Effects of Fire and Drought on Ecological Processes Via Plant-Soil Interactions

by

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University Program in Ecology
Duke University

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Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University Program in Ecology in the Graduate School of Duke University

2018
ABSTRACT

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Abstract

Understanding how biotic organisms are affected by abiotic conditions and, in turn, affect the functioning of their environment is one of the most unifying goals of ecological research. Particularly in the context of a changing environment, a generalizable understanding of the factors which underpin plant functioning has crucial implications for predicting how and why ecosystems may function differently under future climate scenarios. In my dissertation, I assess the implications of two fundamental abiotic drivers - soil resource supply and disturbance regime - on community and ecosystem dynamics. I use a combination of greenhouse and field experiments to iteratively examine how responses to these drivers at the levels of individual and species scale up to influence competitive interactions, biomass regeneration, and productivity.

In chapter two, I quantify a short-lived increase in nitrogen availability following prescribed fire. In chapter three, I then test the extent to which a similar nitrogen pulse is utilized by co-occurring plant species, and relate their nitrogen uptake to regrowth when planted with a stronger or weaker competitor. This chapter demonstrates that species differ significantly in their ability to capture a pulse of nitrogen but that this has no effect on their competitive ability during resprouting. Instead, both functions are correlated in opposite directions with the same root trait, suggesting that they may exemplify opposing life history strategies. In chapter four I examine the role of nitrogen
availability on biomass regrowth in complex field communities to assess how nitrogen supply and disturbance history affect community responses to subsequent disturbances. I found that historical disturbance frequency had a much stronger impact on disturbance response than nitrogen availability, although nitrogen availability mediated the disturbance response of some species. Together these findings suggest that root traits can be used to predict competitive strategies for plants in this frequently-disturbed and pulse-driven ecosystem. If competitive strategies are optimized either following nitrogen pulses or disturbance, species abundances may fluctuate over time and under future conditions depending on the frequency of each abiotic driver. Finally, in chapter five I extend these questions to water-limited conditions: in mesocosms planted with tree saplings that form associations with either arbuscular or ectomycorrhizal fungi, I compare the resistance and recovery of belowground respiration to drought and rewetting. I find that mycorrhizal association affects respiration resistance to drought, but both mycorrhizal types fail to recover following rewetting. The association of trees with mycorrhizal fungi may, then, have implications for the drought tolerance of host tree species.
Dedication

For Richard, who inspires clarity and creativity in my life and work, and without whom this document would not exist.
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1. Introduction

Climate change is affecting the biodiversity and composition of global ecosystems. Changing precipitation patterns, rising atmospheric carbon dioxide concentrations, increasing temperature, and changing disturbance regimes are affecting biotic assemblages of terrestrial systems directly, by shifting species interactions, and indirectly, by altering biogeochemical cycles that influence plant performance. Predicting the response of terrestrial ecosystem to climate change, however, remains difficult in part because climate change factors interact with local disturbance regimes and community dynamics to cause non-linear ecological responses to climate change (Itter et al. 2017). These can include persistent legacy effects (Anderegg et al. 2015, Kaisermann et al. 2017, Wu et al. 2018) and thresholding behavior (Gutschick and BassirRad 2003, Houlton et al. 2007, Staver et al. 2011) that are difficult to anticipate with current models. These knowledge gaps impose important limitations on our ability to predict future ecosystem functioning (Matthes et al. 2016, Rollinson et al. 2017) that must be addressed in order to anticipate how ecosystems will function under future climate scenarios.

Underlying these knowledge gaps is a poor understanding of how environmental stress impacts the feedbacks between soil biogeochemistry and plant physiology (De Kauwe et al. 2014). Root traits have been proposed as a way to integrate
plant-soil interactions, and particularly to improve our understanding of soil-based ecosystem processes (Clemmensen et al. 2013, Bardgett et al. 2014, McCormack et al. 2015). Because of their dynamic functionality in response to soil conditions (Jackson et al. 1990, Fan et al. 2017) and global change factors (e.g. atmospheric CO2; Nie et al. 2013), roots traits may provide an important link between environmental drivers and ecosystem functioning (Warren et al. 2015) via their effects on population (Schroeder-Georgi et al. 2016) and community dynamics (Fort et al. 2014, Teste et al. 2014, Teste et al. 2017). As a consequence, root traits show promise in helping to explain competitive interactions (Fort et al. 2014) and to disentangle the physiological mechanisms underlying ecosystem processes (Bardgett et al. 2014).

In order to abstract from site- and species-specific root responses, current studies of root functioning rely on trait metrics that are hypothesized to correlate with whole-plant functioning (Bardgett et al. 2014, Laliberté 2016, McCormack et al. 2017). These hypotheses are derived from the ‘plant economics spectrum’ (PES), which predicts relationships between leaf-level trait syndromes and strategies of plant resource acquisition, processing, and allocation (Cornwell et al. 2008, De Deyn et al. 2008, Reich 2014, Díaz et al. 2016). Although the PES posits that these leaf trait-function relationships should extend across plant organs, these predictions are rarely tested belowground (but see Bergmann et al. 2017). Without studies evaluating the extent to which PES theory
can be extended belowground, it cannot yet be fully leveraged to predict shifts in root traits in response to environmental drivers, nor can it be applied to understand the feedbacks between plant physiology and soil biogeochemistry that underlie many ecosystem processes.

The goal of my dissertation research is to quantify the importance of plant resource acquisition strategies – as shaped by their belowground traits – on community (composition, competitive ability) and ecosystem (productivity) dynamics via their impacts on plant functioning; I do this in two ways. First, I explicitly assess the impacts of nitrogen (N) availability (quantified in chapter two) and fire disturbance on plant species (chapter three) and community (chapter four) dynamics. In order to develop hypotheses about the physiological mechanisms underlying plant responses to fire and N, I measure morphological root traits hypothesized to correspond to strategies of resource processing. Second, I compare the resistance and recovery of belowground respiration to drought between arbuscular- and ectomycorrhizal-associated plants (chapter five).

In chapter two, I quantified the effect of fire on N cycling and documented a short-term spike in inorganic N availability immediately following a prescribed burn in the longleaf pine ecosystem. Using soil cores incubated in situ, I measured nitrate (NO$_3^-$) and ammonium (NH$_4^+$) pool sizes for nine weeks throughout the growing season in
burned and unburned sites. To assess the potential for changes in microbial N cycling to account for changes to N pool sizes, I also measured net nitrification and net mineralization rates over the same time period. Finally, I compared soil $\delta^{15}$N before and after the burns to assess the potential for litter-derived ash, which should be depleted in $^{15}$N relative to soils, to account for changes to N pool sizes. I found that burned sites experienced a large spike of NH$_4^+$ immediately following the fire which could not be explained by changes to net mineralization nor by ash inputs. Instead, I proposed that a temporary slowing of plant inorganic N uptake may help to account for the observed N pulse. This chapter has been published in Biogeosciences (Ficken and Wright 2017).

In chapter three, I examined the consequences of a similar N pulse for N assimilation and competitive ability of the dominant understory species. First, using $^{15}$N as an isotopic tracer in a greenhouse study, I compared N assimilation patterns (N uptake rate and the maximum leaf N content) of four understory species when exposed either to a pulse of high N availability, or to low, background N availability. I identified one species that was a strong competitor for N when it was delivered as a pulse, and one species that was a strong competitor for N when it was under low background availability. Next, to evaluate the repercussions of these divergent N acquisition strategies on competitive ability, I grew pairs of these species and, after clipping aboveground biomass to simulate a fire, exposed them to continuous high, pulse high,
or continuous low (control) N availability and measured biomass regrowth. I found that both functions – pulsed N uptake and biomass regrowth – were correlated with the same morphological root trait, but the trait had contrasting relationships with each function. Specific root length, a measure of how long and thin roots are – was positively correlated with N capture but negatively correlated with regrowth. As a consequence, N uptake did not influence biomass regrowth, as I had expected based on predictions from the PES. This suggests that N assimilation and biomass regrowth are largely independent functions, and may constitute divergent competitive strategies. Chapter three is in review at Ecology.

In chapter four, my goal was again to examine the scaling implications of the N pulse documented in chapter two. In this chapter, I applied N to field communities exposed to frequent or infrequent fires, and measured biomass regrowth over the growing season. I compared regrowth parameters (short-term resprouting rate, maximum growing season growth rate, and maximum growing season biomass accumulation) among dominant species and among N and fire treatments. I found that fire history had a much stronger impact than N availability on the response of the individual and the community to subsequent disturbances: fire history strongly impacted resprouting, regrowth, and maximum biomass accrual of many species, while N mediated this effect only for a subset of species. Regrowth of some species was
enhanced under frequent burns, while regrowth of others was enhanced under infrequent burns. As a consequence, fire history influenced the biomass accrual of individual species, but not overall productivity. Together, findings from chapters two through four suggest that, despite interspecific physiological differences, N assimilation has relatively little impact on competitive dynamics and biomass regeneration. My results suggest that root traits are important in predicting both the N acquisition strategy and the disturbance response of individual species. N uptake from a pulse and rapid resprouting may constitute alternative and mutually exclusive strategies in this frequently-disturbed, pulse-driven ecosystem. As a consequence, the response at the community-level is highly dependent on the response of the constituent species. Overall, productivity during fire years may be driven by the competitive resprouting species, whereas productivity during non-fire years may be driven by N pulse competitors.

In chapter five, I extended my research on resource acquisition to a different system. In this chapter, I imposed an extreme drought on two tree saplings with contrasting mycorrhizal associations. Plant associations with mycorrhizal fungi are often viewed as strategies to enhance plant nutrient acquisition (Phillips et al. 2013), and they may also provide important benefits for water acquisition (Brunner et al. 2015, Hagedorn et al. 2016). I compared the belowground respiration of maple (associated with arbuscular mycorrhizal fungi) and oak (associated with ectomycorrhizal fungi)
saplings before, during, and after an experimental drought. In this way, I compared the resistance and recovery of roots, mycorrhizal fungi, and free-living microbes from each mycorrhizal system. I found that oak mesocosms were less resistant to drought than maple mesocosms, and that respiration from both mesocosms had low recovery following rewetting. In contrast to respiration, potential enzyme activity of C, N, and phosphorus degrading enzymes exhibited high recovery following rewetting. Findings from this chapter suggest that mycorrhizal association may be an important trait to understand the drought-response of tree saplings.

This goal of my dissertation is to understand the implications of plant resource acquisition strategies on community- and ecosystem-level processes. I found that fires in longleaf pine forests resulted in large pulses of plant-available N, and that co-occurring understory species in this system differed in their ability to assimilate this pulse. Despite the expected importance of N availability for plant productivity in temperate forests, I found that plant performance in greenhouse mesocosms was not impacted by N supply. Instead, my analyses of root traits suggested that belowground carbon storage and remobilization were more important for resprouting potential. These findings were confirmed in complex field communities, where I found that an individual’s exposure to previous fire history was a much more important driver of resprouting patterns than current N availability. Together, my work suggests that nutrient availability plays a
limited role in plant performance following fire disturbance. More work is needed to understand the relationship between nutrient availability and plant performance in other systems, and following other types of disturbance.
2. Contributions of microbial activity and ash deposition to post-fire nitrogen availability in a pine savanna

2.1 Introduction

Temporal heterogeneity in resource supply is ubiquitous across ecosystems (Schimel and Bennett 2004, James and Richards 2006, Archer et al. 2014) and such resource pulses can be important if they contribute disproportionately to the overall resource budget of an ecosystem (McClain et al. 2003). Because they vary in magnitude and frequency, nutrient pulses across ecosystems differ in their potential to influence community and ecosystem dynamics. Despite compelling modelling-based evidence suggesting that nutrient pulses can influence ecological dynamics including species richness (Tilman and Pacala 1993), physiological nutrient uptake constraints (Bonachela et al. 2011), and stoichiometric coupling (Appling and Heffernan 2014), it can be difficult to predict when and where temporal nutrient heterogeneity will occur. This uncertainty makes it difficult to assess the conditions under which temporal heterogeneity in nutrient supply might influence community- or ecosystem-level functioning.

Nutrient dynamics in pyrogenic systems may be especially variable in time because fire is a major disturbance that influences nitrogen (N) availability (Wan et al. 2001). There is a consensus that, across ecosystems, pulses of soil N, in particular ammonium (NH$_4^+$), occur in response to fire (Wan et al. 2001, Huber et al. 2013). However the duration and magnitude of these pulses vary strongly by fuel composition,
and consequently among forest and fire types (Wan et al. 2001). In northern conifer forests, stand-replacing fires can result in increased soil NH$_4^+$ concentrations detectable more than one year following the burn (Smithwick et al. 2005b, Turner et al. 2007). In contrast, in pine forests of the southeastern US (e.g. longleaf pine savannas), where prescribed fires only consume the understory vegetation, NH$_4^+$ concentrations following fires are more variable; some studies have documented no change in soil N pools following fire (Christensen 1977, Richter et al. 1982), while others have documented immediate increases (2 days) that quickly dissipate (Lavoie et al. 1992). This suggests a need for localized studies with temporal data resolution appropriate for that ecosystem to evaluate the mechanism behind changes in soil N availability following fire.

Pine savannas in the southeastern US are often managed with prescribed fires in the absence of recurring natural fires to maintain habitat for endangered species (Sorrie et al. 2006) and nutrient losses or pool redistributions from these fires can be substantial (Wilson et al. 1999, Wilson et al. 2002, Boring et al. 2004). In addition to large quantities of carbon (C) released through fuel consumption (Boring et al. 2004), prescribed fires can release up to 50% of the phosphorus (P) and up to 75% of the N that was stored in the understory biomass (Wan et al. 2001, Carter and Foster 2004). These nutrients can be lost through volatization, or redistributed as ash in low intensity fires. Despite the ecosystem-level nutrient losses associated with fires, short-term pulses of increased N
availability in the soil are also observed following prescribed forest fires across vegetation types (Wan et al. 2001, Certini 2005, Smithwick et al. 2005a, Schafer and Mack 2010) including in longleaf pine (*Pinus palustris* Mill.) savannas (Boring et al. 2004). In longleaf pine savannas, because these nutrient pulses occur during a period of rapid post-fire plant regrowth, they may influence successional patterns (Shenoy et al. 2013), plant diversity, and ecosystem productivity.

The mechanisms driving these ephemeral increases in N availability following fire remain poorly resolved, and so it remains difficult to predict how a specific fire will influence local N availability and turnover. Fire can decrease N availability if N is volatized and lost from the system in high intensity fires (Certini 2005, Lavoie et al. 2010). On the other hand, fire can increase N availability if it spurs microbial turnover of organic matter (Wilson et al. 2002, Certini 2005), returns nutrient-rich ash to the system (Boring et al. 2004), or decreases the vegetation demand for N. Short term increases in soil N availability may not translate to longer-term ecosystem retention if N is lost through leaching or as gaseous products during turnover.

