microbial community after a wind disturbance, I expect (H1) that plant-soil feedbacks in wind-damaged areas will be different from those in intact forest areas. Since plant-soil partnerships are context-dependent, I also expect (H2) that any alteration to plant-soil feedbacks in wind-damaged areas will mainly be due to interactions between the soil biotic community and the abiotic environment. However, based on the importance of many post-disturbance abiotic changes, namely light availability, to plant community development, I expect that (H3) biotic PSF will be context-dependent, and therefore be weaker in the more variable field settings compared to the controlled greenhouse.

To achieve these goals, this experiment must separate soil factors that are often confounded in field experiments, such as soil biotic and abiotic conditions. Common methods such as ground-inserted pots/containers are susceptible to many of the same pot-effects seen in greenhouse studies. In addition, they are not feasible for studies in remote and difficult terrain. In this study, I instead attempt to separate variables using a two-stage experiment that takes advantage of the priority effect of fungal colonization (Kennedy and Bruns 2005; Kennedy *et al.* 2009). The priority effect is a phenomenon in which the first fungal community to establish on a plant's roots has a colonization advantage over fungi that arrive later. Fungal partners that are introduced early on are more likely to develop and drive the stable root community. In a greenhouse inoculation phase, seedlings are introduced to soil biotic communities via a small amount of soil inoculum. After a few months, the inoculated seedlings are assumed to be "conditioned" with the targeted root biotic community. The pre-conditioned seedlings are then planted in the field for the second, field transplant phase of the experiment.

## 4.2 METHODS

### Overview

This study examines the comparative effects of soil biotic conditioning and abiotic environmental conditions on seedling growth and survival in tornado-damaged forest areas. In order to specifically separate the effects of soil biotic conditioning from other environmental variables, I used a two-part field transplant experiment (Figure 4.1). First, seedling roots were grown in soil inoculum gathered from beneath common mature trees (Figure 4.1B). This first period of greenhouse growth served to condition seedling roots to one particular soil origin type. Second, inoculated seedlings were then transplanted into field plots (Figure 4.1C). This second period of field growth introduced real environmental variation, mainly the suite of interacting environmental factors that differ between intact and wind-damaged forests. By inoculating and conditioning seedlings in a separate process, I am able to separate soil inoculum type from other environmental factors in our analysis.

Site Description

This study took place in three sites in the Chattahoochee National Forest in northeast Georgia, USA: Boggs Creek Recreation area (BC), Martin Branch Road (MB), and Timpson Creek Road (TC, Figure 4.2). Each of these sites experienced severe tornado damage from an EF-3 tornado with maximum winds of 120 mph on April 27, 2011 (NOAA 2011). Baseline species composition and damage profile for all sites were surveyed in the months immediately following the tornado, as part of a separate study on post-tornado recovery (Peterson, unpublished data). The three sites (BC, MB, and TC, Figure 4.2) all contain relatively similar tree species composition and experienced a range of damage from mild (0-25% existing basal area downed) to severe (75-100% existing tree basal area downed).

Boggs Creek Recreation Area is a publicly accessible area for fishing, hiking, and camping located within the Chattahoochee National Forest in northeast Georgia (34.6793°, -83.8956°, Lumpkin County GA). Martin Branch Road (34.7495°, -83.7495°, White County GA) and Timpson Creek Road (34.8687°, -83.4761°, Rabun County GA) refer to sections of the Chattahoochee National Forest accessible by forest service road. All three sites are southern Appalachian forests generally dominated by oaks (*Quercus alba*, *Q. rubra*, *Q. prinus*, *Q.*

*coccinea*) and pines (*Pinus strobus* and *P. virginiana*). Red maple (*Acer rubrum*), tulip poplar (*Liriodendron tulipifera*), black gum (*Nyssa sylvatica*), and sourwood (*Oxydendrum arboreum*) are also common. Minor differences in species composition separate the sites: Boggs Creek is the most oak-dominated site, with higher percentages of *Q. alba*, *Q. prinus*, and *Q. rubra*. Timpson Creek contained a slightly different composition of oaks than the other two sites; Timpson Creek contained more *Q. coccinea* and *Q. velutina* than *Q. prinus* and *Q. rubra*. Further site characteristics can be found in Table 4.1.

Boggs Creek was overall the steepest site; plot elevations ranged from 576 to 626 meters, with 10 to 15 degrees slopes leading down to the creek. Martin Branch was the lowest elevation site, with plot elevations ranging from 554 to 587 meters above sea level and slope from 2 to 12 degrees. Timpson Creek was the highest elevation site, with plot elevations ranging from 652 to 736 meters and slope from 7 to 16 degrees.

Both Boggs Creek and Timpson Creek plots were dominated by Ultisol soils, with some Inceptisols in lower elevation areas. Martin Branch plots mainly consisted of Inceptisols. Within each site, soils ranged with topography: Boggs Creek plots contained Wickham fine sandy loam along the creek banks, Tallapoosa soils and Ashe and Edneyille stony loams along slopes, and Tallapoosa cobbly fine sandy loam along ridges (NRCS). Martin Branch sites contained Tusquitee loams and stony loams on ridgetops, and a mix of Edneyville, Ahse, and and Porter stony loams on slopes. Timpson Creek soils were mostly Taluda and Ashe stony soils.

All three sites had similar climatic normal. Average low in winter (BC: -2.94 °C, MB: -2.05 °C, TC: -2.44 °C), high in summer (BC: 29.27 °C, MB: 29.50 °C, TC: 28.94 °C), and yearly precipitation (BC: were all comparable. For Boggs Creek, average temperature ranges from -2.94 °C in winter to 29.27 °C in summer, and average yearly precipitation is 157.7 cm (NCDC 2011).

Soil Collection

In April 2014, soil samples were gathered from beneath mature trees of 4 common canopy tree species (*Acer rubrum*, *Nyssa sylvatica*, *Pinus strobus*, and *Quercus prinus*). Species were chosen based on seed availability and germination success. Two focal trees > 10 cm DBH of each species were chosen for each site, one in an intact forest area, and one in an area of severe tornado damage (initial basal area downed > 75%). Those in tornado-damaged areas were also significantly impacted by the storm (either uprooted or top snapped). Soil was gathered from the top 5-10 cm, in three equidistant points 1.5 m from the base of the tree, then aggregated into one soil sample. Soil samples for each individual focal tree were kept separate. Further basic characteristics of the focal trees can be found in Table 4.2.

Part 1: Greenhouse

In March 2014, seedlings of the four focal species (*A. rubrum*, *N. sylvatica*, *P. strobus*, *Q. prinus*) were germinated in flats of steam-pasteurized greenhouse soil (Fafard soil mix). When first true leaves emerged, seedlings were then transplanted into conetainers of steam-pasteurized greenhouse soil inoculated with 30 mL of live field soil. Each seedling spp x soil inoculum type combination was replicated 8 times, for a total of 768 seedlings. Utensils and containers were sterilized with ethanol between handling soil types. Seedlings were grown in these conetainers in a shadehouse for 6 months. Final heights were measured in November 2014.

Part 2: Field Transplants

From November 2014 to February 2015, seedling conetainers were transplanted in the field. Within each site, 8 circular 1.25 m radius common garden plots were established, one at each of the focal trees. Each of the 24 focal tree plots received 32 conetainer seedlings, composed of the 4 species of seedlings each inoculated with 8 different soil inoculum types. Soil inoculum types were reciprocally planted only within each site (i.e. soil inoculum from trees in Boggs Creek was only transplanted in Boggs Creek plots). All focal tree plots were then surrounded by deer-proof fencing to prevent herbivory.

From February to November 2015, seedling height and diameter were measured four times: at planting, in spring, in summer, and at collection. Abiotic environmental characteristics of each plot were also taken during this time period. At the four cardinal directions surrounding the tree, I measured soil temperature (˚C, analog thermometer), soil volumetric water content (FieldScout time domain reflectometer), and canopy openness (densiometer) three times. Soil samples were taken from the cardinal directions to be analyzed for total carbon and nitrogen content at the UGA Stable Isotope Laboratory. In June 2015, I assessed nitrogen mineralization using ion strips according to published procedures (Qian and Schienau 1996). Anion and cation strips were placed in the four cardinal directions at each plot and recovered after two weeks. Nitrate and ammonium were extracted from the strips using potassium chloride, then sent to the UGA Stable Isotope Laboratory for analysis. Hemispherical canopy photographs were taken at the cardinal directions of each plot in September and October 2015. Canopy openness was calculated from the photos using the program CIMES-FISHEYE (Gonsamo *et al.* 2011).

Analysis

I constructed four separate statistical models that could explain our growth and survival data. These were designed to determine whether seedling growth and survival were explained by competing sets of factors: mainly, soil inoculum types or field location types. Soil inoculum type included both inoculum source damage and inoculum source species. Inoculum source species was described as same-species or different-species soil inoculum, with all three different-species types analyzed as one category (i.e. for an *Acer rubrum* seedling, seedling growth in inoculum from *Nyssa sylvatica*, *Pinus strobus*, and *Quercus prinus* trees would all be included in "different-species" inoculum growth). Field location types include categorical data, such as the whole-plot wind damage status and tree species of the focal plot (also described as same-species or different-species), as well as continuous data on environmental characteristics gathered from cardinal directions within the plot (canopy openness, soil moisture and temperature, soil C:N ratio, and N mineralization rate). Because inoculum type and field location type are necessarily nested in the field portion of the experiment, I analyzed these factors separately and then compared models using Akiake's Information Criterion (AIC). All analyses were done using the statistical program R (R Core Team 2015). Outliers were removed using an automated process in the 'outliers' package. Two C:N data points were removed from Timpson Creek Intact *P. strobus* and replaced with the plot average. One plot (Martin Branch Wind-damaged *N. sylvatica*) experienced substantial seedling mortality and was removed from seedling survival analysis.

First, I addressed the effect of soil inoculum alone (source species and source damage) on seedling growth (Question 1). Models 1 and 2 test this first hypothesis separately in greenhouse and field settings. The data for Model 1 came solely from the first, greenhouse stage of the experiment.

In Model 1, [seedling growth in greenhouse] *is a function of* [soil inoculum source species] x [soil inoculum source damage] x [seedling species]

Model 2 mirrors Model 1, but in a field setting.

In Model 2, [seedling performance (growth or survival) in field] *is a function of* [soil inoculum source species] x [soil inoculum source damage] x [seedling species]

For both of these models, a significant [soil inoculum source damage] x [soil inoculum source species] interaction would support the first hypothesis that biotic PSF will differ in soils from intact- and wind-damaged forest areas. Comparing Models 1 and 2 addresses the context-dependency of biotic PSF (Question 3). If soil inoculum affects seedlings differently in greenhouse and field settings, it would support the third hypothesis.

Together, Models 2, 3 and 4 and focus on teasing out effects of soil inoculum type and field location type during the field-transplant stage of the experiment (Question 2). The third and fourth models address whether seedling performance in the field can be explained by field location and abiotic characteristics. Model 3 contrasts with Model 2 by focusing on plot-level species and wind damage instead of soil inoculum source species and soil inoculum source damage. Model 4 is slightly different from the rest, as it uses plot-level environmental characteristics (continuous data) gathered in the field.

In Model 3, [seedling performance (growth or survival) in field] *is a function of* [focal tree plot species] x [plot wind damage] x [seedling species]

In Model 4, [seedling performance (growth or survival) in field] *is a function of* [canopy openness] + [C:N ratio] + [N-mineralization] + [soil moisture] + [soil temperature]

In order to test Hypothesis 2, models with the same dependent variable (growth or survival) were  compared via Akiake's Information Criterion (AIC) in R. Models with lower AIC values were determined to be the best fit. If AIC determines Model 2 to be the best fit, this would support Hypothesis 2.