In addition to difficulties associated with assessing the relative importance of each mechanism influencing post-fire N availability, logistical challenges remain to accurately measure N availability. First, changes to soil N availability are likely to occur rapidly following fire. Since microbial turnover occurs on a span of hours to days, and
plants in fire-adapted systems begin re-sprouting within days to weeks, changes to N availability in pyrogenic systems are also likely to be ephemeral. Previous studies of post-fire N dynamics in longleaf pine savannas have relied on monthly or less-frequent soil samples (Wilson et al. 2002, Lavoie et al. 2010), but this sampling resolution may be too coarse if changes in N dynamics occur rapidly following fire, or are transient. Secondly, net N cycling rates are often calculated as the difference in pool size between two time points. When measured in the field, repeated sampling of the same soil core would control for spatial heterogeneity in starting conditions, but would likely distort estimates of N dynamics because soil disturbance can increase rates of C mineralization and microbial respiration. Instead, to avoid disturbance associated with repeated sampling of the same core, nutrients are assumed to be distributed homogeneously in a small sampling area. Thus, it is assumed that cores collected in close proximity to each other are comparable, and can be considered replicates. However, nutrient pool sizes can vary by orders of magnitude within a meter (Jackson and Caldwell 1993), and so these assumptions, while practical, are problematic and often invalid. As such, field estimates of net cycling rates calculated from the difference between two nearby cores may be influenced by the idiosyncrasies of N spatial heterogeneity, and may not accurately represent local or larger-scale N dynamics. Without using expensive tracers, field-based sampling protocols to estimate net nutrient cycling remain imperfect and
researchers must collect extensive soil replicates to overcome the issues associated with environmental heterogeneity.

In this study, our broad aim was to evaluate alternative mechanisms driving post-fire changes in N availability while addressing the above mentioned methodological and analytical challenges to estimating net cycling rates. We measured soil N status every week for nine weeks during the 2014 growing season in five longleaf pine savannas sites in North Carolina. Our study is the first that we know of to provide high-resolution temporal (i.e. weekly) data on the effects of prescribed fire on soil N dynamics in longleaf pine savannas. We then used a Bayesian hierarchical linear model to account for heterogeneous in situ N availability. The goals of our study were (1) to evaluate the short-term effects of fire on soil inorganic N availability and (2) evaluate whether changes in N pool sizes following fire could be attributed to changes in net microbial cycling rates or ash deposition.

2.2 Methods

2.2.1 Study site and fire characteristics

Our study was carried out in a longleaf pine savanna on Fort Bragg Military Reservation (35.1391°N 78.9991°W) near Fayetteville, NC, USA. This area is characterized by deep, sandy and sandy loam soils from the Candor and Blaney series, which lack an O horizon. Mean monthly temperature ranges from 6.9 - 26.0 °C, and
mean annual precipitation is 127.5 cm. This area includes numerous microtopographical gradients represented by numerous low riparian wetlands in an upland matrix. The uplands are well-drained and savanna-like, with an open canopy of longleaf pine (*Pinus palustris* Mill.) and an understory dominated by wiregrass (*Aristida stricta* Michx.; Sorrie et al. 2006). Several hardwood species and *Pinus serotina* replace *P. palustris* in the wetlands lining streambeds; in these areas, the soil is often saturated and the ground covered with *Sphagnum* L. sp. Separating the uplands from the wetlands, the ecotones have dense, shrubby vegetation dominated by Ericaceous species, including *Vaccinium formosum* Andrews, *V. fuscatum* Aiton, *V. tenellum* Aiton, *Lyonia lucida* (Lam.) K. Koch, *L. mariana* (L.) D. Don, and *Gaylussacia frondosa* (L.) Torr. and Gray ex. Torr.

Since the 1980s, prescribed burns have been used as a management tool to maintain the longleaf pine savannas on the reservation; since the mid-1990s, these burns have occurred on 3-year rotations to promote longleaf pine regeneration and maintain habitat for rare and endangered species (Sorrie et al. 2006). Prescribed burns are set primarily during the growing seasons, when wildfires also occur. To maintain control of the prescribed burns, they are performed as low-intensity backing fires (ignited along a road or other fire break and allowed to burn into the wind). This fire return interval mimics the historical fire return interval of 1 to 3 years (Frost 1998), with burns occurring on average every 2.2 years (Stambaugh et al. 2011). Fort Bragg is composed of burn
parcels (hereafter “sites”) with independent burn histories. Permanent vegetation sampling transects spanning the topographic gradient have been maintained in 32 sites since 2011; the burn regime in the majority of these sites has been experimentally altered, with a subset of sites being maintained in 3-year burn intervals (Ames et al. 2015).

From these sites, we selected three sites scheduled to burn in 2014, and three not schedule to burn in 2014 for use in a before-after-control-impact experiment. To avoid any artefacts associated with different historical burn characteristics (e.g. historical burns occurring in wetter or dryer years; historical burn intensity and frequency), we limited the number of burned sites to those with similar burn histories (i.e. all on the same 3-year burn rotation). However, one site not scheduled to burn until 2016 experienced a wildfire in July 2014, and another site scheduled to burn did not. The site that burned prematurely due to a wildfire was grouped with other burned sites, despite its shortened fire return interval (one year) relative to the other burned sites (three years). Previous work has found no significant difference in vegetation cover after 40 years of management with either a 2- or 3-year burn interval (Brockway and Lewis 1997); because biomass is a strong determinant of fire intensity, we did not anticipate that a site experiencing a shortened burn regime for one year would have substantial effects on fire dynamics. The site that failed to burn was considerably different from the
remaining sites (% soil moisture and % soil organic matter were both more than double that of the other sites), and as such was dropped from further analyses. Thus, we were left with 5 study sites: B1, B2, B3 experienced burns in 2014; C1 and C2 were control sites that remained unburned. Our study sites, renamed here for clarity, correspond to sites 3, 11 (wildfire site), and 15b (B1-3), and 9 and 32 (C1 and C2) described in Ames et al. (2015). At each site, we established a 1x1 m sampling area approximately 5 m upslope of the ecotone. This topographic location was chosen to minimize the effects of extremely well-drained, hydrologically disconnected (as found in the uplands) or saturated, anoxic (as found in the wetlands) soils on microbial processing. At the onset of the experiment, all vegetation within sampling plots was identified to species and the percent cover was estimated.

Burns occurred in treatment sites B1, B2 (wildfire), B3 on 4 July, 9 July, and 7 July 2014, respectively (Julian days 185, 190, and 188). Using metal tags marked with Tempilaq temperature-sensitive paint (Air Liquide America 296 Corporation, South Plainfield, NJ, USA), we collected data on aboveground fire temperature at B1 (6 tags) and B3 (8 tags). Tags were installed on metal polls approximately 50 cm above the soil surface. They were installed surrounding our plots approximately 3 m apart from each other. We did not collect fire temperature data at B2 because it was not initially scheduled to burn.
2.2.2 Soil analyses

From May 30 through July 25, 2014, soil cores were collected weekly (nine weeks) from each site for pool size measurements (Figure 1). We collected pool size cores for a minimum of three weeks after a prescribed burn. As such, our site-specific sampling allowed us to collect data before and after burns and detect any immediate changes in N concentration in response to the burn. Each week, three cores (each 5 cm diameter x 12 cm deep and adjacent (~10 cm) to each other) were randomly collected from each site, for a total of 27 cores collected for pool size measurements at each site over the nine-week sampling period. (In burned sites, the number of cores collected prior to versus following prescribed fires differed between sites depending on when the burns occurred. In B1, N=15 cores were collected prior to burns and N=12 cores were collected following prescribed burns. In B2 and B3, N=18 cores were collected prior to burns and N=9 cores were collected following prescribed burns.) These cores were used to compare the pool sizes of nitrate ($\text{NO}_3^-$) and $\text{NH}_4^+$ during the growing season (Figure 1). After collection, all cores were stored on ice, immediately transported back to the laboratory, and kept at 4 °C until they were analyzed for inorganic N, soil moisture, soil organic matter, pH, and $\delta^{15}$N. All cores were homogenized by passing through a 2 mm sieve. Frequent fires in this ecosystem consume aboveground vegetation and litter, preventing the development of an O horizon in these soils (Boring et al. 2004). As such,
Siev ing removed coarse root fragments, rather than partially decomposed organic matter. Subsamples (~10 g) from each core were extracted within 48 hours of collection with 2 M KCl for inorganic N concentrations. The samples were shaken for 30 minutes, centrifuged, and the extract was then filtered out and stored frozen until analysis on a Lachat QuikChem 8500. Additional soil subsamples were oven dried for gravimetric soil moisture analysis, and combusted at 450 °C to measure organic matter content. Finally, we measured soil pH in 2:1 dH₂O:soil ratios with a bench top pH probe.

Figure 1: Schematic illustrating the sampling design across nine weeks during the growing season. Each week, two sets of three replicate cores were installed at each study site. One set was immediately removed for pool size estimates; the second set incubated in situ for one week to estimate net cycling rates.

To measure net cycling rates, we also installed three PVC collars (5 cm internal diameter x 12 cm deep) in each site every week (N=27 cycling rate cores collected at each
site over nine-week sampling period; the number of cores collected prior to versus following the prescribed burns differed between sites depending on when burns occurred, as above). These collars were installed adjacent to the soil cores collected for pool size measures (Figure 1), and were incubated in situ for one week, after which time they were collected and analyzed as above for inorganic N pool sizes, soil moisture, soil organic matter, and pH. After allowing for error in the initial pool size of the incubated soil core (see below for model details), net nitrogen cycling rates were calculated based on the difference in extractable NO\textsubscript{3} and NH\textsubscript{4} in the incubated cores and the unincubated cores. That is, while traditional methods assume that the N pool sizes in the initial and incubated soil cores are equivalent, our analyses calculated net cycling rates allowing for differences in initial conditions between the two cores.

### 2.2.3 $\delta^{15}$N analysis and mixing models

To assess plant-derived ash inputs to soil N pools after fire, we analyzed soils from a subsample of time points for $\delta^{15}$N. Plants generally discriminate against $^{15}$N in favor of $^{14}$N uptake (Hogberg 1997, Craine et al. 2015), and, as such, vegetation tends to be depleted in $^{15}$N relative to soil. If appreciable plant-derived N was deposited on burned sites as ash, we expected to see a decrease in $\delta^{15}$N while observing an increase in N pool size. Although ash is deposited on surficial soils, heavy rains occur frequently during June-August in this region of North Carolina (11.5, 14.8, and 11.5 cm,
respectively; State Climate Office of NC), and the well-drained, sandy soils could leach nutrients through the soil profile. Moreover, because plants begin resprouting within days of a fire (personal observation), we did not want to discount the potential for nutrients to be redistributed by roots. As a consequence of our uncertainty regarding the vertical distribution of deposited $^{15}$N, we subsampled the full soil cores (0-12 cm) for $^{15}$N analyses. The enrichment of the sample in $^{15}$N is reported on a per mille basis (%$\text{o}$) and was calculated as follows:

$$
\delta^{15}\text{N (‰)} = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000
$$

(1)

where $R_{\text{sample}}$ is the ratio of $^{15}$N:$^{14}$N in the sample, and $R_{\text{standard}} = 0.0036$, the ratio in atmospheric $N_2$.

We subsampled cores collected at each site for pool size estimates from the two sampling weeks pre-burn and the two sampling weeks post-burn, for a total of four consecutive sampling weeks surrounding each burn (for B1-3, N=6 unburned and N=6 burned samples; for C1 and C2, N=12 samples). For unburned sites, we subsampled $\delta^{15}$N for four weeks corresponding to the same consecutive weeks surrounding the burn dates in burned sites (hereafter burn season). For example, site B1 burned on July 3, 2014 corresponding to our sixth sampling week. For this site, and for the unburned site C1, we therefore measured $\delta^{15}$N from soil subsamples in the 4$^{th}$-7$^{th}$ sampling weeks. Because our sites were not truly paired, we chose time points for $\delta^{15}$N analyses in the unburned
sites based on the burn dates of the closest burn site. In this way, we allowed ourselves
to detect any ash drifting between sites. Soils were oven dried at 40°C until a constant
weight, then ground finely, encapsulated in tin capsules, and combusted on a Carlo-
Erba Elemental Analyzer coupled to a mass spectrometer at the Duke Environment
Isotope Laboratory.

We used two end-member mixing models to estimate the mass of ash-N
deposited onto the system. We used δ^{15}N and N concentrations from pre-burn soil, and
published δ^{15}N signatures of ash (-0.81 δ15N; Huber et al. 2013) as the end-members, and
post-burn soil δ^{15}N and N concentrations as the resulting mixture from the two end-
members. We solved for the mass of ash-N needed to be deposited in order to account
for the observed shift in soil δ^{15}N signature.

To assess whether ash inputs could be detected in post-burn soil cores using the
natural abundance of ^{15}N, we also calculated the mass N needed to be deposited to
observe a shift in soil isotopic signature of the minimum external precision (0.1‰ δ^{15}N at
1 standard deviation).

### 2.2.4 Model development and statistical analyses

#### 2.2.4.1 Pool sizes

To assess the spatial distribution of nutrient (NO$_3^-$ and NH$_4^+$) and soil organic
matter (SOM) availability, we calculated the coefficient of variation (CV) for each site
prior to prescribed burns. To understand how fire and soil variables affect N pool sizes, we fitted a Bayesian hierarchical linear model; this is akin to a multiple regression that also allows for variability in the relationship between true soil N pool sizes ($\mu$) and measured pool sizes ($\gamma$) which might occur, for example, through analytical error. Any effect of soil environmental conditions on N pool sizes would occur on $\mu$, not $\gamma$. Each core was modelled independently, and we accounted for site blocking effects by including random intercepts for each site. For core $i = 1…n$ at site $j = 1…5$, observed N pool size (NH$_4^+$ or NO$_3^-$) was modelled as a function of random site effects, percent soil moisture (SM), SOM, soil pH, and the number of days since the previous burn, days since fire (DSF) as follows

$$y_{0ij} \sim N(\mu_{0ij}, \sigma^2)$$  \hspace{1cm} (2a)

$$\mu_{0ij} = \beta_{0ij} + \beta_{1i}SM_i + \beta_{2i}SOM_i + \beta_{3i}pH_i + \beta_{4i}DSF^{-1}_i$$  \hspace{1cm} (2b)

$$\sigma \sim \text{unif}(0,100)$$  \hspace{1cm} (2c)

For NO$_3^-$, we added an additional predictive parameter, $\beta_{5i}NH_4^+_{i}$ to allow for NO$_3^-$ concentrations to additionally vary with nitrification substrate (NH$_4^+$) availability. We expected the effects of a burn to diminish with time, so we transformed DSF to DSF$^{-1}$, so that as DSF increased, DSF$^{-1}$ $\to 0$. Full models included all main effects and no interactions.
2.2.4.2 Cycling rates

We built a hierarchical state-space model within a Bayesian framework to understand how fire and soil variables affected N cycling rates. As in the models of N pool sizes above, our cycling rate models allowed for variation in the relationship between true ($\mu$) and measured ($z$) cycling rates, modeled below as $\tau$. In addition, we also allowed for error associated with the assumption that the N concentrations in initial cores ($y_0$; as from equation 2a) were equal to the initial concentrations of the incubating cores ($y_1$). By including these errors into our model, we essentially relaxed the assumption that paired cores (un-incubated and incubated cores) were true replicates and had equal initial N concentration and edaphic conditions (SOM, pH, SM). We removed four core pairs (of 135) that exhibited NH$_4^+$ or NO$_3^-$ concentrations below the detection limit in the initial concentration. For core $i = 1...n$ at each site $j = 1...5$, cycling rate was modelled as

$z_{i,j} \sim N(\mu_{i,j}, \tau)$ \hspace{1cm} (3a)

$\mu_{i,j} = y_{1,i,j} - y_{0,i,j} = \beta_{1,i} SM_i + \beta_{2,i} SOM_i + \beta_{3,i} pH_i + \beta_{4,i} DSF^{-1}_i + \beta_{5,i} D_i + \beta_{6,i} y_{0,i,j}$ \hspace{1cm} (3b)

$\tau = \sigma^{-2}$ \hspace{1cm} (3c)

$\sigma \sim \text{unif}(0,100)$ \hspace{1cm} (3d)
To model cycling rates, we included incubation length (D, in days) and initial NH₄⁺ or NO₃⁻ concentration as additional predictors. Again, full models included all main effects and no interactions.

Prior to all analyses, we removed two cores from B1 (burned) and two from C2 that had pool size values below the analytical detection limit (four of 135 cores). All models were built with the rjags package (version 3.15) in R version 3.2.1 (Team 2011). All predictors, including random site site effects, were modelled with uninformative normally distributed priors. Models were run with three chains and run with 200,000 iterations after a 100,000-iteration burn in period. Convergence was tested by examining chain density and trace plots to ensure proper chain mixing, and by calculating the Gelman-Rubin diagnostics using the gelman.diag() in the coda package (version 0.18.1) to ensure scale reduction factors for each predictor was <1.05. All values are reported with 95% credible intervals (CI).

2.3 Results

2.3.1 Site conditions and fire characteristics

Although plant community composition varied, shrubs *Gaylussacia frondosa* (L.) Torr. & A. Gray ex Torr., *Clethra alnifolia* L., and the native bamboo *Arudinaria tecta* (Walter) Muhl. were dominant in all of our study sites. Study sites were dry, low in organic matter, and acidic (Figure 2). Prescribed burns in all three sites thoroughly consumed all or most of the aboveground biomass. Aboveground understory vegetation
in B1 and B3 was completely consumed. In B2 some scorched leaves remained on the woody vegetation, but the herbaceous understory species were completely consumed. Fire temperatures were similar between B1 and B3: average maximum fire temperature at B1 was 612 °C ±18 and at B3 was 635 °C ±18. Fire temperature was not measured in B2.

![Figure 2: Mean soil conditions prior to and following burns at each focal site. Mean soil moisture (a), pH (b), soil organic matter (c) and total inorganic N (i.e. NH4+ and NO3-; d). Total inorganic N is reported per gram of dry soil (gds). Values are reported ±95% CI.](image)

2.3.2 Pool sizes

To assess the fine-scale spatial variability in NH$_4^+$ and NO$_3^-$ concentrations, we compared the CV of NO$_3^-$ and NH$_4^+$ pool sizes within each site prior to prescribed burns. Both NO$_3^-$ and NH$_4^+$ pool sizes were highly spatially heterogeneous, despite similar mean concentrations across sites prior to prescribed burns (Figures 3-4). Spatial variability in NO$_3^-$ pool size was high within each site, but each site exhibited similar variation in NO$_3^-$ pool size. CV-NO$_3^-$ ranged from 42.3 in B1 to 57.6 in C2. NH$_4^+$ pool sizes were also highly variable across sites, and there was a considerable range of the spatial heterogeneity across sites. CV-NH$_4^+$ ranged from 57.0 in B2 to 114.2 in C2.

![Histogram of soil NH$_4^+$ concentrations prior to prescribed burns. Solid vertical lines indicate the median concentration across all sites; dashed vertical lines indicate the site-specific median concentration.](image-url)
In the first week of sampling, initial pool sizes of inorganic N were similar between sites. Over the pre-burn season, sites had greater NO₃⁻ than NH₄⁺ availability (3.06 ±0.16 µg NO₃⁻ per gram of dry soil [gds⁻¹] and 0.86 ±0.07 µg NH₄⁺ gds⁻¹). However, the ratio of NH₄⁺ : NO₃⁻ increased following prescribed burns and sites B2 and B3 both experienced a shift in the dominant inorganic N form to NH₄⁺ immediately after a burn.

There were observable increases in NH₄⁺ pool sizes immediately after a burn relative to the same time points in unburned control sites and time points in burned sites immediately prior to the burn (Figure 5). Three days post-burn in Site B1, NH₄⁺ had increased from 0.83 ±0.15 µg NH₄⁺ gds⁻¹ in the pre-burn season to 6.10 ±1.08 µg NH₄⁺ gds⁻¹.
One day post-burn in Site B2, NH$_4^+$ had increased from 0.91 ±0.12 µg NH$_4^+$ gds$^{-1}$ in the pre-burn season to 7.79 ±2.09 µg NH$_4^+$ gds$^{-1}$. The pattern of NH$_4^+$ pool size change in Site B3 was qualitatively different than changes observed in Sites B1 and B2. In Site B3, there was an approximate exponential increase in NH$_4^+$ pool size that plateaued, but did not diminish, by the end of our sampling, more than 3 weeks post-burn. Three days following a burn in Site B3, NH$_4^+$ pool size had increased from 0.84 ±0.16 µg NH$_4^+$ gds$^{-1}$ in the pre-burn season to 21.9 ±6.31 µg NH$_4^+$ gds$^{-1}$. Site effects ($\beta_0$) had the strongest overall effect on NH$_4^+$ pool sizes, although this effect was not significant at C1. In burned sites B1-3, $\beta_0$ was -26.9 (95% credible interval (CI) = -46.11– -6.57), -22.03 (95% CI = -38.91 – -4.78), and -24.16 (95% CI = -41.78– -6.12), respectively. At C2, $\beta_0$ was -23.23 (95% CI = -40.38 – -5.69). After random site effects, pool sizes of NH$_4^+$ were most strongly correlated with days since fire (DSF; $\beta_{DSF} = 12.50$, 95% credible interval (CI) = 5.34-19.66; Figure 6) and pH ($\beta_{pH} = 6.16$, 95% CI = 1.79-10.46; Figure 6). Because we fit our pool size models to the inverse of DSF (i.e. DSF$^{-1}$; see Methods), the positive correlation between NH$_4^+$ and DSF indicates decreasing pool sizes as time since fire lengthens. Pool sizes of NH$_4^+$ were larger for less acidic soils and in recently burned soils. Pool sizes of NH$_4^+$ were slightly greater in soils with more organic matter ($\beta_{SOM} = 0.2$, 95% CI = 0.01-0.30), but did not vary with soil moisture ($\beta_{SM} = -0.11$, 95% CI = -0.30-0.07; Figure 6).
Figure 5: An increase in NH$_4^+$, but not NO$_3^-$, concentration occurred following prescribed fires in burned sites. Changes in pool sizes of (a) NH$_4^+$ in burned sites, (b) NH$_4^+$ in control sites, (c) NO$_3^-$ in burned sites, and (d) NO$_3^-$ in control sites. The x-axis for A and C depicts time (in days) centered on the date of burn; days immediately before the burn are negative x-values while days immediately following a burn are positive; the burn date is at 0 and is demarcated with a vertical dotted line. The x-axis for sites B and D depicts time in Julian Days. Prescribed burns in B1, B2, and B3 occurred on Julian Days 185, 190, and 188, respectively. Pool sizes are reported in µg N per gram dry soil (gds). Error bars (±95% CI) for individual time points may be obscured by the point.
Figure 6: Days Since Fire (DSF) was the strongest predictor of NH$_4^+$ availability after site effects. Parameters are days since fire (DSF), soil pH, soil moisture (SM), soil organic matter (SOM), and substrate availability (i.e. NH$_4^+$ reported per gram of dry soil, gds). Thin black lines show 95% CI and thick lines show 50% CI. CI may be obscured by the mean point. For visual clarity, posterior distributions of site effects are not included here. See text for details.

In contrast to observed pulses of NH$_4^+$ availability following fire, we did not find strong fire-associated increases in NO$_3^-$ pool size (Figure 5). NO$_3^-$ availability ranged from 0.1 to 8.56 µg NO$_3^-$ gds$^{-1}$, and on average was 2.85 ±0.15 µg NO$_3^-$ gds$^{-1}$. Site effects on NO$_3^-$ pool sizes much weaker than for NH$_4^+$ and were only significant at B1 ($\beta_0 = -4.81$, 95% CI = -9.46 – -0.08). DSF was not related to NO$_3^-$ pool sizes ($\beta_{\text{DSF}} = 0.44$, 95% CI = -1.22 - 2.10; Figure 6). Pool sizes of NO$_3^-$ varied with soil organic matter content ($\beta_{\text{SOM}} = 0.03$, 95% CI = -0.01 – 0.06).
95% CI = 0.01-0.07), NH$_4^+$ pool size ($\beta_{\text{NH}_4^+} = 0.06$, 95% CI = 0.02-0.10), and pH ($\beta_{\text{pH}} = 1.46$, 95% CI = 0.39-2.49; Figure 6).

We also examined whether there was a long-term legacy of fire detectable in sites that had not experienced prescribed burns in that growing season (i.e. across pre-burn time points in burned sites, and all time points from unburned sites). In these areas that had not experienced recent fires, DSF had no effect on NH$_4^+$ ($\beta_{\text{DSF}} = 0.70$, 95% CI = -60.54-61.46) or NO$_3^-$ availability ($\beta_{\text{DSF}} = 1.79$, 95% CI = -60.31-63.75). Soil moisture ($\beta_{\text{SM}} = 1.55$, 95% CI = 0.65-2.45) and, to lesser extents, NH$_4^+$ availability ($\beta_{\text{NH}_4^+} = 0.19$, 95% CI = 0.45-0.72) and soil organic matter ($\beta_{\text{SOM}} = 0.03$, 95% CI = 0.01-0.07) were positively correlated with NO$_3^-$ pool sizes.

### 2.3.3 Cycling rates

Net N cycling rates were generally low and temporally heterogeneous across the growing season (Figure 7). They varied between net production and net consumption between sampling points (Figure 7). Across the whole growing season, net mineralization in unburned sites was 0.19 (±0.16) µg gds$^{-1}$ day$^{-1}$; in burned sites, it was 0.26 (±0.05) µg gds$^{-1}$ day$^{-1}$. Net mineralization rates were appreciably more variable the week following a burn, but patterns between sites were inconsistent (Figure 7). For the first week following burns in sites B1-3, net mineralization rates were -1.72 (±0.32), 0.31 (±0.10), and 2.54 (±0.46) µg gds$^{-1}$ day$^{-1}$ respectively. Despite the change in net
mineralization pattern following a burn, there was no consistent effect of DSF on net mineralization rate (Figure 8; $\beta_{DSF} = 0.34$, 95% CI = -0.50-1.19). Of the measured edaphic variables, net mineralization rates were correlated only slightly with soil moisture (Figure 8; $\beta_{SM} = 0.31$, 95% CI = 0.14-0.48).

Net nitrification rates were temporally heterogeneous throughout the full growing season, but were not appreciably more variable immediately following burns. Net nitrification rates were very low across the growing season in burned (-0.08 ±0.05 µg gds$^{-1}$ day$^{-1}$) and unburned sites (-0.04 ±0.04 µg gds$^{-1}$ day$^{-1}$; Figure 7). Measured edaphic parameters were poorly correlated with observed net nitrification rates, although there was a slight positive relationship between net nitrification rates and soil moisture (Figure 8; $\beta_{SM} = 0.07$, 95% CI = 0.03-0.11).
Figure 7: Changes in net mineralization rates cannot account for observed pulse of NH$_4^+$ after prescribed fire. Changes in (a) net mineralization in burned sites, (b) net mineralization in unburned sites, (c) net nitrification in burned sites and (d) net nitrification in unburned sites. The x-axis for A and C depicts time (in days) centered on the date of burn; days immediately before the burn are negative x-values while days immediately following a burn are positive; the burn date is at 0 and is demarcated with a vertical dotted line. The x-axis for sites B and D depicts time in Julian Days. Prescribed burns in B1, B2, and B3 occurred on Julian Days 185, 190, and 188, respectively. Cycling rates are reported in µg N per gram dry soil (gds) per week. Error bars (±95% CI) for individual time points may be obscured by the point.
Figure 8: Soil characteristics were poor predictors of net cycling rates. Estimates of parameter posterior mean effects with bars showing 95% credible intervals (CI) for net mineralization and net nitrification rates. Parameters are days since fire (DSF), soil pH, soil moisture (SM), soil organic matter (SOM), the incubation length (Inc.), and initial substrate availability (S0) per gram of dry soil (gds). For visual clarity, posterior distributions of site effects are not included here. See text for details.

2.3.4 Total soil $\delta^{15}$N and ash deposition

Soil N concentration was relatively stable throughout the burn season, and was similar between burned (0.35%N ±0.09) and unburned sites (0.38%N ±0.07; Table 1).

Mean total soil N varied between sites (Table 1; Figure 9). On average, B2 had the lowest total soil N content (0.18%N ±0.02), and total soil N ranged from 0.08% at C1, to 0.98% at
Across all burned and unburned time points, confidence intervals overlapped between burned and unburned conditions at each site, indicating no persistent change in total N over the full growing season (Table 1).

Figure 9: Prescribed burns resulted in an increase in the relative availability of inorganic N, but no change in total soil N. Panel (a) shows the mean percent of inorganic N (i.e. NH$_4^+$ and NO$_3^-$) in soil under in unburned and burned periods. Panel (b) shows the mean total N soil content in burned and unburned periods, reported per gram of dry soil (gds). Error bars are ±95% CI.
Table 1: Mean soil inorganic N pool sizes, $\delta^{15}$N values, and total N for each study site, as well as estimates of the mass of ash-derived N needed to be deposited to account for changes to soil $\delta^{15}$N. Inorganic N pool sizes are reported per gram of dry soil (gds). All values are reported ±95% CI. Sample sizes for inorganic N pool sizes differ between sites depending on when the prescribed burns occurred; see Methods for details on sample sizes. Soil %N and $\delta$ 15N values were collected on a subset of time points; sample sizes for these variables are N=6 for burned site means, and N=12 for control (unburned) site means. Ash-derived N values are the estimated masses of N needed to be deposited onto each site to result in the observed post-burn $\delta$15N. Note that Christensen (1977) measured 1.15 ±0.49 g N m$^{-2}$ deposited in ash fall in fire in a longleaf pine savannas.

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<th>Burned Sites</th>
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<td>B1</td>
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<td>$\text{NH}_4^+$ (µg gds$^{-1}$)</td>
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<td>0.91 (0.24)</td>
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<td>Postburn</td>
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<td>$\text{NO}_3^-$ (µg gds$^{-1}$)</td>
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<tr>
<td>Preburn</td>
<td>3.63 (0.78)</td>
<td>2.61 (0.63)</td>
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<td>Postburn</td>
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<tr>
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Across the full growing season, soil $\delta^{15}$N in burned sites was 2.76‰ (±0.36) and in unburned sites was 2.00‰ (±0.36). The response of $\delta^{15}$N to burning varied between sites. Soil $\delta^{15}$N in unburned Sites C1 and C2 was on average 1.22‰ (±0.52) and 1.79‰ (±0.36; Table 1), respectively. In burned sites, there were shifts in $\delta^{15}$N, although the direction of shift varied between sites. Soils in B1 were depleted in $^{15}$N after a prescribed
burn relative to before the burn; $\delta^{15}$N shifted from 3.98‰ ($\pm0.64$) to 3.22‰ ($\pm0.50$). In Site B2 the soil $\delta^{15}$N decreased from 2.67‰ ($\pm0.57$) before the prescribed burn to 2.45‰ ($\pm0.24$) after the burn. Finally, there was a slight enrichment in soil $^{15}$N in Site B3 following fire; $\delta^{15}$N shifted from 1.37‰ ($\pm0.36$; Table 1) to 2.59‰ ($\pm0.93$; Table 1). The 95% CIs surrounding the mean $\delta^{15}$N (and %N) overlapped for all burned sites, indicating the soil $\delta^{15}$N at each site was statistically indistinguishable pre- and post-burn.