## 4.4 RESULTS

Model 1: Soil inoculum influence in a greenhouse setting

Overall, seedling growth in the greenhouse was influenced by soil inoculum source species (the species of the focal tree from which soil inoculum was gathered) but not by soil inoculum source damage (whether that focal tree was located in a tornado-damaged area or an intact forest area). In particular, *Nyssa sylvatica* seedlings growing in same-species soil inoculum (13.68 +/- 0.55 cm) were taller than those grown in soil inoculum from another species (8.68 +/- 0.49 cm; $F(1,208)= 30.8902$; $p<0.001$; Figure 4.3). In contrast, *Pinus strobus* and *Acer rubrum* seedlings grown in same-species soil inoculum (*Acer*: 6.86 +/- 0.19 cm; *Pinus*: 5.94 +/- 0.17 cm) were shorter than those grown in inoculum from another species (*Acer*: 7.58 +/- 0.20 cm, $F(1,304)=6.62$, $p<0.05$; *Pinus*: 6.78 +/- 0.10 cm, $F(1,229)=18.34$, $p<0.001$; Figure 4.3; Table 4.03). *Quercus alba* seedling heights did not differ with soil inoculum origin.

Model 2: Soil inoculum influence in a field setting

After seedlings were transplanted into field plots, seedling growth rates continued to be influenced by soil inoculum source species, but not inoculum source damage. This effect, however, was only true for *Nyssa sylvatica* seedlings, whose field-based growth rates were greater for those grown in soil inoculum from different-species origins (0.0089 +/- 0.00012 cm/day) than in soil inoculum from the same-species (0.00025 +/-0.0003 cm/day; $F(1,139)=6.28$, $p<0.05$; Figure 4.5). In addition, *Nyssa sylvatica* seedlings grew faster in soil inoculum from wind-damaged areas (0.00096 +/- 0.00012 cm/day) than in soil inoculum from intact forest areas (0.00042 +/- 0.00022 cm/day; $F(1,139) = 6.28$, $p <0.05$; Figure 4.5; Table 4.04). Other seedling species' field-based growth rates were not affected by soil inoculum origin.

Soil inoculum type did not influence seedling survival rates in the field, however. Neither soil inoculum source species nor source damage affected the survival rate of seedlings of any species (Figure 4.4; Table 4.05).

Model 3: Focal tree influence in a field setting

Seedlings were less likely to survive when planted in a wind-damaged plot compared to an intact-forest plot (LR $\chi 2 = 5.31$, $p < 0.05$). This effect was likely driven by *Nyssa sylvatica* seedlings (LR $\chi 2 = 18.42$, $p < 0.001$; Figure 4.6; Table 4.06).

Overall, seedling growth rate was not influenced by focal tree identity or wind-damage. Only *Pinus strobus* seedlings had a lower growth rate when planted in intact forest plots (0.001 +/- 8.44e-05 cm/day) than those planted in wind-damaged plots (0.0013 +/- 0.0001 cm/day; $F_{(1,155)} = 11.72$, $p < 0.001$; Figure 4.7; Table 4.07).

Model 4: Environmental factors influence in a field setting

Overall, seedling survival was significantly negatively correlated with Carbon:Nitrogen ratio (coefficient = -0.04 +/- 0.02, LR $\chi 2 = 8.16$, $p < 0.01$; Table 4.08) and positively correlated with soil moisture (coefficient = 0.12 +/- 0.06, LR $\chi 2 = 3.90$, $p < 0.05$). Mineral nitrogen content, soil moisture, and canopy openness were all negatively correlated with seedling survival, but not significant.

Specifically, both *Nyssa sylvatica* and *Acer rubrum* seedlings were significantly less likely to survive in areas with high C:N ratios (*Acer*: coefficient = -0.10 +/- 0.03, LR $\chi 2 = 10.16$, $p < 0.01$; *Nyssa*: coefficient = -0.07 +/- 0.03, LR $\chi 2 = 4.75$, $p < 0.05$; Table 4.08). *Nyssa sylvatica* seedlings also had lower survival in plots with high soil temperatures (coefficient = -0.14 +/- 0.06, LR $\chi 2 = 4.92$, $p < 0.05$) and more open canopies (coefficient = -6.98 +/- 2.34, LR $\chi 2 = 9.48$, $p < 0.01$). *Pinus strobus* seedling survival increased with greater soil volumetric water content (coefficient = 0.43 +/- 0.13, LR $\chi 2$ = 10.85, p<0.01). *Quercus prinus* seedling survival, however, was not significantly correlated with any of the measured environmental variables.

With all species taken together, seedling growth rate in the field was not significantly correlated to any of the measured environmental variables. When analyzed separately, only *Acer rubrum* and *Pinus strobus* seedling growth rates were correlated with any of the variables. Specifically, *Acer* seedlings grew faster in plots with greater mineral nitrogen and higher soil temperatures (Nitrogen: $1.29e^{-03}$ +/- $6.39e^{-04}$, F(1,143)= 4.044, p <0.05; Temperature: $9.03e^{-05}$ +/- $2.90e^{-05}$, F(1,143) = 9.68, p< 0.01; Table 4.09). Pinus strobus seedlings grew faster in areas with greater canopy openness ($1.88e^{-03}$ +/- $8.38e^{-04}$, F(1,153)= 5.02, p <0.05).

Hypothesis comparison (AIC)

For field-based seedling growth rate, the soil inoculum origin model had the lowest AIC value and was determined to be the best fit (Model 2, -6369.266, Table 4.10), followed by the Plot Identity model (Model 3, -6358.278). The environmental variable model had the highest AIC value and determined to be the worst fit (Model 4, -5918.822).

A different pattern was seen for seedling survival in the field, however. The environmental variable model had the lowest AIC value and was determined to be the best fit (Model 4, 874.26), followed by the soil inoculum origin model (Model 2, 1117.107). The plot identity model had the highest AIC value and was determined to be the worst fit (Model 3, 1084.96, Table 4.10).

## 4.5 DISCUSSION

### Overview

Four years after a tornado, plant soil interactions for common southern Appalachian seedlings appear to be the same in both intact- and tornado-damaged forest areas. While some plant-soil feedbacks were apparent in the field, they appear to be secondary determinants of seedling performance. Seedling survival in the field was more affected by abiotic environmental characteristics regardless of soil inoculum origin.

In addition, PSF calculated from seedlings grown in the greenhouse did not match PSF calculated from field-transplanted seedlings. This experiment was designed to understand the importance of environmental context on the nature of PSF. In this experiment, inoculum affected seedlings differently in the greenhouse than in the field, indicating that the plant-soil interactions for these species are highly context-dependent.

### Effects of soil inoculum in greenhouse and field

Significant 'home vs. away' PSF indicate that mature trees of these species harbor soil microbial communities that differentially affect their conspecific seedlings. Greenhouse and field stages, however, produced different PSF for each given species, indicating that these microbial relationships are context-dependent. For fast-growing species *Pinus strobus* and *Acer rubrum*, conspecific soil microbial communities inhibited seedlings in the greenhouse but had no effect in the field. *Nyssa sylvatica* was the only species to exhibit significant PSF in the field phase of the experiment. *Nyssa*'s soil microbial community improved same-species seedling performance in the greenhouse but inhibited it in the field. While this study did not aim to address specific mechanisms, the PSF patterns seen here could potentially be explained by a combination of mycorrhizal type and pathogen susceptibility.

This experiment contained two EM-associated and two AM-associated tree seedlings. Within those categories, there was a faster-growing and a slower-growing species. Fast-growing pioneer species such as *Acer* and *Pinus* may allocate resources to growth over pathogen protection, making them vulnerable to same-species soil microbial communities. Ectomycorrhizal-associated tree genera such as *Pinus* and *Quercus* are vulnerable to a wide array of pathogens as seedlings through "damping off syndrome." The significant negative feedbacks from greenhouse *Pinus* and *Acer* seedlings were not apparent in the field transplant phase, however. *Pinus* seedlings in particular may have gained some pathogen protection from EM neighbors in the EM-dominated field sites. A recent study comparing PSF between AM and EM trees in temperate forests demonstrated that EM species benefit more from conspecific neighbors, potentially due to protection from pathogens (Bennett *et al*. 2017).

*Nyssa*, as a slow-growing AM-associated species, may benefit from conspecific soil in the greenhouse simply because there is a higher likelihood of encountering beneficial mutualists that it relies on. The dominant tree species in this area of southern Appalachian Mountains associate with ectomycorrhizal fungi. For the three sites used in this study, EMF-associated trees comprise from 72% to 78% of total mature tree basal area. Ecological dominance of ectomycorrhizal-associated species may suppress species richness of arbuscular mycorrhizal fungi, provide additional belowground networks for EMF-associated species, or create a biogeochemical context that is unfavorable to AM trees (Becklin *et al*. 2012; McGuire 2007). Since ectomycorrhizal fungi are more likely than arbuscular mycorrhizal fungi to act as multi-host generalists instead of specialists (Smith and Read 2010), it may be that *Nyssa* and other AM trees such as *Acer rubrum* have fewer and more patchy distributions of suitable fungal partners. In the field, however, the benefit of encountering appropriate mutualists could be outweighed by greater vulnerability to pathogens in own-species inoculum, creating the negative feedback. A change in nutrient status, temperature, or water could leave *Nyssa* seedling roots more vulnerable to attack, or increase the activity of same-species pathogens that were dormant in the greenhouse.

Why were soil inoculum effects different between greenhouse and field?

Greenhouse studies of plant-soil feedbacks may overestimate, or misrepresent, plant-soil feedbacks experienced in field conditions. As field-based studies of PSF increase in commonality, researchers are beginning to purposefully compare PSF in field and greenhouse situations. Several other studies have found a striking disparity between greenhouse and field PSF, including results very similar to this study. One comparative study with grassland species (Heinze *et al*. 2016), for instance, found that some grassland species with self-reinforcing (positive) feedbacks in the greenhouse exhibited self-regulating (negative) feedbacks in the field, as did *Nyssa sylvatica* in this study. The difference between greenhouse and field could be explained by the increased range of conditions—both biotic and abiotic—experienced in the field. In addition, the field environment introduced competition with neighboring vegetation, which could alter plant-soil interactions. Results from another grassland study (Schittko *et al.* 2016) suggests that the presence of herbivores and other trophic-level interactions could also influence PSF in the field compared to greenhouse. Schittko *et al*. found significant PSF in the greenhouse but none in the field, similar to *Pinus* and *Acer* in this study.

Plants experienced a much broader range of biotic and abiotic conditions in the field compared to the greenhouse. In particular, the range of temperature and soil moisture conditions experienced could play a role in the disparity between greenhouse and field, as noted in Heinze *et al.* (2016). I attempted to minimize the differences between greenhouse and field phases by using an outdoor shadehouse that experienced more typical daily and seasonal temperature fluctuations than an enclosed greenhouse. Despite this, the shadehouse and field areas may have been far enough apart by latitude and elevation to provide sufficiently different abiotic conditions. In addition, field-planted seedlings experienced far greater seasonal variation in soil moisture than seedlings in the greenhouse, who were regularly watered. Seedlings may be more able to resist pathogenic fungal infection under wet conditions, even if fungi themselves are more prevalent (Hersh *et al*. 2012).

Comparison to previous study

The inoculum effects seen in the greenhouse stage of this study also differed from the results of our previous greenhouse study. These differences could be due to the increasing time-since-disturbance, or simply due to a number of changes in experimental design. The previous greenhouse study used a large proportion of whole soil in seedling pots, which confounds soil abiotic characteristics with soil biota. In addition, the two experiments contained different combinations of tree species, due to difficulties with seed germination. Lastly, the soils for the previous greenhouse experiment were collected one a year after the tornado, whereas the current experiment took place three years after the tornado. Within that time, the aboveground vegetative community composition and structure have shifted in response to the disturbance and canopy gaps have begun to close. It is highly unlikely that the soil microbial community remained the same in the two years separating the greenhouse studies.