We used pre-burn soil $\delta^{15}$N isotopic signature in mixing models to calculate the mass of ash-N needed to be deposited on our sites to achieve both the minimum and the empirically measured shift in soil $\delta^{15}$N. To achieve a shift in soil $\delta^{15}$N of the minimum external precision, sites B1, B2, and B3 would need 11, 5, and 20 g N m$^{-2}$ ash-N, respectively, deposited following fire. To achieve the measured shift in soil $\delta^{15}$N, sites B1 and B2 would need 100 and 11 g N m$^{-2}$ added through ash deposition; B3 would need 175 g N m$^{-2}$ to be removed from fire (Table 1). We also calculated the same values using fresh leaf $\delta^{15}$N from leaves collected from our sample site (-2.9‰ ±0.1; J. Wright, unpublished data), rather than published ash $\delta^{15}$N values (Table 2).
Table 2: The mass of N needed to be deposited at each site in order to achieve a shift in soil $\delta^{15}$N of the minimum external precision, and the observed empirical shift in soil $\delta^{15}$N. These values were calculated from mixing models with fresh leaf $\delta^{15}$N as one end member. Across 1,827 samples from 67 plant species, the mean $\delta^{15}$N was -2.9‰ (±0.1; J. Wright, unpublished data). Units are in g N m$^{-2}$. Minimum external precision is 0.1‰ $\delta^{15}$N at 1 standard deviation.

<table>
<thead>
<tr>
<th>Burned Sites</th>
<th>B1</th>
<th>B2</th>
<th>B3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum shift</td>
<td>8</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Empirical shift</td>
<td>316</td>
<td>8</td>
<td>-108</td>
</tr>
</tbody>
</table>

### 2.4 Discussion

In this study, we collected weekly measurements of soil inorganic N availability to document short-lived changes in N dynamics following fire and throughout the growing season of a pyrogenic forest in the southeastern US. As far as we know, this is the first study to pair estimates of N pool sizes and cycling rates at high temporal resolution in a longleaf pine savanna. Prior to prescribed burns, there was high variability in N availability, particularly for $\text{NH}_4^+$ pool sizes. This heterogeneity reinforces the need for a methodological approach that considers initial edaphic conditions when carrying out in situ experiments on paired soil cores. To address this, we relaxed the assumption that initial and incubating cores were true edaphic replicates; we used a Bayesian statistical framework to allow for variability in the relationship between true versus measured inorganic N concentrations in our soils.
Immediately following prescribed burns, we found sharp increases in NH$_4^+$ pool sizes in all of our study sites. However, the magnitude and duration of this increase varied between sites. Unlike studies in southeastern US pine savannas with monthly or less-frequent temporal sampling protocols, our weekly sampling allowed us to capture highly ephemeral changes in soil inorganic N pools. Furthermore, we found no changes in cycling rates and no evidence that ash deposition could account for the large increases in N availability following fire. Instead, we propose that an ephemeral dampening of plant uptake could contribute to the observed increases in inorganic N following fire.

2.4.1 Changes in N dynamics across the growing season

Throughout the growing season, inorganic N availability and net cycling rates were low, as is common in longleaf pine savannas (Binkley et al. 1992). In unburned conditions over the growing season, there was greater NO$_3^-$ availability than NH$_4^+$. This pattern is consistent with previous work, which documented relatively high NH$_4^+$ availability in the winter, followed by decreasing NH$_4^+$ availability throughout the growing season (Christensen 1977). Net nitrification rates were low across the growing season, may have been inhibited by the low soil pH (Ste-Marie and Paré 1999). Net mineralization in our study was higher than measured over the summer months in previous studies (Wilson et al. 1999), so rather than low mineralization rates, our low
soil NH$_4^+$ : NO$_3^-$ ratios may be a result of preferential plant or microbial uptake of NH$_4^+$ over NO$_3^-$. 

We observed sharp increases in soil inorganic NH$_4^+$, but not NO$_3^-$, immediately following fire across three longleaf pine savanna sites in North Carolina (Figure 5). Although a global meta-analysis found that post-fire soil NO$_3^-$ concentrations peak ten months after NH$_4^+$ concentrations (Wan et al. 2001), studies in southeastern US ecosystems found no change in soil NO$_3^-$ up to 30 days (pine savanna; Boring et al. 2004), 320 days (pine forest; Lavoie et al. 2010) and 500 days (shrubland; Schafer and Mack 2010) following fire. Across the full growing season, we measured NH$_4^+$ pool sizes of burned sites that were nearly 5x that of unburned sites. The direction of the effect of fire was consistent across our study sites, however the magnitude of increase was highly site-specific. Within a site, increases in NH$_4^+$ availability immediately following fire ranged from 5x to more than 25x the pre-burn levels. This NH$_4^+$ pulse was short-lived, and only in B3 was the increased NH$_4^+$ pool size sustained for longer than one week. As a consequence, a decreased sampling frequency would not have detected the ephemeral changes in soil NH$_4^+$ pool size in B1 and B2.

It remains unclear why, however, sites experience such variability in the magnitude of NH$_4^+$ response following fire. Although we cannot rule out the possibility that our high-intensity sampling influenced nitrogen cycling and pool sizes, we saw no
evidence of increasing inorganic N availability, or increasing variability in N availability, in our control sites, which experienced the same levels of sampling disturbance without fire. However, differences in post-fire vegetation regrowth in sites B1, B2, and B3 (C. Ficken, unpublished data), may suggest an important role of plant uptake in regulating soil N concentration. While B1 and B2 exhibited rapid vegetation resprouting following fire, regrowth in B3 was patchy. Vegetation began resprouting in B1 and B2 six days after fire, but not until 18 days after fire in B3. Moreover, in 2012, the last year all sites were sampled prior to the 2014 burns, B3 had the smallest standing biomass stocks of all three burned sites (J. Wright unpublished data). If plant N uptake remained low following fire in B3, this might explain the persistent increase in N availability in this site. However, biomass stocks after three months of regrowth in 2014 were also substantially different between B1 and B2, despite these sites exhibiting similar patterns of NH$_4^+$ availability over time. In unburned years, Mitchell et al. (1999a) found that annual net primary productivity (ANPP) in a longleaf pine savanna was positively correlated with local moisture availability and biomass estimates in this heterogeneous system are highly dependent on local woody versus herbaceous cover, as well as annual variability in environmental conditions. Factors controlling unburned ANPP may differ from those controlling biomass regeneration, and given the
spatiotemporal heterogeneity of this system, teasing apart these drivers may require a large-scale manipulative experiment.

2.4.2 Assessing mechanisms of N pulses following fire

A nitrogen pulse may occur following fire through (1) an increase in microbial mineralization, (2) ash inputs, or (3) a decrease in plant uptake. Fire may stimulate microbial turnover of organic matter if additions of C or N from ash deposition or root exudation (southern shrubland; Schafer and Mack 2010) enhance microbial activity. Soil surface blackening after fire may increase soil temperature and stimulate immediate and prolonged N transformations after fire (Ojima et al. 1994, Booth et al. 2005).

Wilson et al (2002) found significant increases in microbial biomass following fire in a longleaf pine savanna. Although we did not directly measure microbial biomass, we found no changes in net microbial mineralization associated with the observed increase in pool size, although cycling rates were increasingly variable following fire in burned sites (but not in unburned sites). Indeed, the increase in mass of NH$_4^+$ following fire was much greater than the mass of NH$_4^+$ produced on a daily basis by net microbial mineralization, reinforcing the conclusion that changes in microbial cycling rates could not account for the observed increase in pool sizes.

We also found no indication that the newly available NH$_4^+$ substrate led to a delayed increase in net nitrification rate. This, along with high soil C:N (unburned sites-
47:1; burned sites- 53:1) relative to other longleaf pine soils (Lucash et al. 2007), might suggest that autotrophic nitrifying microbes are competitively inferior to heterotrophic microbes under post-fire conditions in our study sites. Alternatively, an unmeasured increase in gross nitrification might have allowed for a commensurate increase in microbial immobilization of NO$_3^-$ following fire. In general, however, the lack of change in microbial N cycling rates suggests that changes in microbial activity fail to account for the observed increases in NH$_4^+$ availability following fire.

Direct additions of N into the soil from ash may provide an alternative mechanism for the observed increase in mineral N availability. To test this, we examined changes in both total N and $\delta^{15}$N immediately before and after burns. N from ash additions is primarily organic (Christensen 1977, Raison 1979, Huber et al. 2013), and is thought to increase N pools by stimulating microbial activity. We found no change in total nitrogen (i.e. %N) or soil organic matter before and after prescribed burns. These results support the findings of a global meta-analysis of fires in forested systems, which found no effect of fire on total N (Wan et al. 2001).

We used the natural abundance of $^{15}$N as an isotopic tracer of ash additions. However, fractionation during volatization preferentially releases $^{14}$N, resulting in ash material that is enriched in $^{15}$N relative to fresh plant matter, and an increase in $\delta^{15}$N signature in ash relative to what would be observed in fresh plant material (Saito et al.
2007, Stephan et al. 2015). A study in a subalpine grassland reported foliar $\delta^{15}$N values (-2.9‰) and N concentrations in ash (11.63 ±0.80 mg N g-1 ash; Huber et al. 2013) comparable to foliar $\delta^{15}$N values (-2.9‰; ±0.1; J. Wright, unpublished data) and ash-N concentrations of our system (8.75 ±0.90 mg N g-1 ash; Christensen 1977). Huber et al. (2013) also reported $\delta^{15}$N values in ash of -0.81‰, which we used in mixing models.

Using this isotopic signature of ash, we found that a minimum of 5 to 20 g ash-N m$^{-2}$ would need to be deposited in our burned sites in order to observe a detectable shift in soil isotopic signature. Because these minimum values are greater than the mass of ash-N deposition previously reported in a longleaf pine system (1.15 g m-2; Christensen 1977), this method may not be ideal for detecting ash inputs in systems with low aboveground vegetation stocks. Nevertheless, we estimated that 100, 11, and 175 g ash-N m$^{-2}$ would need to be deposited on sites B1-3 to account for our measured shifts in soil $\delta^{15}$N. These deposition levels are highly unlikely to have occurred at our sites, since they would require substantial aboveground vegetation accumulation and our system is burned every three years. However, it is unclear how quickly surface inputs can be expected to distribute throughout the soil profile. In the sandy soils of our system, frequent heavy summer rains or active root growth may quickly redistribute surface inputs. While one study of longleaf pine savannas found that changes (losses) in total soil N following fire were concentrated at the soil surface (Binkley et al. 1992), another
study of subalpine woodlands and grasslands detected no changes in total N in surface soils following burning (Huber et al. 2013). Our work is in agreement with that of (Christensen 1977), who found significant differences in $\delta^{15}$N with depth, but no change following fire.

Given the uncertainties surrounding the redistribution of surface inputs down the soil profile, we cannot conclusively rule out the potential to surface additions to contribute to the observed NH$_4^+$ pulse. Nevertheless, considering the unrealistic mass of ash-N needed to be deposited onto surface soils to account for our measured shifts in $\delta^{15}$N, we conclude that ash inputs are unlikely to fully account for the increase in measured soil inorganic N availability.

Finally, changes in plant and microbial immobilization could cause an increase in soil inorganic pool sizes. Prescribed fires in longleaf pine savannas are low intensity, and sharp increases in soil temperature with depth, particularly in dry soils, are unlikely to substantially damage the soil microbial community below 5 cm (Hartford and Frandsen 1992). In fact, previous work in longleaf pine savannas has documented increases in microbial biomass following fire (Wilson et al 2002). In contrast to the microbial community, prescribed burns in our study system generally topkill a majority of the aboveground herbaceous and woody biomass with stem diameters less than 10 cm (Just et al. 2015). If fire damage temporarily halted or slowed the plant uptake of inorganic N,
we would expect to see an accumulation of soil N if microbial immobilization did not increase sufficiently to deplete the pool. However, N accumulating in excess of demand can only partly explain observed increases in inorganic N availability, since the pulse of N we detected following fire was many times greater than what was produced by net mineralization and net nitrification. Nevertheless, a change in plant sink strength may have contributed to post-fire NH$_4^+$ pulse.

Previous work found no evidence that plant species in an African savanna re-translocated nutrients from root biomass to resprouting shoot biomass following a fire (Vijver et al. 1999), indicating that soil pools can be an important source of N for regenerating biomass. Indeed, the biomass of resprouting vegetation following fire has been shown to be highly enriched in $^{15}$N relative to pre-burn biomass (Huber et al. 2013, Schafer and Mack 2014a), an indication that the source of N in resprouting biomass is also enriched (Evans 2001). Root biomass is an important component of short-term N retention in grassland ecosystems (de Vries and Bardgett 2016). In fire-prone systems, fire-tolerant plants could play an important role in preventing N leaching losses if they are able to resume N uptake quickly following fire.

We propose that plant-demand for inorganic N may have a strong influence on soil N pool sizes in this system, and a temporary decrease in this demand may have contributed to the observed post-fire NH$_4^+$ pulse. Preference for NH$_4^+$ by plants
inhabiting acidic soils, where nitrification is limited by low pH and NO$_3^-$ availability is consequently low (Kronzucker et al. 1997, Ste-Marie and Paré 1999, Houlton et al. 2007, Wang and Macko 2011), could help to explain the relatively large pool sizes of NO$_3^-$ relative to NH$_4^+$ during the growing season (Vitousek 1977), and this pattern is consistent with previous seasonal trends in a longleaf pine savannas (Christensen 1977). Furthermore, an increase in soil NH$_4^+$ pool size after fire without a stable increase in net microbial mineralization rates could occur if there is a decrease in plant uptake.

Similarly, a muted effect of fire on NO$_3^-$ pool size may occur if plants have diminished uptake of this inorganic N form, and plant uptake exerts a relatively weaker control on soil NO$_3^-$ pools. Plant control on ecosystem N status has been well documented in northeastern US hardwood forests, where a defoliation event resulted in substantial N losses from the ecosystem (Likens et al. 1969, Aber et al. 2002).

If post-fire patterns in N availability were related to plant uptake, we would expect differences in the magnitude and duration of soil N change following fire to be related to plant N-demand and regrowth following fire. In stand-replacing fires in temperate forests, where vegetation is killed, relatively persistent increases in N pools should occur following fire. We similarly would expect smaller and more ephemeral changes in N pools in systems in which plants are only top-killed. Indeed, stand-replacing fires have been shown to result in changes to soil N pools that persist more
than one year following the fires (Smithwick et al. 2005b, Turner et al. 2007). In contrast, elevated inorganic N immediately following fires in grassland decreases throughout the growing season (Augustine et al. 2014). Low-intensity fires in grass-dominated glades adjacent to oak-hickory forest sites in a Kentucky study resulted in increases in post-burn soil NO$_3^-$ pool sizes, but no increase in lysimeter-detected NO$_3^-$ leaching losses below 10 cm (Trammell et al. 2004). Although the study did not examine microbial biomass, they found no effect of fire on net N mineralization, suggesting role of plant uptake in patterns of N loss and retention post-fire. Such instances of plant control of N availability provide an important setting in which to examine the role of nutrient availability– and nutrient pulses in particular– on plant community composition and ecosystem productivity. Differences in the ability of species to capture this ephemeral resource may help explain differences in post-fire resprouting patterns and biomass regeneration following fire.

2.4.3 Ecological implications of fire-associated N pulse

To put the fire-associated pulse of inorganic N that we observed into context, we compared its mass to N inputs in the longleaf pine savannas ecosystem. Although the pulse of soil N following fire is most likely a redistribution of N from other ecosystem pools, it is conceptually helpful to understand the magnitude of this pulse relative to other components of the N cycle in longleaf pine savannas. The increase in soil inorganic
N following fire (0.98 g N m\(^{-2}\) 10 cm\(^{-1}\)) was approximately 10x the daily total net inorganic N production (i.e. net mineralization + net nitrification; 0.11 g N m\(^{-2}\) 10 cm\(^{-1}\) day\(^{-1}\)).

These ephemeral increases in soil inorganic N availability occur during an important ontological stage of plant development as longleaf pine understory species begin resprouting within a few days following fire (CD Ficken, personal observation). The mass of mineral N released following fire was more than twice the mass of N longleaf pine understory species assimilate into their biomass over a full growing season (0.45 g N m\(^{-2}\); unpublished data). However, the extent to which plants or microbes have access to this ephemeral nutrient pulse remains unclear. Previous studies have documented rapid plant uptake of N tracers by intact plants (Likens et al. 1969, Aber et al. 2002), suggesting that plants may have access to this N pulse during regrowth.

**2.4.4 Conclusions**

We have documented large pulses of mineral N following prescribed burns in a longleaf pine savanna in North Carolina. Our weekly sampling revealed that while one site experienced a persistent increase in NH\(_4^+\) pool size, other sites experienced only very short-lived pulses that would not have been detected with monthly sampling. The marked differences in the duration of the NH\(_4^+\) pulse that we observed may explain why previous studies with less-frequent soil sampling showed no change in mineral N
following fire. However, the factors that influence the magnitude of the system’s response to fire are still unknown. We propose here a role for plant uptake in regulating post-fire N availability, and encourage future work to explore the relationships between N availability and plant biomass dynamics immediately following fire. Due to the rapid changes in N availability following fire, as well as the fast resprouting of understory species, we recommend that responses of the local plant community be considered when determining an appropriate sampling regime for biogeochemical responses to disturbance. In systems in which the plant community responds rapidly, soil samples should be collected quickly and frequently to capture post-disturbance plant-nutrient and biogeochemical dynamics.

The pulses documented here were not associated with increases in microbial activity, and, although inconclusive, our data also do not support the hypothesis that the observed inorganic N pulse could be attributed to ash inputs to the system. We propose that a temporary slowing of plant uptake may contribute to the observed mineral N pulse. Although plants begin resprouting a few days after a fire, the extent to which plants access the newly available NH₄⁺ remains unclear. Given the magnitude of this pulse relative to other N transformations in the system, and particularly relative to the mass of N estimated to be assimilated into the understory biomass annually, these fire-associated N pulses may be important sources of plant-available N as the vegetation
regrows. As such, they may also play an important role in structuring plant recovery from disturbance and shaping community diversity in this system.
3. Testing for niche differentiation: nitrogen uptake and biomass regrowth have contrasting relationships with root traits

3.1 Introduction

Understanding the processes that govern plant competition and coexistence is an enduring goal of ecology (Chesson 2000), with many theories proposing that coexistence can be maintained through niche differentiation (e.g. Grime 1977, Tilman 1982, Vellend 2010). Physiological characteristics that optimize performance in a particular niche typically necessitate tradeoffs in other characteristics, thus limiting performance in different niches or under different environmental conditions. In systems with temporally fluctuating resources, niche differentiation may occur if species specialize on and show enhanced fitness during periods of either high resource availability, termed pulse periods, or inter-pulse periods (Goldberg and Novoplanksy 1997, Chesson et al. 2004). Such tradeoffs in environmental optima allow species with varied strategies to coexist in close proximity to each other, despite sharing a common limiting resource (Tilman and Pacala 1993, McKane et al. 2002, James and Richards 2006). Despite a rich theoretical literature illustrating the potential for resource partitioning to promote species coexistence, relatively few studies experimentally link niche partitioning to tradeoffs in plant functioning (but see McKane et al. 2002, February et al. 2011, Shriver 2017), and fewer still do so in a generalizable framework.
Plant functional traits provide a framework to identify the physiological tradeoffs proposed to underlie competitive and life history strategies (Lavorel and Garnier 2002, Westoby and Wright 2006). Comparing functional traits among coexisting species can therefore enhance our understanding of the resource use niches inhabited by each species. For example, traits related to root morphology and depth distributions are likely related to resource foraging and capture (McMurtrie et al. 2012), so divergent distribution profiles may indicate that species are accessing spatially independent resource pools (Gebauer and Ehleringer 2000, Paula and Pausas 2011). The plant economics spectrum (PES; Wright et al. 2004, Reich 2014) posits first that a plant’s resource use strategy (RUS) can be identified based on a number of co-varying functional traits, and secondly that its RUS corresponds to multiple metrics of overall plant functioning (e.g. productivity, respiration). Resource acquisitive plants are expected to capture and process all resources relatively quickly and to have high productivity when resource availability is high (akin to ruderal plants, or those with a high R*; Grime 1977, Tilman 1982), whereas resource conservative plants are expected to exhibit more efficient resource capture and processing, making them relatively more productive when resource availability is low (Reich 2014). Accordingly, a plant’s RUS should influence not only its growth, but also its relative competitive ability under different environmental conditions. For example, differences in leaf and root structural
traits among grasses have been shown to indicate underlying physiological tradeoffs that lead to an inverse relationship between drought tolerance and nutrient acquisition (Balachowski and Volaire 2017), and water use and leaf structural traits predicted plant survival during an extreme drought (Lloret et al. 2016). Overall, examining relationships between plant traits and plant functioning may improve our empirical understanding of co-existence mechanisms by providing a physiological basis to explain niche differentiation.

Although most commonly tested at the leaf-level, the trait syndromes which correspond to functioning are hypothesized to vary across plant organs (e.g. leaves, wood, roots; Reich 2014). However, mixed evidence exists for extrapolating these relationships from leaves to roots (Roumet et al. 2006, Orwin et al. 2010, Bergmann et al. 2017) perhaps because organ functionality, lifespan, and environment all fundamentally differ above- and belowground (Mommer and Weemstra 2012). When resource competition occurs belowground, measures of root traits may provide a more direct assessment of RUS, and overall competitive interactions, than leaf traits. For example, nitrogen (N) influx into roots is regulated by different uptake proteins when N is in high versus low supply, and transporter production is upregulated in response to N supply (Hawkesford et al. 2012). Moreover, since N influx and the density of each transporter type on roots may vary according to root structure (Maire et al. 2009), morphological
traits may be an appropriate proxy for N uptake strategy and plant performance during N pulse or inter-pulse period. Given that N pulses are produced in response to many abiotic and biotic drivers (e.g. Lipson et al. 1999, James and Richards 2006, Archer et al. 2014), temporal N partitioning may be a generally important component of niche differentiation identifiable through root functional traits.

In this study, we test whether contrasting N uptake strategies constitute a mechanism of niche differentiation by influencing plant competitive ability (biomass regrowth) under different N availability regimes. While competitive ability is often assessed by comparing growth rates of mature individuals, many systems experience forms of disturbance that remove much of the above-ground biomass through fire, wind throw, or herbivory, necessitating regrowth from below-ground meristems. To examine the physiology underlying these functions (N uptake and biomass regrowth), we assess their relationships to root functional traits and the PES. Longleaf pine forests, from which we chose focal species, are typically N-limited (Wilson et al. 1999) but experience frequent low-intensity fires which result in ephemeral elevated soil concentrations of inorganic N (Ficken and Wright 2017). In addition to temporarily elevating soil inorganic N concentrations, fires also reset aboveground competition for space and light by removing standing biomass, but do not kill plants (i.e. the fire-adapted plant community largely resprouts after fire). Specifically, we test (1) whether species differ in
N uptake under different N supply regimes, (2) whether differences in N uptake strategy influence biomass regrowth under different N supply regimes and when grown with a competitor, and (3) which root traits are associated with each function. To address these questions, we performed two greenhouse experiments. After identifying two species with contrasting N uptake strategies in the first experiment, we measured the biomass regrowth of individuals when grown either with a conspecific (i.e. same N uptake strategy) or heterospecific (i.e. contrasting N uptake strategy), and exposed to different N supply regimes. Based on predictions of the PES, we expected that species which captured more N when availability was high would also have faster regrowth following disturbance when disturbance was coupled with a N pulse. However, we also expected that this relative competitive advantage would be contingent upon resource supply, and thus could be interpreted as a mechanism promoting coexistence. That is, we predicted that the conservative species would outcompete the acquisitive species when N supply was low or constant.

### 3.2 Methods

#### 3.2.1 Experiment 1 – N uptake

**3.2.1.1 Propagation**

To compare the ability of different species to take up elevated nitrogen, we propagated four co-occurring species (*Ilex glabra, Lyonia lucida, Pinus palustris, and*
Vaccinium formosum) common to longleaf pine (P. palustris) forest understories. I. glabra specimens were grown from stem cuttings collected from longleaf pine forests on Fort Bragg Military Reservation (35.1391°N 78.9991°W) in August 2014. Cuttings from terminal stems (5 cm length) were initially planted in perlite, then transplanted in September 2014 to 1 gallon pots (3.79 L) and filled with 1:1 mixtures of sand and field soil collected from a longleaf pine forest in fall 2014. Bare root tubelings of L. lucida, P. palustris, and V. formosum were purchased in October 2014 and planted into 1 gallon pots (3.79 L) filled with 1:1 mixtures of sand and field soil. All plants were grown in the greenhouse (12h daylight) and misted daily.

3.2.1.2 $^{15}$N fertilization treatments

Five individuals of each species were assigned either into a background or pulse N fertilization treatment group. Background and pulse N fertilization levels were based on field measures of soil inorganic N concentration before and after a fire-induced pulse of inorganic N. Field measures of background soil inorganic N were approximately 5 µg N per gram of dry soil (gds); field measures of inorganic N up to one week following fire was approximately 20 µg N gds$^{-1}$ (Ficken and Wright 2017). Each individual in the background fertilization group therefore received 32.25 mg 98atom% $^{15}$NH$_4$$^{15}$NO$_3$ in 1 L water to achieve a fertilization rate of 12.5 mg N L$^{-1}$ H$_2$O. Individuals in the pulse fertilization treatment received 137 mg 98atom% $^{15}$NH$_4$$^{15}$NO$_3$ in 1 L water to achieve a
fertilization rate of 50 mg N L\(^{-1}\) H\(_2\)O. We applied fertilizer in one application directly to soil at the base of the plant and allowed it to drain through.

3.2.1.3 Sample collection and trait measurements

We collected leaf samples immediately before tracer application and at 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 days post-application. Each leaf sample consisted of three leaves collected from each plant without respect to leaf age or location. Immediately after collection, we placed leaf samples into coin envelopes and dried at 70°C for 10 days.

We recorded the weight of dried samples, and then ground leaves finely in a Geno/Grinder tissue homogenizer (SPEX SamplePrep; New Jersey, USA). We packaged subsamples of ground leaf material in tin capsules, and analyzed them for N mass (%) and isotopic composition (atom% \(^{15}\)N) on a Carlo-Erba Elemental Analyzer coupled to a Thermo-Finnegan Delta Plus XL CF-IRMS at the Duke Environmental Isotope Laboratory.

We harvested above- and belowground biomass of all individuals immediately after final leaf collections were completed on day 12. Aboveground biomass was dried at 70°C for at least 10 days and weighed. We carefully washed root systems of soil, then scanned roots on an Epson Expression scanner, and analyzed traits using WinRhizo software (Regent Instruments). We recorded root length, surface area, and the number of root tips. These morphological traits are expected to influence the capacity for a plant
to explore the soil and forage for resources. After scanning, root systems were dried at 70°C for 10 days and weighed. We additionally calculated the Root:Shoot ratio (RS) and specific root length (SRL; root length in m per gram of belowground biomass) for entire root systems.

### 3.2.1.5 Statistical analyses

To compare plants across multiple traits, we performed a principal components analysis (PCA) with the prcomp function in the stats package in R (Team 2011) on root traits (length, root surface area, root tips, SRL, RS) as well as aboveground and belowground biomass and initial leaf %N. We performed PERMANOVA tests with the adonis function in the vegan package in R to test for differences in multivariate trait spaces between species and between fertilization treatments.

We estimated uptake rate and cumulative $^{15}$N assimilated by day 10 by fitting linear (background $^{15}$N) and logistic (pulse $^{15}$N) models to the $^{15}$N assimilation time series for each individual with the nlsList function within the nlme package (v. 3.1-121) in R (Team 2011). Ten days of uptake was chosen to approximate the duration of the N pulse observed in field studies. Assimilation of $^{15}$N in the pulse $^{15}$N treatment was fit to

$$^{15}N = \frac{A}{1 + e^{-k(x-t_0)}}$$

where $A$ is the upper asymptote, $k$ is the rate parameter, and $t_0$ is the midpoint. The maximum uptake rate occurs when $x = t_0$ and is given by $Ak/4$.

Anticipating that species may differ in how quickly they can upregulate the production
of N uptake transporter proteins (Hawkesford et al. 2012), and thus may differ in how quickly they can ramp up N assimilation rate, we modeled our pulse uptake data with a three-parameter logistic curve, rather than a Michaelis-Menten curve. We tested for significant differences between species with ANOVA tests in R.

Using the lm function in the base R package, we examined the relationship between root traits and the mass of $^{15}$N assimilated by day 10. For individuals in the pulse fertilization treatment, we also examined relationships between root traits and the mass of $^{15}$N assimilated by day 10, as estimated during model fitting. We regressed each trait independently against the cumulative mass of $^{15}$N assimilated by day 10 for pulse and background treatments separately, and chose best models with AIC.

### 3.2.2 Experiment 2 – Biomass regrowth

#### 3.2.2.1 Propagation and fertilization treatment application

Based on the results from Experiment 1, we categorized *Ilex glabra* as a resource-acquisitive species, and *Vaccinium formosum* as a resource-conservative species, and target only these two species in this experiment. To test whether species’ RUS (identified in experiment 1) influenced their relative competitive ability (biomass regrowth, this experiment), we grew pairs of *Ilex glabra* and *Vaccinium formosum* in all pairwise combinations under different N supply regimes (press, pulse, or control; see next paragraph). Pairs were grown for six months under greenhouse conditions in 1 gallon
pots (3.79 L) in 1:1 field soil:sand, and watered as needed. After discarding pots in which individuals had not survived six months post-transplanting, we were left with 26 (V. formosum – V. formosum), 29 (I. glabra – I. glabra), and 24 (V. formosum – I. glabra) replicates of each pair. In April 2017, we clipped aboveground biomass of each individual to simulate biomass loss from fire disturbance. Plants were clipped 2 cm above the soil surface. Aboveground biomass was dried until a constant mass and weighed.