Importance of soil inoculum in the field, compared to other factors

In the field-transplant portion of the study, seedling survival was most affected by broad environmental characteristics (Model 4), while seedling growth was most affected by soil inoculum origin (Model 2). At face value, this suggests that major trends in which seedlings survive or perish is determined, not by soil biotic interactions, but by resource availablity. Soil biotic interactions, therefore, would determine more fine-scale growth dynamics among the survivors. Overall, this result is not surprising, given the overwhelming importance of light availability and nutrient cycling to plant community development in secondary succession.

The specific abiotic factors that were significant in the model, however, were not the ones expected to be the biggest drivers of seedling growth. Most surprisingly, canopy openness was not a major determinant of seedling growth for any species. Instead, C:N ratio was most correlated to seedling survival, especially for *Nyssa sylvatica* and *Acer rubrum*. It is possible that the canopy openness (from photos at 1.5 meters height) overestimated the variation in light environment seedlings experienced between plots, due to shading by understory species that wouldn't have been captured in the hemispherical photos. Regardless, I expected more soil characteristics such as nitrogen mineralization or soil moisture to play a larger role in post-disturbance seedling survival than a fairly static soil characteristic like C:N ratio. This result, combined with large differences in survival between plots, suggests that the correlation between seedling survival and C:N ratio may merely be an artifact of the nested experimental design and a plot effect.

The lack of a clear mechanism casts doubt on a strict interpretation of the AIC scores. In addition, the AIC scores were overall very high, and their values were close together. Even if the three effects modeled in this experiment (inoculum, plot identity, environmental characters) do differentially influence seedling growth and survival in the order suggested by the comparison, those differences may not be large enough to make a substantial difference in the seedling's life. Instead, the most important factors driving seedling growth and survival may be either muddled by stochasticity or not measured in this experiment.

Importance of wind damage

One result was consistent in both phases of the experiment: In both the greenhouse and the field, plant-soil interactions in soils from wind-damaged areas were the same as those in soils from intact forest areas. Wind damage had little to no effect on the soil inoculum. Only *Nyssa sylvatica* seedlings showed a significant change due to wind damage. *Nyssa* seedlings grew somewhat taller in intact forest soil inoculum, but regardless of the soil's species origin; it had no effect on the 'home vs. away' feedback comparison. This *Nyssa* wind effect was also only visible in the field transplant phase, indicating another instance in which the function of soil biota differed by environmental context.  This result is consistent with historically low ability of *Nyssa* to recruit seedlings after a disturbance, even compared to other late-successional species (Abrams 2007).

In the field phase, seedlings actually performed better in the intact forest plots than in wind-damaged plots. This is an unexpected result; it is usually assumed that seedlings benefit from the higher light environment and more abundant resources in a disturbed habitat. The specifics of these field locations could have created more harsh environments than expected, however. Steep slopes and bare soil in some areas led to visible soil erosion in the plots. Evidence of wildlife activity was also abundant, including scat, digging, and herbivory. Although I constructed fences to protect the plots from large animal interference, smaller animals could still enter.

*Nyssa sylvatica* shows consistently unique plant-soil interactions

This is the second experiment I have conducted in which *Nyssa sylvatica* seedlings exhibited more striking and more specific relationships with soil inoculum than other seedling species. The details of the interactions between *Nyssa sylvatica* and the soil have not been thoroughly studied, but we may be able to infer some potential mechanisms based on knowledge of *Nyssa sylvatica*'s natural history.

Although *Nyssa sylvatica* is a common tree in all study sites, it is the least dominant of all species used in the studies. *Nyssa* persists throughout a wide geographic and ecological range, but rarely if ever reaches dominance (Abrams 2007), possibly due to slow growth even after disturbances and a reliance on bird-dispersed seeds. Despite its ability to tolerate a broad range of ecological conditions, its slow growth rates prevent it from competing with fast-growing species such as *Acer rubrum* that take advantage of canopy release after disturbances (Abrams 2007). In fact, Abrams draws a specific contrast between *Nyssa sylvatica* and *Acer rubrum*; although both have the ability to persist in a variety of conditions, *A. rubrum*'s opportunistic growth allows it to dominate forests while *N. sylvatica* lingers in the subcanopy.

As a stress-tolerating, subordinate canopy tree, *N. sylvatica*'s negative plant-soil feedbacks in the previous greenhouse study and in the field portion of this study are consistent with the trend of less abundant species having more negative plant-soil interactions (Klironomos 2002; Mangan *et al.* 2010). In a study of generalist pathogens and temperate forest trees, *Nyssa sylvatica* seedlings were very vulnerable to a few key generalist pathogens if they occurred in combination (Hersh *et al.* 2012). If species-conditioned soils are more likely to contain key combinations of soil pathogens, it could lead to the species-specific PSF seen in these two experiments, even if individual pathogen distributions aren't species-specific. The Hersh *et al.* (2012) study also showed that soil moisture played a large role in seedling vulnerability to pathogens; seedlings were better able to survive fungal attack in higher soil moisture, even though fungal prevalence was also greater. Since greenhouse pots were regularly watered, a difference in soil moisture between greenhouse and field, could help explain the disparity in *Nyssa*'s feedbacks between the two experiment phases.

As noted earlier, *Nyssa's* AM mycorrhizal type could also be a large component of its plant-soil feedbacks. As an AM-associated tree species, *Nyssa* has a relative disadvantage in pathogen protection (Bennett *et al*. 2017), diversity of the local fungal associate pool (Becklin *et al*. 2012), and distribution of appropriate fungal partners. In addition, AM fungi may be more vulnerable to disturbances, since they are host obligate and do not disperse easily (Smith and Read 2010). Although *Acer* is also an AM-associated tree species, its fast growth rate and high competitive ability may buffer it from mycorrhizal-specific soil effects that limit *Nyssa.*

<u>Consequences for PSF as a factor in ecosystem development</u>

For a given tree species, seedlings do not appear to experience different species-specific relationships in intact and wind-damaged areas. This suggests that PSF likely do not have a major influence on species performance and distributions immediately following a severe tornado. Regardless, this work provides a different perspective than theory, which suggests that post-disturbance plant-soil feedbacks would be minimized to a "blank slate" (Kardol *et al*. 2013; Reynolds *et al*. 2003). This work states instead that PSF are "more of the same" within a few years post-disturbance. Secondary succession following isolated disturbances surrounded by relatively intact forest may allow rapid recolonization of at least the most common soil microbial populations. Alternatively, soil microbes may be more resilient than expected, or remain unaffected by the damage to their host plants.

Plant-soil feedbacks may still be important for ecosystem development overall, or for other disturbance types. Even if species-specific plant-soil interactions do not drastically change following a disturbance, community-level interactions are likely still important drivers of primary succession and ecosystem development (Kardol *et al.* 2006; Van der Putten *et al*. 1993), through nutrient-mediated feedbacks, reliance on mutualists, and pressure from pathogens. Disturbances other than wind have more direct effects on soil characteristics and communities. Even severe cases of wind damage only modestly and indirectly affect soil characteristics. In this study, the conditions of intact and wind-damaged forest areas were more similar to each other than they were to the greenhouse conditions. Fire, flooding, mechanical disruption, and agricultural disturbances are more likely to drastically change soil biotic communities in ways that have lasting effects on plant-soil interactions.

Future directions

The experimental design used here uses a robust method necessary for field studies to separate confounding soil factors. By pairing greenhouse and field phases, we are better able to experimentally isolate the relative influence of biotic soil communities from other environmental factors. This method, however, does not characterize the soil biotic community itself. Sequencing fungal communities from the soil as well as collected plant roots would provide necessary data to assess actual mechanisms of the plant-soil interactions. This experiment uses plant performance as a proxy for soil community similarity. Direct assessment of the soil microbial community would allow explicit comparisons of root and soil fungal communities across species and damage types.

In this experiment, there were larger differences between feedbacks in greenhouse and field settings than between intact forests and wind-damage forest areas. These results provide more evidence that greenhouse-assessed plant-soil feedbacks should be calibrated with field studies. In order to apply plant-soil feedback research into real ecosystems and plant communities, more plant-soil studies should use field transplant experiments.

## Conclusions

While plant-soil feedbacks may influence some species performance overall, their influence does not appear to vary between intact and wind-damaged forest areas. Plant-soil interactions in a recovering Appalachian forest four years after a tornado appear to be nearly the same as plant-soil interactions in an intact forest. In this system, seedling success is likely first determined by broad abiotic conditions relating directly to resource acquisition, and is only secondarily, and minimally, influenced by species-specific interactions with soil pathogens, mutualists, or other microbiota.

## 4.6 Tables and Figures

Table 4.01. Species distribution of total tree basal area within each study site

| | Site Name | | |
|---|---|---|---|
| Tree Species | Boggs Creek | Martin Branch | Timpson Creek |
| *Pinus strobus* | 20% | 39% | 40% |
| *Quercus alba* | 10% | 4% | 14% |
| *Quercus prinus* | 21% | 6% | — |
| *Liriodendron tulipifera* | 9% | 8% | 8% |
| *Acer rubrum* | 5% | 10% | 7% |
| *Oxydendrum arboreum* | 5% | 6% | 5% |
| *Quercus rubra* | 10% | 3% | — |
| *Pinus virginiana* | 9% | 4% | — |
| *Quercus coccinea* | — | 2% | 10% |
| *Carya spp.* | 3% | 4% | 2% |
| *Quercus velutina* | — | 2% | 4% |
| *Tsuga canadensis* | 4% | 1% | — |
| *Nyssa sylvatica* | 2% | 1% | — |

Table 4.02. Individual Plot Characteristics

| Site | Species | Damage | Canopy Openness | Est. % Damage | Aspect (deg) | Slope (deg) | DBH |
|---|---|---|---|---|---|---|---|
| Boggs Creek | *Acer rubrum* | Intact | 0.07 | 0.00 | 264.19 | 13.11 | 16.30 |
| Boggs Creek | *Nyssa sylvatica* | Intact | 0.09 | | | | 10.20 |
| Boggs Creek | *Pinus strobus* | Intact | 0.11 | 42.80 | 277.2 | 12.34 | 40.90 |
| Boggs Creek | *Quercus prinus* | Intact | 0.06 | 0.55 | 114.16 | 15.31 | 36.40 |
| Martin Branch | *Acer rubrum* | Intact | 0.12 | 0.00 | 256.25 | 5.68 | 22.20 |
| Martin Branch | *Nyssa sylvatica* | Intact | 0.22 | 44.97 | 10.42 | 4.9 | 23.90 |
| Martin Branch | *Pinus strobus* | Intact | 0.20 | 44.97 | 10.42 | 4.9 | 43.50 |
| Martin Branch | *Quercus prinus* | Intact | 0.13 | 0.00 | 268.02 | 12.41 | 67.30 |
| Timpson Creek | *Acer rubrum* | Intact | 0.05 | 0.00 | 230.9 | 8.78 | 14.50 |
| Timpson Creek | *Nyssa sylvatica* | Intact | 0.09 | 8.55 | 256.93 | 9.84 | 13.40 |
| Timpson Creek | *Pinus strobus* | Intact | 0.10 | 0.00 | 261.57 | 6.56 | 37.00 |
| Timpson Creek | *Quercus prinus* | Intact | 0.08 | 0.55 | 104.61 | 10.48 | 35.20 |
| Boggs Creek | *Nyssa sylvatica* | Snapped | 0.20 | 90.80 | 270.64 | 15.21 | 15.80 |
| Boggs Creek | *Acer rubrum* | Canopy snapped | 0.18 | 89.10 | 295.4 | 11.84 | 22.20 |
| Boggs Creek | *Pinus strobus* | Uprooted | 0.23 | 62.71 | 295.73 | 10.36 | 52.50 |
| Boggs Creek | *Quercus prinus* | Uprooted | 0.22 | 94.25 | 295.4 | 11.84 | 20.50 |
| Martin Branch | *Acer rubrum* | Snapped | 0.21 | 78.21 | 278.28 | 1.76 | 25.80 |
| Martin Branch | *Nyssa sylvatica* | Uprooted | 0.27 | 81.61 | 278.67 | 8.41 | 11.70 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Martin Branch | *Pinus strobus* | Canopy snapped | 0.22 | 73.56 | 278.28 | 1.76 | 82.70 |
| Martin Branch | *Quercus prinus* | Bent, snapped | | 82.25 | 256.23 | 9.46 | 9.00 |
| Timpson Creek | *Acer rubrum* | Canopy snapped | 0.24 | 85.06 | 48.88 | 6.9 | 31.30 |
| Timpson Creek | *Nyssa sylvatica* | Bent | 0.29 | 97.70 | 303.02 | 6.96 | 7.60 |
| Timpson Creek | *Pinus strobus* | Snapped | 0.23 | 98.85 | 45.38 | 7.99 | 55.60 |
| Timpson Creek | *Quercus prinus* | Broken branches | 0.18 | 67.31 | 32.25 | 16.63 | 20.00 |