The day after clipping, we applied press, pulse, or control fertilizer treatments to pots. By including an unfertilized control, our goal was to differentiate between the effect of fertilization (press and pulse vs control) and the effect of fertilization rate (press vs pulse). Treatments were imposed every other week over a two-month period, for a total of 10 fertilizer applications. Pots in the pulse treatment received 0.230 g N in one L of water in the first week, followed by nine weeks of water only; pots in the press treatment received 0.023 g N L⁻¹ each week for 10 weeks; pots in the control treatment received 10 weeks of water only. Pots in the press and pulse treatment therefore received the same mass of N delivered either all at once (i.e., as a pulse) or slowly over the full experimental period (i.e., as a press). Pots were watered in between fertilization treatments as needed, but never within 2 days of fertilizer application.
3.2.2.2 Sample collection and trait measurements

We re-harvested above- and belowground biomass of all individuals one week after the final fertilization treatment, that is after 12 weeks of regrowth. Aboveground biomass was dried at 70°C for at least 10 days, and weighed. Immediately after harvesting, we carefully washed root systems of soil, scanned subsamples roots on an Epson Expression scanner, and analyzed traits using WinRhizo software (Regent Instruments). Subsamples included all coarse and fine roots of one root branch. We recorded the fresh weight of root subsamples, and recorded root length, surface area, and the number of root tips for all roots – root traits expected to influence a plant’s foraging capacity. We calculated the RS, SRL, and root dry matter content (RDMC; dry/fresh mass) for root subsamples. After scanning, root subsamples and bulk root systems were dried at 70°C for 10 days and weighed.

3.2.2.3 Statistical analyses

To assess the role of competitive environment on biomass regrowth, we compared the mass of aboveground regrowth across species-neighbor pairs with ANOVA tests. Hereafter, we refer to “Species” as the focal species in the pair (I. glabra or V. formosum); we refer to “Neighbor” as the secondary plant (Self or Other). We performed separate ANOVAs for each fertilization treatment to avoid testing for three-way interactions (Species x Neighbor x Fertilization treatment). To assess the role of N
fertilization rate on biomass regrowth, we compared the mass of aboveground regrowth across neighbor-treatment pairs for each species. Post-hoc pairwise comparisons were performed with Tukey’s tests.

We built linear regression models to examine the correlation between the mass of resprouted biomass and each individual root trait (root length, surface area, and the number of root tips), neighbor and N fertilization treatment. Best models were chosen to minimize AIC values.

### 3.3 Results

#### 3.3.1 Experiment 1 – N uptake

Species varied in plant traits directly and indirectly involved in soil resource acquisition (Appendix A; Figure 22), but trait values were similar within each species. Species differed significantly in total biomass (F=22.34, \( p<0.001 \)), Root:Shoot ratio (RS; F=42.11, \( p<0.001 \)), root tips (F=7.76, \( p<0.001 \)), root surface area (SA; F=5.77, \( p=0.003 \)), and specific root length (SRL; F=21.05, \( p<0.001 \); Appendix A). Although species differed significantly in initial leaf %N (F=2.93, \( p=0.047 \)), Tukey’s tests revealed that only *V. formosum* had marginally significantly higher concentrations of leaf %N than *L. lucida* (\( p=0.064 \)), and leaf %N did not differ between any other species (Appendix A). *L. lucida* individuals had the greatest above- and belowground biomass while *I. glabra* individuals had the least above- and belowground biomass. RS ranged from 0.20 for *V.*
formosum to 1.15 for *P. palustris*. *P. palustris* had the largest RS and also the fewest root tips (Appendix A), likely because this species produces a substantial tap root.

We performed a PCA on plant and root traits to examine how plant individuals varied in the trait space they occupied (Figure 10). Based on PERMANOVA, traits differed significantly between species (*F*=6.64, *p*=0.001), and marginally between N fertilization treatments (*F*=3.31, *p*=0.054). The first two principal component (PC) axes accounted for 66% of the variation between individuals. PC1 described primarily measures of root morphology (PC1 eigenvalues: root length = -0.48, SRL = -0.47), whereas PC2 described root system size and initial leaf %N (PC2 eigenvalues: belowground biomass = -0.45, leaf %N = 0.45).
Figure 10: PCA of traits of focal species. Species differed significantly in multivariate trait space (PERMANOVA F=6.64, p=0.001). AG and BG Biomass refer to aboveground and belowground biomass, respectively; RS is root:shoot ratio; root SA is root surface area (cm$^3$); SRL is specific root length (m g$^{-1}$); leaf %N is N concentration prior to 15N addition.

Species’ assimilation curves differed substantially between background and pulsed N conditions, and between species (Figure 11a,d). Across all species, plants assimilated significantly more N by day 10 when exposed to a N pulse versus background N availability (F=12.21, p=0.001). Under background N availability, species did not differ in the mass of N assimilated by day 10 (F=2.06, p=0.146), although V. formosum assimilated substantially more N under background availability (0.25 ±0.10 mg
$^{15}$N g$^{-1}$) than the other species (Figure 11b). Under background availability, initial leaf %N was the strongest predictor of N uptake ($p=0.014$, $R^2 = 0.29$; Figure 12).

Figure 11: Assimilation patterns of N by focal species under background (a,b,c) and pulsed (d,e,f) N supply. Panels a and d show the accumulating mass of 15N in leaves over time across focal species. Solid lines (± SE envelopes) show the mean 15N accumulating over time. The dotted lines indicate 10 days after initial label application. Panels b and e compare the mass of 15N assimilated by day 10 across focal species (μg g$^{-1}$ leaf biomass). Panels c and f compare the maximum assimilation rates across focal species (μg hour$^{-1}$).
Figure 12: Uptake of $^{15}$N uptake was correlated with leaf %N and with specific root length (SRL). A) Leaf %N was the best predictor of uptake under background (open circles, solid line; $p=0.014, R^2 = 0.29$) and pulsed N availability (filled circles, dashed line; $p<0.001, R^2 = 0.72$). B) SRL was positively correlated with N uptake under pulsed availability ($p<0.001; R^2 = 0.64$), but not under background availability ($p=0.640$).

When exposed to a N pulse, species strongly differed in the mass of N they assimilated by day 10 ($F=7.63, p=0.002$; Figure 11d,e), and their maximum assimilation rate ($F=4.58, p=0.024$; Figure 11f). *I. glabra* had a significantly higher maximum assimilation rate than *P. palustris* ($p=0.025$) and a marginally higher assimilation rate than *L. lucida* ($p=0.057$; Figure 11f). *I. glabra* assimilated significantly more N by day 10 than all other species ($p<0.05$ for all pairwise comparisons; Figure 11d,e). By day 10, *I.*
*I. glabra* had assimilated 1.64 ±0.42 mg $^{15}$N g$^{-1}$ leaf biomass. This was more than 3 times the mass of $^{15}$N assimilated by *L. lucida* (0.47 ±0.13 mg $^{15}$N g$^{-1}$), *P. palustris* (0.32 ±0.04 mg $^{15}$N g$^{-1}$), and *V. formosum* (0.38 ±0.09 mg $^{15}$N g$^{-1}$). Under pulsed N availability, like under background availability, leaf %N was a good predictor of N uptake (p<0.001, $R^2$ = 0.72; Figure 12a). SRL was also strongly positively correlated with N uptake under pulsed availability (p<0.001; $R^2$ = 0.64; Figure 12b).

### 3.3.2 Experiment 2 – Biomass regrowth

There was no difference in regrowth between control, press, or pulse N treatments (p=0.103), but across all treatments *I. glabra* regrew more biomass than *V. formosum* (df=1, F=4.35, p=0.040). Additionally, neither focal species differed in regrowth when paired with a competitive versus an acquisitive neighbor species, under either press or pulse N treatments (Figure 13). In contrast, under control N availability, there was a highly significant effect of neighbor (F=14.76, p<0.001), and both *I. glabra* and *V. formosum* regrew more biomass when paired with a heterospecific than when paired with a conspecific neighbor (Figure 13; Tukey’s test: p$_{I. glabra}$=0.079; p$_{V. formosum}$=0.043).
Figure 13: Comparison of biomass regrowth of *I. glabra* and *V. formosum* when paired with a conspecific (“Self”, solid triangles) or heterospecific (“Other”, open circles) neighbor. Under control N availability, *I. glabra* (*p*=0.079) and *V. formosum* (*p*=0.043) regrew more biomass when paired with a heterospecific than with a conspecific and there was no effect of neighbor under either N fertilization treatment level (Press or Pulse).

SRL was the best predictor of biomass regrowth across all treatments (focal species, neighbor, and N treatment). In contrast to the relationship between SRL and N uptake, however, SRL was negatively correlated with biomass regrowth (Figure 14).
Biomass regrowth was also negatively correlated with the log number of root tips ($R^2 = 0.18$, $p<0.001$), and with specific root area ($R^2 = 0.16$, $p<0.001$).

![Figure 14: Correlation between biomass regrowth and SRL. $R^2 = 0.24$, $t=-5.42$, $p<0.001$.](image)

### 3.4 Discussion

Understory species from a longleaf pine forest differed significantly in their capacity to assimilate pulsed N and exhibited qualitatively different assimilation patterns under background N supply (Figure 11). These findings suggest that N uptake during pulse versus inter-pulse periods may constitute contrasting RUS exhibited by species in this system. However, we found no evidence to support the hypothesis that
this temporal N partitioning and niche differentiation influenced a plant’s relative competitive ability under different N supplies. That is, biomass regrowth of was unrelated to the relative complementarity between RUS and N supply. This may largely be explained because N uptake and biomass regrowth did not co-vary, in contrast to our initial predictions based on the PES. Furthermore, our results indicate that the same root trait – specific root length – may underlie tradeoffs between N assimilation and resprouting capacity. This finding suggests that root morphological traits may improve our understanding of plant functioning in response to different performance metrics, but they also highlight limitations of applying RUS concepts in frequently-disturbed or pulse-drive systems.

3.4.1 Resource use strategy and productivity

We tested whether contrasting temporal N partitioning as a form of niche differentiation influenced relative competitive ability following disturbance, and thus whether N partitioning could promote species coexistence. We expected that biomass regrowth would be contingent upon the relative matching of a species’ RUS to the resource supply. Under background N availability, for example, we expected that the resource conservative species would regrow more biomass than the acquisitive species; and we expected the converse pattern in response to elevated N. We also expected the greatest regrowth to occur when the RUS of the focal species matched the N supply and
when the focal species was paired with a neighbor whose RUS did not match the N supply. Thus, although we found evidence of temporal N partitioning, we also found that resprouting was not dependent on this measured niche dimension. However, we found that under low N availability both species regrew more biomass when paired with a heterospecific rather than with a conspecific, regardless of N use strategy. If species niches differ in dimensions that are unrelated to N uptake, this finding supports longstanding views that species coexistence is promoted when their resource use niches do not overlap (Gross et al. 2007) and that non-overlapping niches also promote higher overall resource use efficiency (Hooper and Vitousek 1998, Hooper et al. 2005, Cardinale et al. 2011). That these patterns were eliminated under both high N treatments (press and pulse) suggests that any limitation in biomass regrowth due to N availability may have been eliminated in our press and pulse treatments. However, it is important to note that biomass regrowth in the press and pulse treatment did not differ from biomass regrowth in the control treatment, as we would expect if resource limitation was alleviated.

To provide mechanistic insight into the physiology underlying patterns of N uptake and biomass regrowth, we wanted to test which root traits were correlated with each. In accordance with the PES, which predicts that plant nutrient capture and processing should co-vary, we expected N uptake and biomass regrowth to co-vary, and
both to be positively correlated with resource-acquisitive root traits. In support of the PES, N uptake was positively correlated with specific root length (Figure 12b). However, in contrast to predictions of the PES, biomass regrowth was negatively correlated with specific root length (Figure 14). As such, biomass resprouting may be decoupled from competition for N. Rather than both functions being indicative of a consistent and co-varying resource capture-processing relationship, our results suggest that N uptake and rapid regrowth may constitute opposite lifestyle specialties on a tradeoff axis, both underpinned by specific root length.

Indeed, although soils exhibit substantial increases in N concentrations after fire (Ficken and Wright 2017), smaller-scale increases in N concentration may occur with greater regularity than those from fire. For example, ephemerally elevated N may occur when precipitation alleviates microbial substrate limitation (James and Richards 2006) and in response to localized animal excretions (Cech et al. 2010, Christenson et al. 2010). N uptake of smaller, relatively frequent pulses may provide a greater competitive advantage than N uptake of larger, infrequent pulses (Grover 2011), so the relatively inferior resprouting capacity of N pulse specialists could be offset by high maximum N uptake rates and efficient growth during non-fire years. A tradeoff between N uptake and resprouting capacity may consequently indicate that plants optimize their growth either immediately following fire or in response to non-destructive N pulses.
3.4.2 Root trait-function relationships and the PES

We also tested whether N uptake and biomass regrowth functions were both indicative of an acquisitive RUS and could be explained by root traits. We therefore tested whether the trait syndromes proposed by the PES, which broadly hypothesize that resource uptake and processing should co-vary, can be generalized to a frequently-disturbed system, and also whether observed leaf trait-function relationships can be extrapolated to root traits. We found that specific root length was strongly correlated with both functions. This differs from de Vries and Bardgett (2016), who found that species-level root traits were poor predictors of N uptake in herbaceous communities after root biomass and leaf-level traits were accounted for. Fine roots are more important for water and nutrient uptake than larger, structural roots (McCormack et al. 2015), so plants with a root system composed of longer and thinner roots (high SRL) would have greater root surface area over which a pulse of N could be detected and taken up than plants with a lower SRL. The positive relationship between SRL and N uptake is consistent with predictions stemming from the PES, which predicts that plants with high SRL, indicative of an acquisitive RUS, will also be highly responsive to ephemeral, labile nutrients (Reich 2014, Laliberté 2016).
Traits that promote a rapid uptake of an ephemeral resource may confer a competitive advantage if the uptake of that resource influences fitness. However we found that SRL was negatively correlated with biomass regrowth. This contradicts PES predictions of trait-function relationships for resource aquisitive species suggesting either that PES predictions do not hold for belowground traits, or are not applicable for resprouting. Although a positive correlation between leaf N content and photosynthesis forms the basis of the PES (Reich et al. 2001), photosynthetic productivity may not be directly analogous to resprouting if resprouting is limited by retranslocation of stored resources, such as carbon (Paula and Pausas 2011), rather than by the capture of new resources (Bilbrough and Caldwell 1997). We did not include total belowground biomass as a potential predictor of resprouting capacity because this trait is impractical to quantify outside of greenhouse experiments, and a larger goal of trait-based ecology is to elucidate trait-function relationships that can be measured and tested in field communities. Nevertheless, we found that individuals with shorter, coarser roots regrew more biomass regardless of N supply rate and regardless of the RUS of its neighbor competitor. If shorter, coarser roots have greater reserves of nonstructural carbon compounds that are retranslocated aboveground in resprouting biomass (Dietze et al. 2014), then we would expect plants with lower SRL to have faster rates of biomass regrowth, as we observed. We would also have expected to observe a positive
relationship between biomass regrowth and RDMC if regrowth was controlled largely by belowground carbon stores, although other studies have found opposing relationships to growth between root tissue density and SRL (Kramer-Walter et al. 2016).

### 3.4.3 Conclusions

Together, our findings suggest a tradeoff in two essential plant functions – N uptake and biomass regrowth – both strongly correlated with the same root trait. They suggest that plants may exhibit two growth strategies in frequently-disturbed systems or those with fluctuating resource availability: plants with high SRL may prioritize growth during periods of high N availability, whereas those with low SRL may exhibit high growth rates following disturbance regardless of N availability. A temporal partitioning of N uptake and a tradeoff between N uptake and biomass regrowth may both contribute to coexistence in environments with variable resource supplies. If tradeoffs in root morphology underlie strategies of growth and biomass production, shifts in patterns of N availability and/or disturbance regimes may predictably alter community-level root traits. We encourage future work to assess the generalizability of our results across ecosystems, particularly by focusing on the extensions and limitations of relating root traits to RUS and biomass regrowth.
4. Fire history but not nitrogen availability mediates plant recovery from subsequent disturbances

4.1 Introduction

Understanding how plant communities recover following disturbance has important implications for successful restoration practices and for improving our understanding of basic successional dynamics. In systems which are frequently disturbed, species which differ in the strategies by which they respond to and recover from disturbance are said to inhabit different persistence niches (sensu Bond and Midgley 2001), which are maintained through tradeoffs between productivity and competitive ability (Grime 1977, Tilman and Pacala 1993) and between recruitment and persistence (Bond and Midgley 2003). Interspecific differences in energy allocation after disturbance (Falster and Westoby 2005) are expected to interact with local biotic and abiotic factors to influence community dynamics (Cardinale et al. 2006, Haddad et al. 2008), suggesting the potential for unexpected ecological responses to altered disturbance regimes (Suding et al. 2004). Understanding the factors that influence community recovery following disturbance can help to predict how ecosystems will function under altered environmental conditions.

Plant performance following disturbance is hypothesized to be driven by the supply of limiting resources (Hodgkinson 1992, Clemente et al. 2005). For individuals which survive disturbance, this is likely mediated by their pre-disturbance traits which
influence a plant’s access to nutrients (Paula and Pausas 2011) and its disturbance tolerance (Grady and Hoffmann 2012). As a consequence, the historical context of a site’s history may influence its response to subsequent disturbances by filtering the species pool (Dale et al. 2001, Coop et al. 2016, Dudney et al. 2017) or the traits of the extant community.

In many systems, fire is a major disturbance that both structures vegetation communities (Boring et al. 2004, Coop et al. 2016) and influences N availability (Neary et al. 1999, Wan et al. 2001). Pyrogenic ecosystems are often composed of species which resprout quickly following fire (Bond and Midgley 2001, Bond and Midgley 2003, Hoffmann et al. 2012). Since plant-available nitrogen (N) is often elevated immediately following fire (Certini 2005, Schafer and Mack 2010, Ficken and Wright 2017), short-term plant regrowth may also be enhanced. If plants resprout during the post-fire period of high N availability, fires may influence community composition through the construction of niche axes related both to disturbance tolerance and to N availability (Chesson and Huntly 1997). The extent to which plants have access to N pulses is not known, although plants can respond to nutrient enrichment within a matter of days (Jackson et al. 1990, Wang et al. 2006) and a study of a chaparral shrub found increased leaf N content after burning (DeSouza et al. 1986).
Nitrogen is actively taken up through two forms of uptake transporters on plant roots. At low N concentrations, the high-affinity transport system is responsible for nitrate or ammonium uptake; at high N concentrations, such as during a post-fire N pulse, low-affinity transporters allow high N influx into roots (Hawkesford et al. 2012). The presence of these transporter systems is partially inducible and can be upregulated in the presence of inorganic N (Hawkesford et al. 2012). This indicates that plants can differ in their physiological response to a N pulse. If N is limiting (Vitousek and Howarth 1991, Pellegrini et al. 2014), and if species differ in their ability to capture a N pulse, N supply rates may affect community dynamics via differences in interspecific performance. Frequent N pulses may then select for individuals which can capitalize on the N pulse by, for example, upregulating the production of low affinity uptake transporters following fire. A pulse-specialist species may thus show enhanced performance in areas which have experienced frequent historical fires, and reduced performance in areas under fire-suppression.

In addition to structuring communities by creating N pulses, fire history can affect vegetation disturbance response through its effects on the extant community (Brockway and Lewis 1997, Dale et al. 2001). Fires directly influence vegetation composition by preventing the growth of tree species (Lehmann et al. 2014) and by reducing forb performance relative to grasses (Briggs and Knapp 2001, Buis et al. 2009).
Fire also indirectly affects community dynamics through its effect on fire-soil and fire-vegetation feedbacks: by reducing the accumulation of a litter layer (Christensen 1977) and soil water holding capacity (Certini 2005), fires select for species which can tolerate soil drying. Recent studies on leaf flammability traits indicate that species which produce a loose litter bed (i.e. rather than species whose litter forms a compact, dense litter layer) can promote fire frequency and spread (Cornwell et al. 2015, Grootemaat et al. 2015), possibly leading to positive feedbacks that support fire tolerant vegetation.

Here, we sought to understand how fire history and N availability together and independently influenced the response of plants to subsequent disturbances. To do so, we clipped biomass of longleaf pine communities to simulate the effects of fire, and tracked regrowth over a growing season. Communities had either been exposed to frequent or infrequent prescribed burns, and we additionally treated them with continuous N, pulsed N, or H₂O-only control amendments. We asked: (1) How do burn history and N availability affect short-term resprouting rates? (2) Over the full growing season, how do burn history and N availability affect regrowth rates and biomass accumulation? We hypothesized that frequent fires would select for species able to capitalize on a N pulse, so species dominant in frequently burned sites would show enhanced resprouting rates following a N pulse. We also hypothesized that plants in frequently burned communities would be better adapted to recovering from
disturbances, so expected them to have higher net growing season productivity compared to plants in infrequently burned communities.

4.2 Methods

4.2.1 Study site

Our study was carried out in longleaf pine – wiregrass forests (Pinus palustris – Aristida stricta) on Fort Bragg Military Reservation (35.1391°N 78.9991°W) near Fayetteville, NC, USA. The area has deep, sandy and sandy loam soils from the Candor and Blaney series (Staff). Mean monthly temperature ranges from 6.0 °C to 27.5 °C in January and July, respectively, and mean annual precipitation is 127.5 cm. The uplands of Fort Bragg are dominated by longleaf pine – wiregrass (Pinus palustris – Aristida stricta) communities and the riparian wetlands lining streambeds are dominated by P. serotina and several hardwood species. Separating the uplands from the lowlands, the ecotones have a dense understory vegetation composed primarily of ericaceous species and other shrubs (see below for specifics).

Since the 1990s, prescribed burns have occurred on three-year rotations to maintain habitat for rare and endangered species on the reservation. While the uplands and ecotones experience regular fires, the low-intensity prescribed burns more rarely spread into the wetter, denser communities along streambeds. Fort Bragg is divided into burn parcels (hereafter “sites”, each ~45 ha), each with an independent burn history.
Between 2011 and 2014, some sites were experimentally managed with annual prescribed burns, while others were kept under fire suppression; after 2014, sites were returned to independent three-year fire rotations.

4.2.2 Experimental treatments

In this study, we targeted two high fire frequency and two low fire frequency sites in which to study vegetation regrowth response to fire simulation (i.e. clipping). High fire frequency sites experienced seven and nine fires in the past ten years and low fire frequency sites experienced one and three fires in the past ten years; all sites burned in either 2016 or 2017. These sites were chosen based on their histories of particularly high or low fire frequencies, their similar times since last fire, and the relative similarity of their dominant vegetation prior to the start of the experiment. At each site, we installed 15 plots on a transect running along the ecotone. Plots were 1 m\(^2\) and spaced approximately 1 m apart. Five plots per site were randomly assigned to each nitrogen (N) amendment treatment: control, continuous, or pulse. In control plots, 1 L H\(_2\)O was applied every other week for 10 weeks; in continuous N plots, 0.6 g N in 1 L H\(_2\)O as applied every other week for 10 weeks (3 g N applied in total); in pulse N plots, 3 g N in 1 L H\(_2\)O was applied the first week, followed by 1 L of water for the remaining four application cycles. The mass of N applied in the continuous and pulse N treatments was
chosen to approximate the increase in N following fire as documented in previous work (from <1 to 20 µg N gds-1; Ficken and Wright 2017)

In early June 2017, we recorded the presence/absence of all species with greater than ~5% cover in our study plots. Aboveground vegetation from each site was separated by species, and clipped 2 cm above the ground. This clipping was performed to simulate aboveground biomass loss during fire. Biomass of individual species with <5% cover was bulked and categorized as “other”. Biomass was dried at 40 °C until a constant weight and weighed. Although we applied N treatments following clipping, an unexpected tropical storm deposited 2-7 cm on the surrounding areas in less than one week (approximately 20 – 70 L per plot). To ensure the N was not washed out of the rooting zone, we reapplied N treatments June 22, 2017 and consider this the first N treatment application. N treatments were applied five times over a ten-week period.

4.2.3 Biomass estimation

We identified the five most common species in our study plots: *Arundinaria tecta*, *Clethra alnifolia*, *Gaylusaccia frondosa*, *Ilex glabra*, and *Pteridium aquilinum*. For all species except *P. aquilinum*, we collected weekly height measurements of each stem. For these species, we used previously published species-specific allometric equations to estimate biomass from height measurements (Hollingsworth 2015). For *P. aquilinum*, we fit allometric growth curves to area and biomass measurements collected from 56
individuals of a range of sizes collected adjacent to our study plots. Using the resulting equation (Appendix C), we estimated biomass for *P. aquilinum* in our study plots from aerial length and width measurements. We estimated biomass for 11 weeks during the growing season (June 22, 2017 – September 9, 2017), with the final measurement coming two weeks after the last N treatment application. After the final biomass estimation, we harvested aboveground biomass of all focal species in each plot. We also bulked and harvested the biomass of non-focal species. All biomass was dried at 40 °C and weighed.

### 4.2.4 Statistical analyses

All statistical analyses were performed with R version 3.3.2 (Team 2011). To examine the community composition of our study plots, we performed nonmetric multidimensional scaling (NMDS) on initial empirical biomass measures with the metaMDS function in the vegan package (v 2.4.4). We performed PERMANOVA tests with the adonis function in the vegan package to test for differences in multivariate trait spaces among sites and between historic fire frequencies.

We examined vegetation regrowth among fire frequency and N treatments in two ways. First, in order to capture any ephemeral response to the N treatments, we examined biomass regrowth within one month of clipping. We refer to this as the short-term resprouting response. To examine short-term resprouting rates, we fit lines to biomass estimates collected within ~1 month of resprouting (33 days). We fit linear
mixed effects models with the lme function in the nlme package (v 3.1). Estimated biomass of each species was the response variable, fire frequency, N treatment, initial biomass, and date were the predictors, and plot was a random effect. To account for potential differences in community composition, we also included the initial biomass for that species as an additional predictor in each model. Beginning with the full models (ie including 3 way interactions), we dropped non-significant terms sequentially and best models were chosen to minimize AIC values.

Second, we examined biomass regrowth over the full growing season, from the end of May through the beginning of September. In order to examine regrowth patterns over the full growing season, we fit logistic curves to biomass regrowth time series with the nlsList function in the nlme package. We fit curves individually to each species present in each plot. From these growth curves, we extracted the maximum biomass estimates (i.e. upper asymptote) and maximum growth rates. We fit linear mixed effects models with maximum biomass or growth rate as the response, fire frequency, N treatment, initial biomass, and date as the predictors, and plot as a random effect. As in our models of short-term resprouting, we began with full models, dropped non-significant terms sequentially, and chose best models to minimize AIC values.
4.3 Results

4.3.1 Pre-treatment community composition and site characteristics

We aimed to pick sites based, partly, on the similarity of their vegetation composition. Prior to manipulation, frequently-burned (134.78 ± 26.19 g m$^{-2}$) and infrequently-burned sites (179.78 ± 15.76 g m$^{-2}$) did not differ in aboveground biomass (t-test $p=0.148$) despite high fire frequency plots having significantly more species per plot (4.5 ±0.3 species m$^{-2}$) than low fire frequency plots (3.6 ±0.2 species m$^{-2}$; t-test $t=2.41$, df=49.6, $p=0.019$). *I. glabra* was the most common species across all study plots and was found at every study site. *I. glabra* occurred in 80% of plots and comprised 37 ±2% of the biomass per plot in plots where it occurred. *G. frondosa* was found in 60% of plots (28 ±4% of biomass), followed by *P. aquilinum* (45% of plots), *A. tecta* (38% of plots), *Aristida stricta* (28% of plots), and *C. alnifolia* (27% of plots). Although fire frequency explained only 10% of variation in initial composition, initial plant community composition differed significantly between historical fire frequencies (Adonis test, $p = 0.001$). Initial plant community composition also differed between sites (Adonis test, $p = 0.001$ for both), and site explained 40% of variation in initial composition.

4.3.2 Effects of N amendments on short-term resprouting rate

N treatment had minimal effects on regrowth dynamics. In the short-term, only the resprouting rates of *G. frondosa* and *P. aquilinum* were affected by N treatment.
N treatment did not impact growing season regrowth rate nor biomass production for any species. Under both low and high fire frequency, N treatment had a significant interaction with date (p<0.001 for both), indicating that *G. frondosa* and *P. aquilinum* individuals resprouted at different rates depending on N supply (Figure 15). Under low fire frequency, *G. frondosa* individuals given a continuous N resprouted faster than those given no additional N (i.e. control treatment; p=0.004) and individuals given a N pulse (p<0.001). *P. aquilinum* individuals also resprouted fastest when given continuous N (versus control p<0.001; versus pulse p=0.003). Under high fire frequency, *G. frondosa* individuals given no N resprouted faster than those given continuous N or pulse N (p<0.001 for both; Figure 15).
Figure 15: Short-term resprouting of *G. frondosa* and *P. aquilinum* individuals exposed to control, continuous, or pulse N treatment and in low-frequency or high-frequency fire history treatments. *G. frondosa* (low and high fire frequency) and *P. aquilinum* (low fire frequency only) individuals resprouted at different rates depending on N treatment in each fire history treatment group (p<0.001 for each). See text for additional statistical details.

### 4.3.3 Effects of fire history on short-term resprouting rates

Fire frequency had a much stronger effect than N treatment on both short-term resprouting and regrowth over the full growing season. Fire frequency significantly affected the short-term resprouting rates of all focal species except *A. tecta* (Figure 16).

Both *C. alnifolia* and *I. glabra* resprouted faster when conditioned to frequent
disturbances compared to infrequent disturbances: when conditioned to high fire frequency, *C. alnifolia* resprouted ~50% faster (1.7 vs 1.2 g m\(^{-2}\) day\(^{-1}\); p=0.038) and *I. glabra* resprouted 10 times faster (11.4 vs 1.1 g m\(^{-2}\) day\(^{-1}\); p<0.001) than their counterparts conditioned to low fire frequency. *G. frondosa* also generally displayed faster resprouting under high fire frequency, although we analyzed low and high fire frequency treatments separately since models of *G. frondosa* biomass resprouting included a significant three-way interaction between fire frequency, N treatment, and date. Nevertheless, *G. frondosa* exposed to continuous N amendment resprouted 4 times faster when exposed to high versus low fire frequency (3.7 vs 0.9 g m\(^{-2}\) day\(^{-1}\); p=0.001). In contrast to *C. alnifolia, I. glabra*, and *G. frondosa*, resprouting of *P. aquilinium* was enhanced under low fire frequency relative to high fire frequency (p=0.001).
Figure 16: Short-term biomass resprouting over time for each focal species conditioned to either frequent or infrequent prescribed fires. Regression lines are shown with ±1 SE error cloud. *Models of G. frondosa biomass included a significant 3-way interaction between fire frequency, N treatment, and date.