Table 4.03. Generalized linear model for Model 1: influence of soil inoculum type on seedling growth in the greenhouse

| | All Species | | *Acer rubrum* | | *Nyssa sylvatica* | | *Quercus prinus* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | F | P | F | P | F | P | F | P | F | P |
| Soil inoculum source species | 5.38 | **<0.05** | .62 | **<0.05** | 30.89 | **<0.01** | 0.24 | 0.62 | 18.34 | **<0.01** |
| Soil inoculum source damage | 0.39 | 0.53 | 2.29 | 0.13 | 1.43 | 0.23 | 1.45 | 0.23 | 2.92 | 0.09 |
| Soil inoculum species x damage | 24.85 | **<0.01** | 1.51 | 0.22 | 0.06 | 0.81 | 2.12 | 0.18 | 2.38 | 0.24 |
| Seedling Species | 50.26 | **<0.01** | | | | | | | | |
| Seedling Spp x Source Spp | 0.49 | 0.53 | | | | | | | | |
| Seedling Spp x Source Damage | 2.05 | 0.11 | | | | | | | | |
| Seedling x Source Spp x Source Wind | | | | | | | | | | |

Table 4.04. Generalized linear model for Model 2: influence of soil inoculum on seedling growth in the field

| | All Species | | *Acer rubrum* | | *Nyssa sylvatica* | | *Quercus prinus* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | F | P | F | P | F | P | F | P | F | P |
| Soil inoculum source species | 5.36 | **<0.05** | 0.19 | 0.66 | 6.28 | **<0.05** | 6.38 | 0.13 | 0.21 | 0.65 |
| Soil inoculum source damage | 2.43 | 0.12 | 0.09 | 0.76 | 5.49 | **<0.05** | 0.43 | 0.51 | 0.90 | 0.35 |
| Soil inoculum species x damage | 0.001 | 0.99 | 0.97 | 0.33 | 1.23 | 0.27 | 0.02 | 0.88 | 0.68 | 0.41 |
| Seedling Species | 6.71 | **<0.01** | | | | | | | | |
| Seedling Spp x Source Spp | 2.48 | 0.06 | | | | | | | | |
| Seedling Spp x Source Damage | 2.52 | 0.06 | | | | | | | | |
| Seedling x Source Spp x Source Wind | 1.10 | 0.35 | | | | | | | | |

Table 4.05. Logistic regression and likelihood ratios for Model 2: influence of soil inoculum on seedling survival in the field

| | All Species | | *Acer rubrum* | | *Nyssa sylvatica* | | *Quercus prinus* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P |
| Soil inoculum source species | 0.37 | 0.55 | 1.41 | 0.23 | 0.70 | 0.41 | 1.02 | 0.31 | 0.02 | 0.88 |
| Soil inoculum source damage | 0.81 | 0.37 | 0.06 | 0.81 | 1.60 | 0.21 | 2.88 | 0.09 | 1.76 | 0.18 |
| Soil inoculum species x damage | 0.18 | 0.67 | 1.34 | 0.25 | 2.02 | 0.15 | 1.77 | 0.18 | 0.39 | 0.53 |
| Seedling Species | 4.36 | 0.23 | | | | | | | | |
| Seedling Spp x Source Spp | 2.79 | 0.42 | | | | | | | | |
| Seedling Spp x Source Damage | 5.50 | 0.14 | | | | | | | | |
| Seedling x Source Spp x Source Wind | 5.35 | 0.15 | | | | | | | | |

Table 4.06. Logistic regression and likelihood ratios for Model 3: influence of plot location on seedling survival in the field

| | All Species | | *Acer rubrum* | | *Nyssa sylvatica* | | *Quercus prinus* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P | LR $\chi^2$ | P |
| Plot Tree Species | 1.68 | 0.19 | 0.25 | 0.62 | 0.72 | 0.40 | 1.89 | 0.17 | 2.05 | 0.15 |
| Plot Wind Status | 5.31 | **<0.05** | 0.34 | 0.56 | 18.42 | **<0.01** | 0.44 | 0.51 | 0.79 | 0.38 |
| Tree Spp x Wind | 0.09 | 0.76 | 0.08 | 0.77 | 4.45 | **<0.05** | 0.17 | 0.68 | 4.70 | **<0.05** |
| Seedling Species | 4.64 | 0.20 | | | | | | | | |
| Seedling Spp x Tree Spp | 3.11 | 0.38 | | | | | | | | |
| Seedling Spp x Plot Wind | 14.49 | **<0.01** | | | | | | | | |
| Seedling x Tree x Wind | 9.32 | **<0.05** | | | | | | | | |

Table 4.07. Generalized linear model for Model 3: influence of plot location on seedling growth rate in the field

| | All Species | | *Acer rubrum* | | *Nyssa sylvatica* | | *Quercus prinus* | | *Pinus strobus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source | F | P | F | P | F | P | F | P | F | P |
| Plot Tree Species | 0.06 | 0.80 | 0.01 | 0.91 | 1.24 | 0.27 | 3.29 | 0.07 | 0.28 | 0.60 |
| Plot Wind Status | 3.12 | 0.08 | 2.97 | 0.09 | 0.002 | 0.96 | 0.55 | 0.46 | 11.72 | **<0.01** |
| Tree Spp x Wind | 0.00 | 0.99 | 0.39 | 0.53 | 0.08 | 0.78 | 0.003 | 0.96 | 0.16 | 0.67 |
| Seedling Species | 6.80 | **<0.01** | | | | | | | | |
| Seedling Spp x Tree Spp | 1.72 | 0.16 | | | | | | | | |
| Seedling Spp x Plot Wind | 2.06 | 0.10 | | | | | | | | |
| Seedling x Tree x Wind | 0.19 | 0.90 | | | | | | | | |

Table 4.08. Logistic regression coefficients for Model 4: influence of abiotic environment on seedling survival in the field

| | All Species | | | *Acer rubrum* | | | *Nyssa sylvatica* | | | *Quercus prinus* | | | *Pinus strobus* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Coeff | LR $\chi^2$ | P | Coeff | LR $\chi^2$ | P | Coeff | LR $\chi^2$ | P | Coeff | LR $\chi2$ | P | Coeff | LR $\chi^2$ | P |
| Mineral Nitrogen | -1.45 +/- 0.91 | 2.51 | 0.113 | -3.58 +/- 1.84 | 3.78 | 0.052 | 1.44 +/- 2.43 | 0.37 | 0.54 | -2.17 +/- 1.84 | 1.37 | 0.24 | -0.61 +/- 1.65 | 0.14 | 0.71 |
| C:N Ratio | -0.04 +/- 0.02 | 8.16 | < **0.01** | -0.10 +/- 0.03 | 10.16 | < **0.01** | -0.07 +/- 0.03 | 4.75 | < **0.05** | 0.002 +/- 0.03 | 0.002 | 0.96 | -0.002 +/- 0.03 | 0.003 | 0.95 |
| Soil Moisture | 0.12 +/- 0.06 | 3.90 | < **0.05** | 0.13 +/- 0.12 | 1.26 | 0.26 | -0.12 +/- 0.12 | 1.09 | 0.30 | 0.12 +/- 0.13 | 0.89 | 0.35 | 0.43 +/- 0.13 | 10.85 | < **0.01** |
| Soil Temperature | -0.05 +/- 0.03 | 2.16 | 0.142 | -0.08 +/- 0.07 | 1.49 | 0.22 | -0.14 +/- 0.06 | 4.92 | < **0.05** | -0.06 +/- 0.06 | 0.76 | 0.38 | 0.08 +/- 0.06 | 1.50 | 0.22 |
| Canopy Openness | -1.81 +/- 1.11 | 2.68 | 0.102 | -0.16 +/- 2.32 | 0.005 | 0.94 | -6.98 +/- 2.34 | 9.48 | < **0.01** | 0.55 +/- 2.39 | 0.05 | 0.82 | -0.17 +/- 2.14 | 0.007 | 0.93 |
| Seedling Species | | 7.50 | 0.058 | | | | | | | | | | | | |

Table 4.09. Regression coefficients for Model 4: influence of abiotic environment on seedling relative growth rate in the field

| | All Species | | | *Acer rubrum* | | | *Nyssa sylvatica* | | | *Quercus prinus* | | | *Pinus strobus* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Source | Coeff | F | P | Coeff | F | P | Coeff | F | P | Coeff | F | P | Coeff | F | P |
| Mineral Nitrogen | $4.12e^{-04}$ +/- $2.83e^{-04}$ | 2.12 | 0.15 | $1.29e^{-03}$ +/- $6.39e^{-04}$ | 4.04 | **<0.05** | $1.46e^{-04}$ +/- $6.67e^{-04}$ | 0.05 | 0.83 | $-1.82e^{-05}$ +/- $6.95e^{-04}$ | 0.02 | 0.89 | $3.14e^{-04}$ +/- $3.50e^{-04}$ | 0.80 | 0.37 |
| C:N Ratio | $-9.93e^{-06}$ +/- $8.88e^{-06}$ | 1.25 | 0.27 | $3.38e^{-06}$ +/- $1.65e^{-05}$ | 0.04 | 0.84 | $-3.32e^{-05}$ +/- $2.41e^{-05}$ | 1.90 | 0.17 | $-1.40e^{-05}$ +/- $1.75e^{-05}$ | 0.64 | 0.43 | $-8.87e^{-07}$ +/- $1.23e^{-05}$ | 0.01 | 0.94 |
| Soil Moisture | $2.33e^{-05}$ +/- $3.31e^{-05}$ | 0.50 | 0.48 | $4.42e^{-05}$ +/- $5.61e^{-05}$ | 0.62 | 0.43 | $-1.64e^{-05}$ +/- $8.35e^{-05}$ | 0.04 | 0.84 | $-4.13e^{-05}$ +/- $7.14e^{-05}$ | 0.33 | 0.56 | $8.38e^{-05}$ +/- $5.19e^{-05}$ | 2.61 | 0.11 |
| Soil Temperature | $2.53e^{-05}$ +/- $1.75e^{-05}$ | 2.09 | 0.15 | $9.03e^{-05}$ +/- $2.90e^{-05}$ | 9.68 | **<0.01** | $-2.65e^{-05}$ +/- $5.14e^{-05}$ | 0.27 | 0.61 | $-4.50e^{-05}$ +/- $3.90e^{-05}$ | 1.33 | 0.25 | $2.50e^{-05}$ +/- $2.37e^{-05}$ | 1.12 | 0.29 |
| Canopy Openness | $7.00e^{-04}$ +/- $6.29e^{-04}$ | 1.24 | 0.27 | $1.85e^{-03}$ +/- $1.12e^{-03}$ | 2.73 | 0.10 | $-2.49e^{-03}$ +/- $1.81e^{-03}$ | 1.89 | 0.17 | $2.69e^{-04}$ +/- $1.32e^{-03}$ | 0.04 | 0.84 | $1.88e^{-03}$ +/- $8.38e^{-04}$ | 5.02 | **<0.05** |
| Seedling Species | | 7.49 | **<0.01** | | | | | | | | | | | | |

Table 4.10. Akiake's information criterion (AIC) scores for model comparisons

| | Model components | AIC (survival) | AIC (relative growth rate) |
|---|---|---|---|
| Model 2 | Soil inoculum source | 1117.11 | **-6369.27** |
| Model 3 | Plot location | 1084.96 | -6358.28 |
| Model 4 | Abiotic environment | **874.26** | -5918.82 |

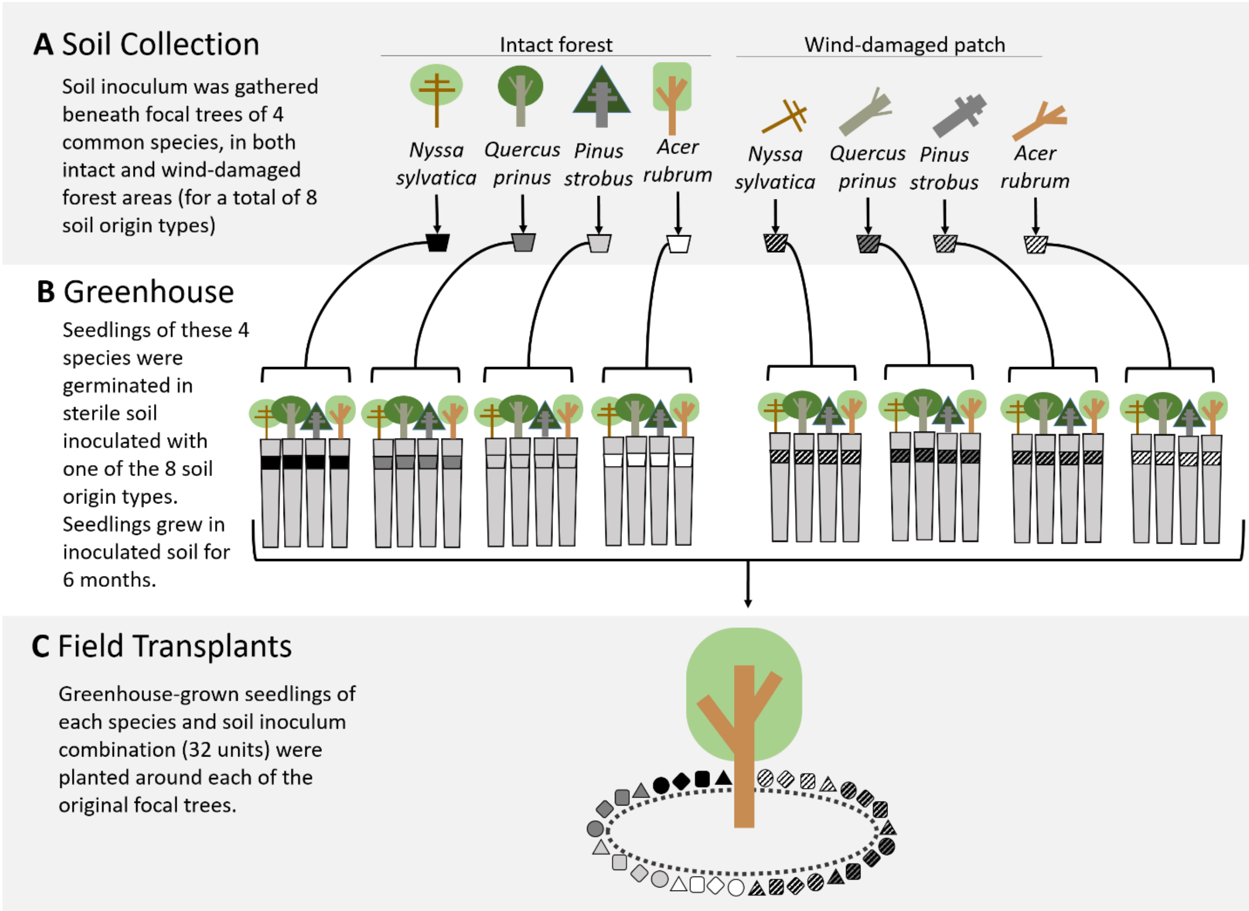


Figure 4.1. Overview of methods and experimental design. This experiment took a two-phase approach. In the first phase, field collected soil (A) was used to inoculate seedlings in the greenhouse (B). After 6 months in the greenhouse, seedling roots were expected to be "conditioned" to the inoculum. Seedlings were then transplanted (C) back into circular study plots surrounding the focal trees from which soil was originally collected. This process took place simultaneously for 3 separate study locations in the Chattahoochee National Forest.

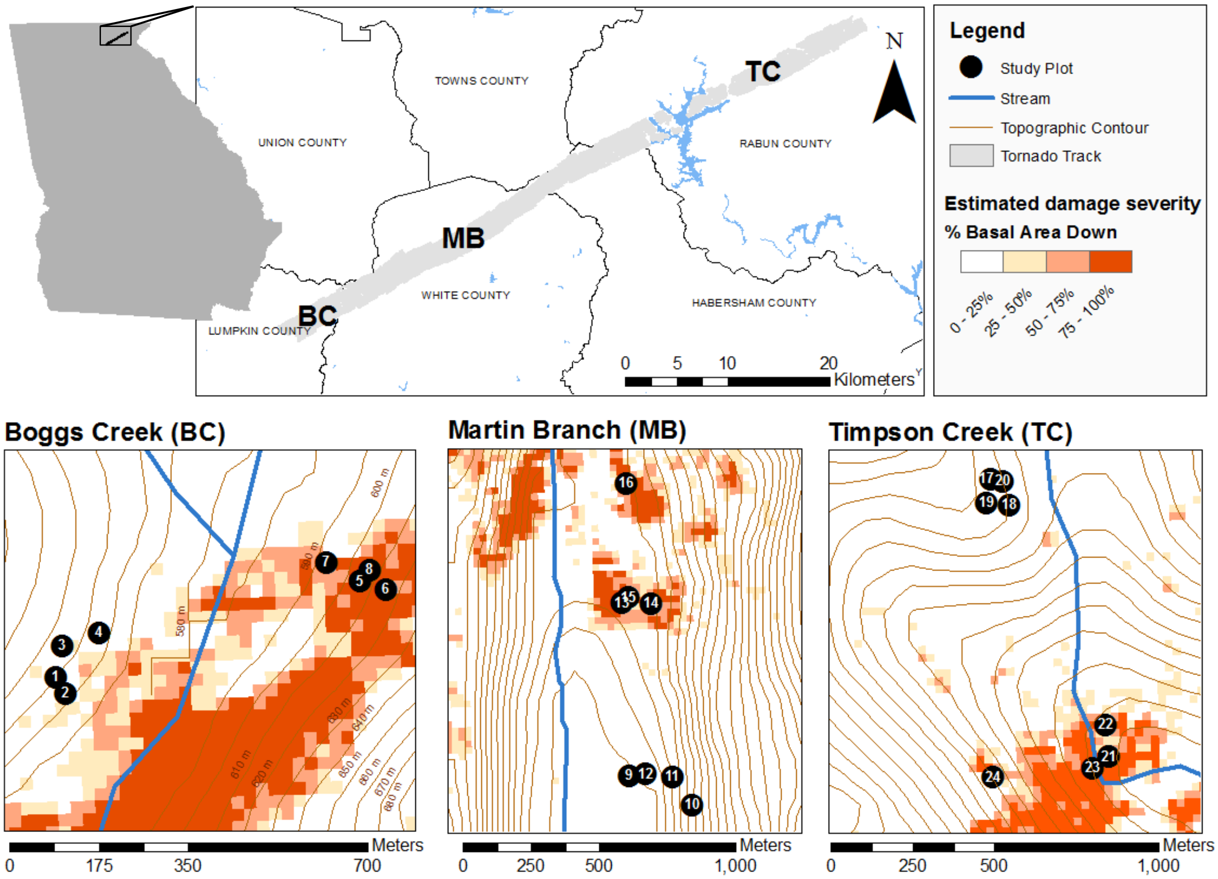


Figure 4.2. Locations of the three study sites and 24 focal tree plots. Estimated damage severity was calculated from aerial photos, and is for visualization purposes only (Cannon et al. 2016). Individual focal tree plot details can be found in Table 4.2.

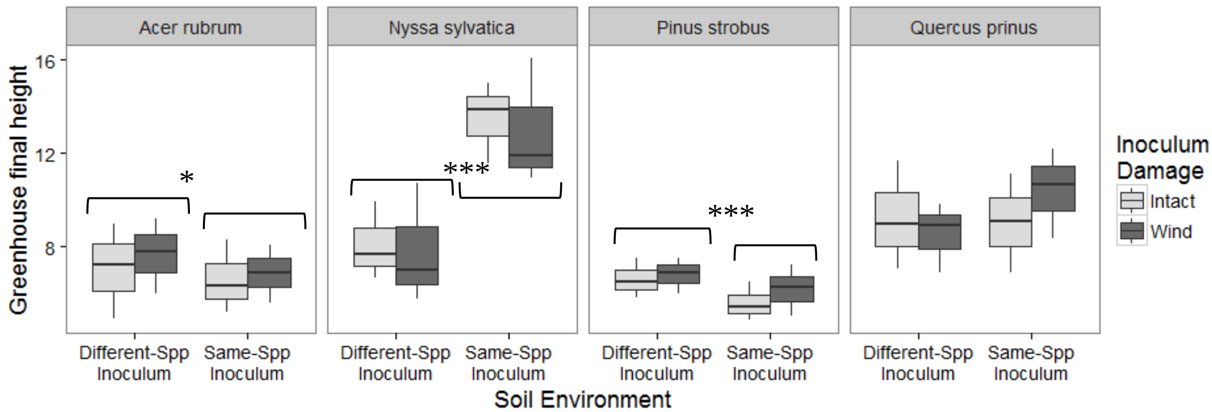


Figure 4.3. Final heights of seedlings after 6 months greenhouse growth in inoculated soil. Inoculum soil was collected from same-species and different-species mature trees found in intact forest (light boxes) or wind-damaged forest (dark boxes). Boxes represent standard error around the median, and asterisks denote statistical significance between bracketed groups (* = p<0.05, *** = p<0.001).

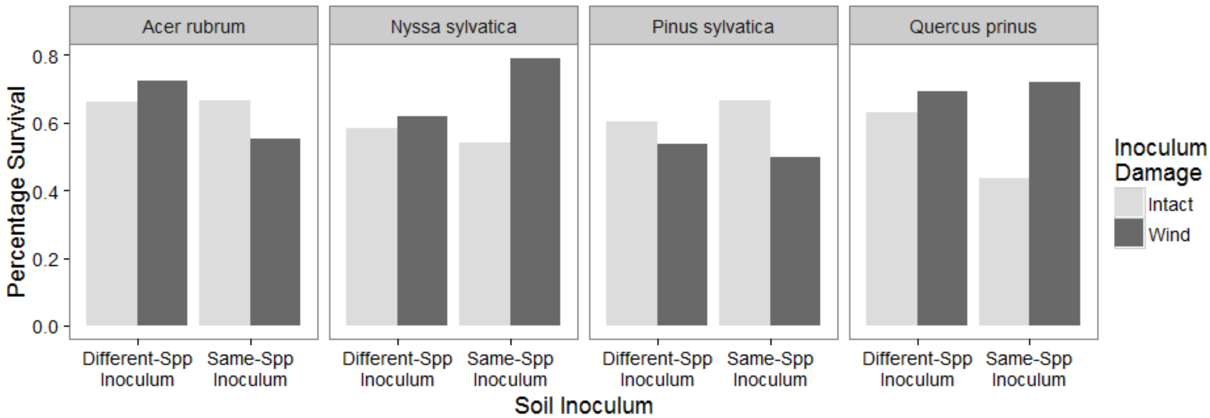


Figure 4.4. Percent survival of seedlings transplanted in the field, based on the type of inoculum seedlings were conditioned with in the greenhouse. Greenhouse inoculum was collected from same-species and different-species mature trees found in intact forest (light boxes) or wind-damaged forest (dark boxes).

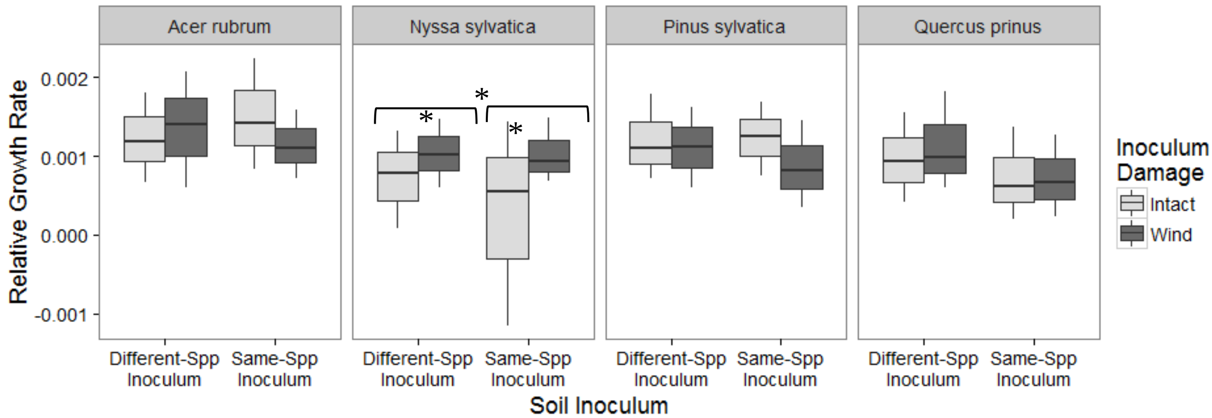


Figure 4.5. Relative growth rate of seedlings transplanted in the field, based on the type of inoculum seedlings were conditioned with in the greenhouse. Greenhouse inoculum was collected from same-species different-species mature trees found in intact forest (light boxes) or wind-damaged forest (dark boxes). Boxes represent standard error around the median, and asterisks denote statistical significance between bracketed groups (* = $p<0.05$).

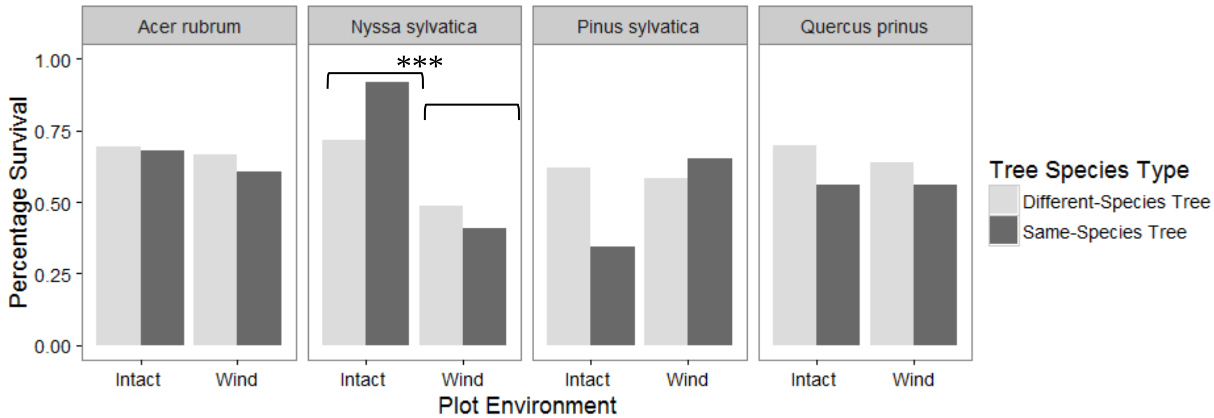


Figure 4.6. Percent survival of seedlings transplanted in the field, based on the type of focal tree plot in which they were planted. Focal trees were same-species (dark boxes) or different-species (light boxes) mature trees in intact forest or wind-damaged forest. Asterisks denote statistical significance between bracketed groups (*** = p<0.001).

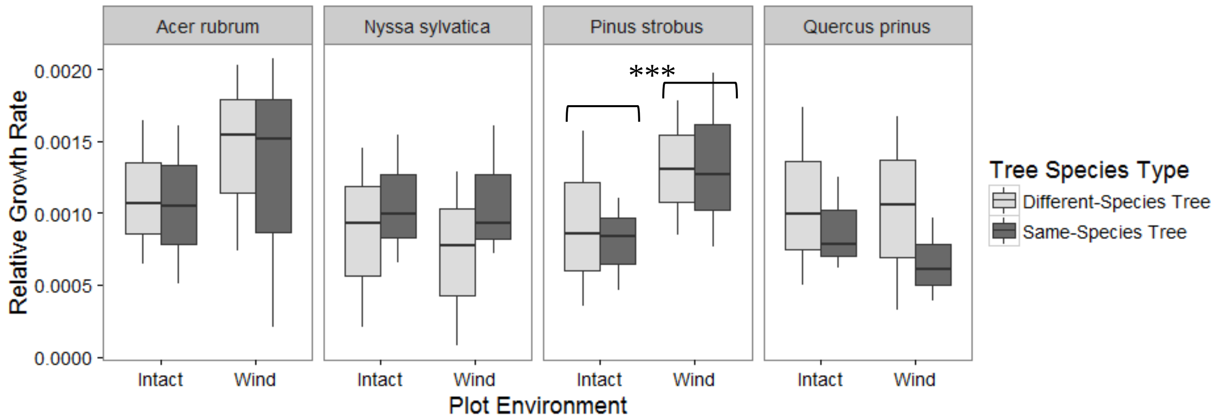


Figure 4.7. Relative growth rate of seedlings transplanted in the field, based on the type of focal tree plot in which they were planted. Focal trees were same-species (dark boxes) or different-species (light boxes) mature trees in intact forest or wind-damaged forest. Boxes represent standard error around the median, and asterisks denote statistical significance between bracketed groups (*** = p<0.001).

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# CHAPTER 5

# CONCLUSIONS

## 5.1 Overview

Overall, these three studies demonstrate the complexity of plant-soil interactions in the temperate forests of Northeast Georgia. Through observational and experimental methods in two temperate forests, I examined the interactions between related trees and seedlings, the patterns caused by these interactions in typical situations, and the changes to these interactions after disturbances. The combination of these studies demonstrates that negative interactions between related trees and seedlings are common in temperate forests, which is consistent with other studies on negative distance/density dependence. Some of these negative distance dependent interactions may be mediated through soil conditioning by biotic and/or abiotic mechanisms. When isolated in a greenhouse, plant-soil interactions for a given tree species can be drastically different in intact forests than in areas immediately impacted by a tornado. Whether the plant-soil feedbacks are magnified after a tornado or diminished, however, depends on the species concerned.

## 5.2 Negative Distance Dependence in a Piedmont Forest

Findings from the second chapter provide evidence for the existence of Negative Distance Dependence (NDD) patterns at the genus level in a mature piedmont temperate forest. Seedling spatial distributions shifted away from same-genus trees in the second year of the study, especially for the smallest size class. In addition, mid-sized seedlings were less likely to survive when greatly influenced by trees of the same genus. Results for all seedling genera combined were consistent with the hypothesis that seedlings in this forest are negatively impacted by the influence of closely related trees. As in other studies, the effects were most evident in the smallest seedling classes, which are also likely to be the youngest. Large seedlings rarely exhibited negative distance dependence in this forest.

Seedling survival had a slightly different relationship with the tree influence metric compared to tree distance alone. Since the tree influence metric includes a measure of tree size in addition to distance, this suggests that seedlings experience the influences of larger, but farther trees more so than small nearby trees. Simply being a seedling's nearest neighbor, then, does not necessarily mean that tree has the largest effect on a seedling.

This work contributes to a growing body of literature demonstrating that Negative Distance Dependence patterns are not limited to tropical forests (Lambers and Clark 2003; Martínez et al. 2013; McCarthy-Neumann and Kobe 2010; Packer and Clay 2000; Reinhart et al. 2012b; Yamazaki et al. 2009). Spatial dynamics created by seedling responses to mature trees occur in many ecosystem types (Comita et al. 2014). Distance-dependent seedling mortality may be a relatively ubiquitous mechanism of maintaining community structure across multiple biomes.

In addition, this work demonstrates that Janzen-Connell type processes can affect individuals at a broader phylogenetic scale than species (Lebrija-Trejos et al. 2014; Liu et al. 2012; Zhu et al. 2015). Individuals related at the genus scale can also influence each other's survival and contribute to genus-level spatial distributions. By extending the concept of Negative Distance Dependence beyond species level, this work questions the need for strong species-specific natural enemies for NDD patterns to emerge.

### 5.3 Plant-soil Feedbacks in a Southern Appalachian Forest

NDD patterns have been documented multiple times in Southern Appalachian forests as well (Godoy et al. 2015; Johnson et al. 2012; Reinhart et al. 2012b). In the third chapter, I was able to confirm that several of these common southern Appalachian tree species have negative-to-neutral plant-soil feedbacks, which may serve as a mechanisms for NDD. These plant-soil feedbacks, however, were largely driven by species-specific pairings, indicating that the composition of the surrounding heterospecific tree community has a large impact on plant-soil feedbacks for a given tree species.

Some theories suggest that, following a severe disturbance, strong negative plant-soil feedbacks should revert to uniformly neutral feedbacks (Kardol et al. 2013; Reynolds et al. 2003). Contrary to published theory, I found that tornado damage did not have a consistent effect on plant-soil feedbacks. Instead, the effect of a tornado on plant-soil feedbacks varied depending on the seedling species itself. Compared to feedbacks from intact forest areas, damaged-area plant-soil feedbacks for *Quercus alba* were more neutral, for *Nyssa sylvatica*'s were more positive, and for *Pinus strobus'* were unchanged. This experiment provided no evidence for a potential 'blank slate' of weakened feedbacks after a disturbance. No clear post-disturbance plant-soil feedback pattern emerged, suggesting that post-disturbance plant-soil dynamics are more complicated and variable than current theory explains.

Contrary to expectations, mycorrhizal colonization alone was not a significant driver of plant-soil feedback differences. Multiple interacting mechanisms may underlie plant-soil feedbacks for each plant species. The varying responses to disturbance of each of these potential mechanisms (e.g. specialist pathogens, mycorrhizal composition, nitrogen availability) would explain the wide variation in post-disturbance feedback changes. The direction and magnitude of any feedback alterations will depend on which organisms are most affected, how specific or general their relationships are, and interactive effects with the changing abiotic and biotic conditions (Corkidi et al. 2002; McCarthy-Neumann and Ibáñez 2013).

### 5.4 Field Assessment of Plant-soil Feedbacks

In order to specifically address the possibilities of interactive effects with the post-disturbance landscape, as well as make stronger conclusions about post-disturbance plant-soil feedbacks, I revisited the same questions with a field experiment. Results from the fourth chapter showed that, within four years of tornado damage, plant-soil feedbacks for common southern Appalachian seedlings in wind-damaged areas were no different from plant-soil feedbacks in intact forest areas. In both the greenhouse and the field, a history of wind damage had little to no effect on the function of soil inoculum. One possibility is that four years is enough time for the soil biotic community to recolonize from existing roots, deeper soil reserves, or airborne spores. With rapid understory growth, a nearby matrix of intact forest, and high microsite variability within the damaged area, abiotic soil conditions may also be similar enough to intact forest within four years to foster a similar soil biotic community.

In this two-part experiment, plant-soil feedbacks calculated from seedlings grown in the greenhouse did not match those calculated from field-transplanted seedlings. Fast-growing species *Pinus strobus* and *Acer rubrum* were inhibited by conspecific soil microbial communities in the greenhouse, but not in the field. *Nyssa sylvatica*'s soil microbial community improved same-species seedling performance in the greenhouse but inhibited it in the field. Only one species (*Nyssa sylvatica*) exhibited negative plant-soil feedbacks in the field. When feedbacks were present in the field, they were secondary determinants of seedling performance, as seedling survival in the field was more closely related to larger-scale abiotic environmental characteristics.

The comparison of greenhouse and field effects of soil inoculum pinpointed the importance of environmental context. In the less controlled field transplant environment, the same soil community interacted in a different way with the seedlings. The behavior, activity, and/or function of the soil inoculum for the seedling species depended greatly on the overall environmental context. Namely, field conditions introduced a much broader range of temperature and moisture conditions compared to the greenhouse, as well as competitive interactions with surrounding vegetation—both of which could help explain some of the disparity between feedbacks in the two areas (Heinze et al. 2016; Hersh et al. 2012).

In both of these two last experiments, *Nyssa sylvatica* stands out as a species that exhibits striking relationships with soil inoculum. *Nyssa sylvatica* is a stress-tolerating, subordinate canopy tree. While it can persist in a wide range of ecological conditions, its slow growth rates prevent it from competing with fast-growing species such as *Acer rubrum* (Abrams 2007). *N. sylvatica*'s negative plant-soil feedbacks are consistent with the trend of less abundant species having more negative plant-soil interactions (Klironomos 2002; Mangan et al. 2010). In forest areas dominated by ectomycorrhizal-associated trees, an arbuscular mycorrhizal-associated tree species such as *Nyssa* may have a relative disadvantage in both pathogen protection (Bennett et al. 2017) and the diversity of the local fungal associate pool (Becklin et al. 2012).

## 5.5 Conclusions

Together, these three research chapters push the boundaries of knowledge on temperate forest plant-soil interactions. While negative distance dependence patterns have been documented in many temperate forests, this work shows that they are evident even at the genus level. When these broader plant-soil relationships can create negative distance dependent patterns, it suggests that highly specialist natural enemies aren't necessary. Consistent with this idea is the finding that the highly variable plant-soil feedbacks after a tornado return to pre-disturbance states within four years—long before the vegetation itself resembles an intact forest. If plant-soil interactions are largely due to combinations of generalists instead of species-specific partners, the rapid recolonization of the most common soil microbial populations could make this return possible. The variability of plant-soil feedback responses to disturbance are contrary to the theory that plant-soil interactions would "neutralize" – feedbacks were either highly variable immediately after a disturbance, or remained the same as intact forest.

Disturbance types that more directly affect soil characteristics and communities may show more drastic and longer-lasting effects on plant-soil feedbacks. Unlike wind damage, the effects of fire, flooding, mechanical disruption, and agricultural disturbances are more likely to directly change soil biotic communities in ways that have lasting effects on plant-soil interactions. While community-level plant-soil interactions help drive primary succession and broad-scale ecosystem development (Kardol et al. 2006; Van der Putten et al. 1993), their contribution to secondary succession, particularly after wind damage, is still largely unknown. As the global climate patterns change, worldwide disturbance regimes also shift. Severe weather events such as fires, floods, and drought are likely to increase, in addition to some wind disturbances. Eastern North America may experience a higher likelihood of strong tropical cyclones (Knutson et al. 2010; Knutson and Tuleya 2004; Solomon 2007) and more frequent tornadoes (Brooks 2013; Diffenbaugh et al. 2013), including large clusters of tornadoes (Elsner et al. 2014), such as the April 2011 supercell studied in this work (NOAA 2011). Examining post-disturbance changes to major ecosystem processes, including plant-soil feedbacks, will help us understand how this shift in disturbance regimes will affect eastern forests. If, as these studies suggests, plant-soil feedbacks do not "neutralize" after disturbances, post-disturbance soil changes may play a larger role in forest regeneration than previously assumed. Exploring how the context-dependent relationships between plants and soil respond to this dynamic environment will help further our understanding of natural regeneration processes as well as predict ecosystem responses to a changing climate.

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## APPENDIX

Appendix Table 1. Characteristics of focal trees

| ID | Tree Species | Wind/ Intact | Damage type | DBH (cm) | % Canopy openness* | Avg soil moisture (%VWC)** | Avg soil temp (˚C)** |
|---|---|---|---|---|---|---|---|
| 1 | Liriodendron tulipifera | Intact | N/A | 56.0 | 18.92 | 26.50 | 15.22 |
| 2 | Liriodendron tulipifera | Intact | N/A | 41.6 | 10.14 | 15.25 | 16.02 |
| 3 | Liriodendron tulipifera | Intact | N/A | 37.3 | 10.81 | 20.25 | -- |
| 4 | Liriodendron tulipifera | Intact | N/A | 41.05 | 10.81 | 20.25 | 15.68 |
| 5 | Liriodendron tulipifera | Wind | uprooted | 57.4 | 52.70 | 12.63 | 16.43 |
| 6 | Liriodendron tulipifera | Wind | uprooted | 60.4 | 60.47 | 10.50 | 16.52 |
| 7 | Liriodendron tulipifera | Wind | crown broken | 56.4 | 36.82 | 11.25 | 15.63 |
| 8 | Liriodendron tulipifera | Wind | uprooted | 40.8 | 33.78 | 28.25 | 15.97 |
| 9 | Nyssa sylvatica | Intact | N/A | 10.8 | 11.82 | 14.13 | 15.54 |
| 10 | Nyssa sylvatica | Intact | N/A | 12.9 | 12.16 | 11.00 | 15.91 |
| 11 | Nyssa sylvatica | Intact | N/A | 9.0 | 12.50 | 17.25 | 16.04 |
| 12 | Nyssa sylvatica | Intact | N/A | 14.0 | 11.49 | 14.38 | 15.81 |
| 13 | Nyssa sylvatica | Wind | snapped | 11.4 | 29.05 | 15.25 | 16.45 |
| 14 | Nyssa sylvatica | Wind | crown broken | 6.8 | 26.69 | 10.25 | 16.23 |
| 15 | Nyssa sylvatica | Wind | bent | 7.3 | 41.89 | 14.00 | 16.94 |
| 16 | Nyssa sylvatica | Wind | crown broken | 48.1 | 38.51 | 11.38 | 14.86 |
| 17 | Oxydendrum arboreum | Intact | N/A | 34.5 | 8.45 | 14.63 | 14.91 |
| 18 | Oxydendrum arboreum | Intact | N/A | 20.7 | 11.15 | 11.00 | 15.3 |
| 19 | Oxydendrum arboreum | Intact | N/A | 26.2 | 10.81 | 12.75 | 16.58 |
| 20 | Oxydendrum arboreum | Intact | N/A | 17.7 | 12.16 | 12.50 | 15.32 |
| 21 | Oxydendrum arboreum | Wind | uprooted | 22.9 | 43.24 | 11.25 | 15.1 |
| 22 | Oxydendrum arboreum | Wind | uprooted | 25.0 | 51.69 | 10.38 | 16.64 |
| 23 | Oxydendrum arboreum | Wind | uprooted | 20.0 | 44.59 | 15.75 | 16.81 |
| 24 | Oxydendrum arboreum | Wind | bent | 12.0 | 42.47 | 9.75 | 15.03 |
| 25 | Pinus strobus | Intact | N/A | 69.7 | 11.15 | 15.38 | 16.05 |
| 26 | Pinus strobus | Intact | N/A | 80.2 | 9.46 | 10.88 | 16.23 |
| 27 | Pinus strobus | Intact | N/A | 78.9 | 13.90 | 12.14 | 15.73 |
| 28 | Pinus strobus | Intact | N/A | 45.0 | 9.12 | 11.88 | 15.55 |
| 29 | Pinus strobus | Wind | crown broken | 84.0 | 32.43 | 12.88 | 16.98 |
| 30 | Pinus strobus | Wind | uprooted | 62.1 | 39.19 | 12.86 | 11.08 |
| 31 | Pinus strobus | Wind | crown broken | 91.2 | 20.95 | 17.25 | 17.36 |
| 32 | Pinus strobus | Wind | uprooted | 44.0 | 35.81 | 11.00 | 15.83 |
| 33 | Quercus alba | Intact | N/A | 58.5 | 11.49 | 15.13 | 14.99 |
| 34 | Quercus alba | Intact | N/A | 68.0 | 15.88 | 10.25 | 17.85 |
| 35 | Quercus alba | Intact | N/A | 43.8 | 12.84 | 19.88 | 15.7 |
| 36 | Quercus alba | Intact | N/A | 52.4 | 10.47 | 7.13 | 11.7 |
| 37 | Quercus alba | Wind | snapped | 37.4 | 21.62 | 11.00 | 14.48 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 38 | Quercus alba | Wind | snapped | 60.0 | 27.03 | 13.13 | 16.81 |
| 39 | Quercus alba | Wind | uprooted | 44.5 | 28.38 | 9.75 | 17.35 |
| 40 | Quercus alba | Wind | uprooted | 49.0 | 39.53 | 14.63 | 15.32 |

* Canopy openness was measured using a handheld concave densiometer. Percent canopy openness describes the percent of vertices on the concave grid that were not covered by vegetation. For each time point, canopy openness measurements were taken at each of the four cardinal directions and averaged together.

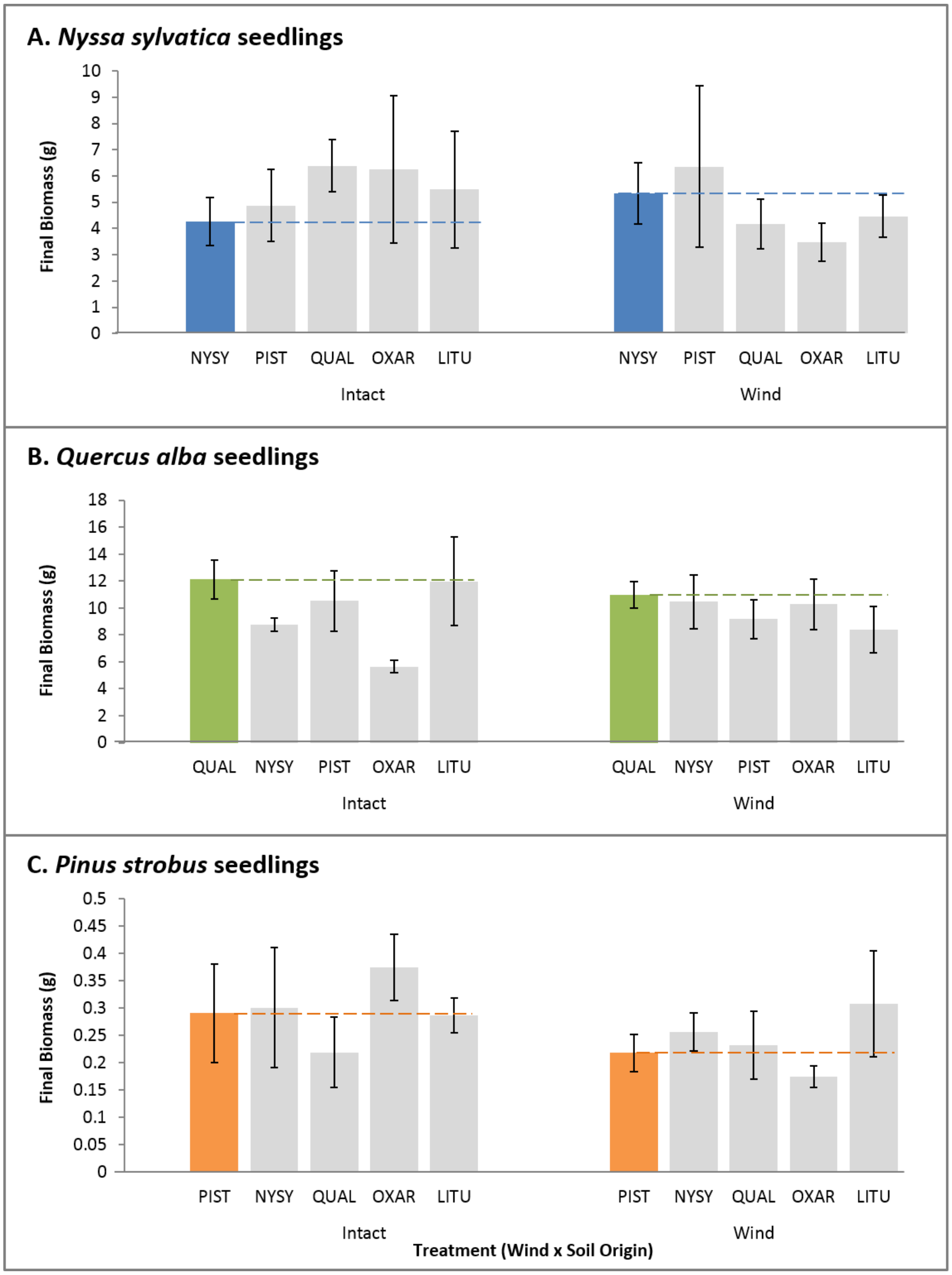
**Soil moisture was taken using a handheld TDR device with 12 cm rods. Soil temperature was taken using a handheld analog soil thermometer. For each time point, soil moisture and temperature were measured at four equidistant points (that did not include tipup mounds) and averaged together.

Appendix Table 2. Analysis of arbuscular mycorrhizal structures in *Nyssa sylvatica* root sections

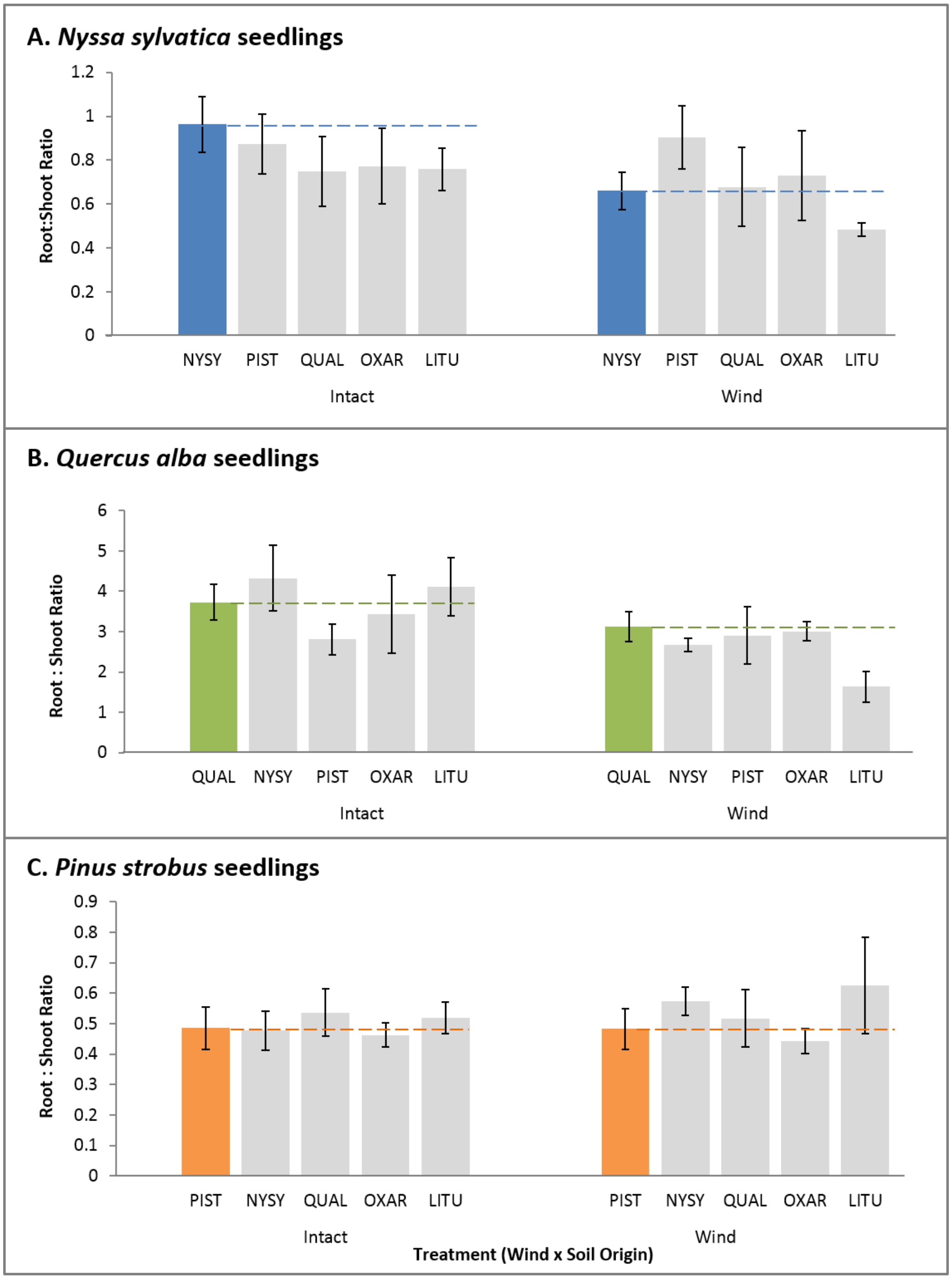
| | Hyphae | | Vesicles | | Arbuscules | | All Structures | |
|---|---|---|---|---|---|---|---|---|
| Source | F | P | F | P | F | P | F | P |
| Soil Origin | 1.53 | 0.24 | 2.06 | 0.15 | 1.57 | 0.23 | 1.53 | 0.24 |
| Wind | 0.73 | 0.40 | 0.48 | 0.50 | 0.73 | 0.40 | 0.73 | 0.40 |
| Soil Origin*Wind | 0.54 | 0.60 | 1.75 | 0.20 | 1.23 | 0.31 | 0.54 | 0.59 |
| block | 3.62 | 0.03 | 1.66 | 0.20 | 1.58 | 0.22 | 3.62 | 0.03 |

Appendix Table 3. Analysis of ectomycorrhizal colonization rate for *Quercus alba* and *Pinus strobus* seedling roots

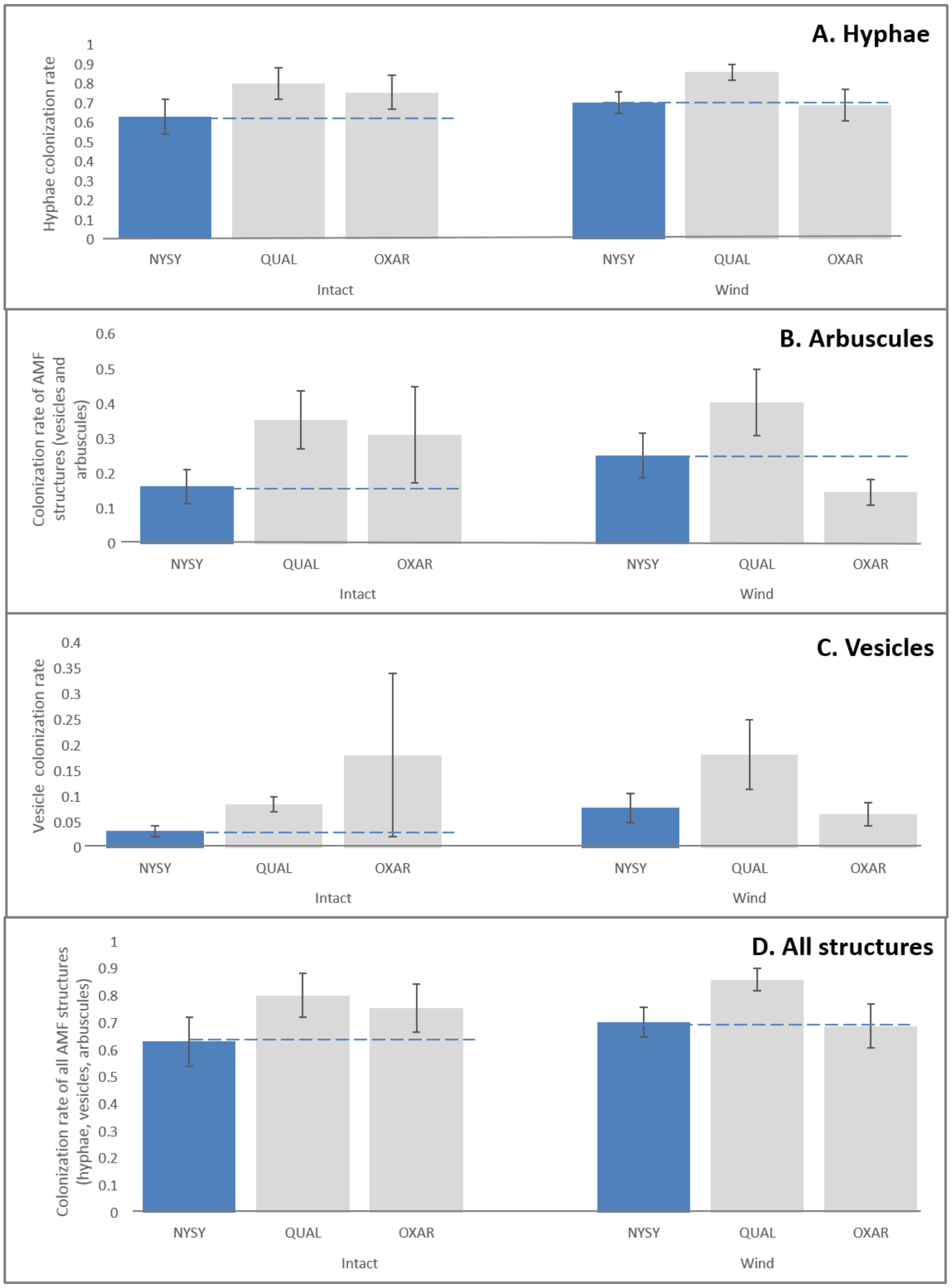
| | *Quercus alba* | | *Pinus strobus* | |
|---|---|---|---|---|
| Source | F | P | F | P |
| Soil Origin | 2.97 | 0.04 | 2.09 | 0.11 |
| Wind | 0.08 | 0.78 | 1.10 | 0.30 |
| Soil Origin*Wind | 0.97 | 0.44 | 1.62 | 0.20 |
| block | 0.49 | 0.69 | 6 | 0.65 |




Appendix Figure 1. Total biomass (g) of *Nyssa sylvatica* (**A**), *Quercus alba* (**B**), and *Pinus strobus* (**C**) seedlings after three months in the greenhouse. Soil was collected from beneath mature trees of five spp ("Soil Origin" treatment), and from intact or wind-damaged forest plots ("Wind" treatment). Species were Nyssa sylvatica (NYSY), *Pinus strobus* (PIST), *Quercus alba* (QUAL), *Oxydendrum arboreum* (OXAR), and *Liriodendron tulipifera* (LITU). (* = p <0.05 ; dots = p <0.1 )




Appendix Figure 2. Root:Shoot ratio of *Nyssa sylvatica* (**A**), *Quercus alba* (**B**), and *Pinus strobus* (**C**) seedlings after three months in the greenhouse. Soil was collected from beneath mature trees of five spp ("Soil Origin" treatment), and from intact or wind-damaged forest plots ("Wind" treatment). Species were Nyssa sylvatica (NYSY), *Pinus strobus* (PIST), *Quercus alba* (QUAL), *Oxydendrum arboreum* (OXAR), and *Liriodendron tulipifera* (LITU). (* = p <0.05 ; dots = p <0.1 )




Appendix Figure 3. Arbuscular mycorrhizal structures found in *Nyssa sylvatica* roots. For each plant, roots were dried, cleared and stained with Direct Blue. Approximately 50 root sections were scored for (A) hyphae, (B) arbuscules, and (C) vesicles. The overall colonization rate (D) combines the scores for all three structure types. *Nyssa* plants grown in *Liriodendron* and *Pinus* soil were not retained for analysis.