4.3.4 Effects of fire history on regrowth over the full growing season

Over the full growing season, species differed in their maximum regrowth rates, but total plot-level biomass did not differ between plots that had burned frequently or infrequently (Figure 17a-c). A. tecta, G. frondosa, and P. aquilinium all showed higher
maximum growth rates when conditioned to low than high fire frequency (Figure 17c). *A. tecta* regrew 8x faster (0.5 vs <0.1 g m\(^{-2}\) day\(^{-1}\); p=0.013) and *G. frondosa* regrew 3x faster (1.1 vs. 0.4 g m\(^{-2}\) day\(^{-1}\); p=0.009; Figure 17c) when individuals had been exposed to low frequency burns than high frequency burns. The best models explaining *P. aquilinium* maximum growth rate retained the interaction between fire frequency and N treatment. In general, however, *P. aquilinium* exposed to continuous N fertilization had nearly 40% faster growth rates when conditioned to low compared to high historical fire frequency (p=0.041). Under low fire frequency, *P. aquilinium* had faster regrowth rates when treated with continuous N fertilization compared to no fertilization (p<0.001) and pulse N (p=0.054).
Figure 17: Full growing season estimated net productivity (a), harvested biomass (b), and maximum growth rate (c) for each focal species. Panel (a) shows biomass estimates derived from allometric equations. Panel (b) shows the biomass harvested at the end of the growing season; dashed lines indicate the mean biomass prior to clipping, at the beginning of the growing season. Panel (c) shows the average maximum growth rate as estimated from logistic growth equations. Panels depict mean ± 1 SE. Significance levels are indicated as follows: p<0.100 (*), p<0.050 (**), p<0.010 (***).

4.3.5 Biomass regeneration

Patterns of net growing season biomass production largely paralleled patterns of maximum regrowth rate (Figure 17). Under low historical fire frequency, *G. frondosa*
(p=0.019) and *P. aquilinum* (p<0.001) had higher net productivity following clipping than when conditioned to high historical fire frequency (Figure 17b). *A. tecta* exhibited marginally higher net productivity under the same conditions (p=0.100; Figure 17b). *G. frondosa* regrew 17.00 ±3.89 g m\(^{-2}\) biomass over the growing seasons under low fire frequency – more than 2x as much as *G. frondosa* under high fire frequency (6.62 ±1.56 g m\(^{-2}\); Figure 17b). *P. aquilinum* regrew ~5x more biomass under low fire frequency (7.28 ±1.54 g m\(^{-2}\)) than under high fire frequency (1.60 ± 0.40 g m\(^{-2}\); Figure 17b). In contrast, *I. glabra* regrew nearly 50% more biomass under high fire frequency (32.60 ±4.33 g m\(^{-2}\)) than under low fire frequency (22.72 ±5.00 g m\(^{-2}\)), but this difference was not significant.

After one growing season, only biomass in high fire frequency plots had recovered to pre-clipping levels (Figure 18). By the end of the growing season, the median biomass in low fire frequency plots (7.58 g m\(^{-2}\); interquartile range (IQR): 2.86-16.89) was only 20% of what was initially present (36.08 g m\(^{-2}\); 8.51-64.96). In contrast, by the end of the growing season the median biomass in high fire frequency plots had recovered to 75% of the initial biomass (6.65 g m\(^{-2}\); IQR:1.36-43.55; Figure 18).
4.4 Discussion

In this study, we examined how plants’ disturbance responses were mediated by different forms of environmental heterogeneity. Previous fire history had a strong and significant effect on species’ responses to subsequent disturbances, whereas the timing of N availability had a more limited impact. Species exhibited high levels of regrowth plasticity between fire regimes – individuals differed in their short-term resprouting
rates and in their regrowth rates measured over the full growing season when conditioned to frequent versus infrequent fires. Individuals also differed in biomass production between fire regimes. However, because some species showed enhanced growth under low fire frequency and others showed enhanced growth under high fire frequency, fire frequency had no net effect on total plot-level productivity. These findings suggest that fire history may drive shifts in community composition via interspecific differences in growth response to disturbance, but that the effects of fire history do not necessarily extend to differences in ecosystem-level productivity among fire management regimes.

### 4.4.1 Short-term resprouting response

Rapid resprouting following disturbance has been proposed as a strategy by which plants tolerate frequent disturbances (Grady and Hoffmann 2012). In support of this, we found that the resprouting of individuals conditioned to frequent disturbances temporarily outpaced that of individuals conditioned to infrequent disturbances. Species for which individuals which did not differ in resprouting rates between disturbance frequencies may prioritize competitive ability over high productivity (Grime 1977, Tilman and Pacala 1993). High resprouting rates have been hypothesized to result from enhanced resource availability post-fire (DeSouza et al. 1986, Hodgkinson 1992). Fires are associated with a short-term increase in plant-available N (Wan et al. 2001, Ficken
and Wright 2017), and interspecific differences in root morphology are associated with different patterns of pulsed N assimilation (Ficken and Wright, *in prep*; chapter 2). We therefore expected that N fertilization would enhance the resprouting rates of our focal species. Surprisingly, we found no effect of N addition on resprouting rates. In a study of a chaparral species, DeSouza et al. (1986) found that burned individuals had higher leaf N content than unburned individuals, suggesting that resprouting stems may have access to additional N from soil or from reserves in belowground storage organs. Other studies have proposed that reduced demand for other resources following fire, and thus their increased availability, may drive enhanced resprouting rates following fire. Resprouting shrubs have been shown to have improved water relations compared to unburned shrubs (Hodgkinson 1992), which, especially if water availability limits growth (Mitchell et al. 1999a), may promote resprouting rates following fire. At the ecosystem-level, evapotranspiration rates did not vary before and after fire in longleaf pine forests (Whelan et al. 2013) which suggests that soil water availability is not affected by fire, we cannot rule out the possibility that soil water availability increased in localized areas.

### 4.4.2 Growing season regrowth and productivity

Disturbances that lead to repeated loss of biomass are likely to negatively impact a plant’s performance (Schutz et al. 2009). Individuals of three woody shrub species (C.
*Alnifolia, G. frondosa, and I. glabra* conditioned to frequent disturbances all initially showed very high resprouting rates, but this did not translate to higher biomass production relative to conspecific individuals conditioned to infrequent disturbances. Repeated burns may deplete belowground C stores needed for regrowth (Schutz et al. 2009), ultimately reducing woody shrub cover (Roques et al. 2001) if plants cannot recover biomass before another burns. Over the full growing season, we found that individuals growing in sites burned with especially low fire frequency showed faster regrowth and higher productivity following clipping than those growing sites burned with especially high fire frequency. This is consistent with previous work in tallgrass prairie showing high post-burn productivity in long-unburned sites (Blair 1997), and with work in longleaf pine forests documenting reduced growth of longleaf pines with frequent fires (Ford et al. 2010). Even in a system which experiences fires every 1–3 years, our findings suggest that repeated frequent disturbances may reduce species’ resilience to biomass loss. This may presage shifts in community composition if the extant community cannot withstand the disturbance frequency and severity.

In pyrogenic systems, soils tend to be nutrient poor and N cycling tends to be tight with few losses (Wilson et al. 2002), so primary production is thought to be limited by N availability (Bustamante et al. 2006, Pellegrini et al. 2014, Pellegrini 2016). As such, we expected that N fertilization would alleviate N limitation and enhance productivity.
following clipping (Blair 1997). In a study of North American and South African savannas, primary production in annually burned sites was enhanced with N addition (Buis et al. 2009). Although here we found that the resprouting of G. frondosa and P. aquilinimum differed significantly across fire and N treatments, we did not find consistent evidence to support this hypothesis. Although pyrogenic systems are thought to be N-limited, our results do not support this finding. Research in southern pine forests suggests that soil moisture may be an important driver of productivity (Mitchell et al. 1999a) and, more specifically, that individual species are differently affected by N, water, and light availability (Mitchell et al. 1999b).

In contrast to N availability, we found that fire history significantly affected understory species productivity over the growing season. The majority of our focal species exhibited enhanced biomass production under low fire frequencies, but infrequently disturbed sites did not show higher plot-level net productivity. This was due to I. glabra which was unresponsive to fire history and was dominant or co-dominant under both fire history treatments. As such, proportionally large changes in, for example, A. tecta had relatively little impact on net plot-level productivity. Previous studies have found only modest effects of fire on annual net primary productivity (Carter and Foster 2004 and citations therein), though results appear to be highly context
dependent, and also appear to differ among which species are examined (Ford et al. 2010, Kirkman et al. 2014, Schafer and Mack 2014b).

4.4.3 Conclusions

If temperate forests are N limited, N availability should have a strong impact on growth and recovery following disturbance. However, we found that N supply had little to no impact on resprouting dynamics. Instead, fire history strongly affected demographic processes by altering resprouting trajectories. Differences in biomass accumulation patterns were observed within species only, and not at the plot level. This indicates that the effects of fire history on vegetation occurred not through a filtering of the community, but instead through physiological plasticity at the species level. These findings suggest that a site’s disturbance history must be considered when anticipating future functioning, such as when developing restoration plans or predicting responses to future climate scenarios.
5. Soil respiration sensitivity to drought differs between an arbuscular and an ectomycorrhizal system

5.1 Introduction

Extreme climatic events, such as heat waves and droughts, are projected to increase in frequency and severity under future climate scenarios (IPCC 2012, 2014). Relatively little research, however, has incorporated extreme events into studies of ecological responses to climate change. Quantifying the impacts of extreme events on the terrestrial carbon (C) cycle is of particular importance due to the sensitivity of the terrestrial C sink strength to extreme events, particularly precipitation anomalies and drought (Reichstein et al. 2013, Zscheischler et al. 2014, Wolf et al. 2016). Soils represent the largest store of C in the terrestrial biosphere (1500 – 2400 Pg; IPCC 2013) and as a consequence, small changes to soil C cycling can have large consequences for atmospheric carbon dioxide (CO₂) concentrations. In particular, soil respiration (Rₚ) is the largest flux of terrestrial C to the atmosphere (100 Pg C year⁻¹) and its increase over time implies a positive correlation with air temperature (Bond-Lamberty and Thomson 2010) or an overall increase in C cycling. But it is not clear that our understanding of the current relationships between soil Rₚ and its abiotic drivers (largely soil moisture and temperature) will remain appropriate under altered precipitation regimes (Vicca et al. 2014). Without studies explicitly targeting extreme environmental conditions, the impacts of such events on ecosystem functions will remain uncertain.
Predicting ecosystem responses to extreme events hinges upon understanding feedbacks between numerous biotic and abiotic components. Extreme events may have direct and indirect, immediate and lagged impacts on a range of functions within an ecosystem (Frank et al. 2015). A single function may respond or not, recover or not (Jentsch et al. 2011, Smith 2011) and the scale of a response may range from an individual to the ecosystem. For example, soil $R_s$ is the total CO$_2$ emitted from soils from microbial, mycorrhizal, and plant (root) communities, and its response to extreme events will represent the net response of all biotic components, as well as their feedbacks with each other. Indeed, models missing key root parameters (e.g. growth) perform poorly in predicting ecosystem-specific C fluxes during drought (Hanson et al. 2004), emphasizing the need to incorporate physiological responses of individual biotic groups to carbon cycling models. Incorporating and improving the mechanistic representation of rhizosphere processes into earth system models is largely expected to improve their accuracy in predicting global C biogeochemistry under future climates scenarios, including extreme events (Warren et al. 2015, Bradford et al. 2016, Schlesinger et al. 2016).

All biotic components of soil $R_s$ will be affected by climate change, but the extent and direction to which each component will change is not clear. Across global forests, heterotrophic $R_s$ is positively correlated with overall soil $R_s$ (Bond-Lamberty et al. 2004).
While the contribution of heterotrophic $R_s$ declines with increasing $R_s$, it generally accounts for over 50% of total soil $R_s$ (Subke et al. 2006). The contributions of mycorrhizae to total soil $R_s$, like that of microbial respiration, are expected to be seasonally linked to both plant phenology and belowground C allocation (Litton et al. 2007, McCormack et al. 2014). Some studies have estimated mycorrhizal $R_s$ to be a smaller proportion than that of free-living microbes or plant roots, although not inconsequential (~10 and ~25% of total soil respiration, respectively; Subke et al. 2006, Heinemeyer et al. 2007). However, plants can allocate up to 85% of net photosynthate to mycorrhizae (Treseder and Allen 2000), suggesting that, seasonally, mycorrhizae have the potential to contribute substantially to overall soil $R_s$ patterns (Talbot et al. 2008).

It is thought that changes to plant functioning (Schlesinger et al. 2016) in response to drought will initiate changes in belowground biogeochemistry. Autotrophic (plant root) $R_s$ is allometrically related to photosynthesis (Heilmeier et al. 1997, Litton et al. 2007), which itself varies in response to temperature, precipitation, and nutrient availability (Chapin 1980). Mycorrhizal $R_s$ is less well-studied (Moore et al. 2015), but we anticipate that changes in mycorrhizal $R_s$ during drought will be driven both directly by declines in soil moisture and indirectly by reductions in photosynthate-C provided to the mycorrhiza by the plant host. In comparison to AM, EM are thought to have both a greater hyphal spread away from the host plant and a greater carbon demand from the
While the former might allow EM to forage further for soil moisture, the latter carbon constraint may result in abrupt declines in EM Rs relative to AM Rs. Microbial Rs varies with temperature and substrate availability, the latter of which is largely determined by microbial and mycorrhizal exoenzyme production (Schimel and Weintraub 2003, Wieder et al. 2013). We expect the that autotrophs will exhibit the steepest declines in Rs with declining water availability (i.e. the greatest drought sensitivity), followed by mycorrhizal and microbial Rs.

AM and EM both appear to increase soil aggregation and buffer plant roots from hydraulic stress (Allen 2007, Querejeta 2017), although the underlying mechanisms are unclear. Both fungal groups can redistribute soil water via their hyphae (Warren et al. 2013, Querejeta 2017). Morphologically, AM hyphae tend to be finer than EM hyphae, which may give them access to water in smaller soil pores, but may also make them more susceptible to desiccation under extreme drought. AM fungi produce glycoproteins that are thought to enhance soil-aggregation and rhizosphere water retention (Rillig 2004), allowing for more water loss from bulk soil before a wilting response is observed in host plants relative to non-mycorrhizal plants (Augé 2001, Augé et al. 2004). EM fungi produce nets of mycelium that alter soil hydraulic properties and increase water retention during drought. Finally, AM and EM fungi often form symbioses with different tree species, which themselves may differ in hydraulic traits,

In addition to uncertainty surrounding the individual responses of roots, mycorrhizae, and microbes to extreme drought, their capacity to recover remains unclear. Rewetting following drought produces a pulse of high CO$_2$ flux (Hagedorn et al. 2016), which, after an extreme rain event, can account for up to 10% of annual net ecosystem CO$_2$ production (Lee et al. 2004). The source of this pulse is debated, but is hypothesized to be driven by increases in microbial activity as labile C substrates – root exudates, lysed microbial cells or newly available OM – increase in availability (Fierer and Schimel 2002, 2003). The degree to which plant roots, free-living microbes, or mycorrhizal hyphae are responsible for CO$_2$ pulses following dry-rewetting cycles is not known. In the absence of plants (and presumably mycorrhizae), CO$_2$ production in laboratory rewetting incubations was strongly correlated with new microbial DNA production (Blazewicz et al. 2014), suggesting that the CO$_2$ flush observed after rewetting is correlated with the proliferation of microbial biomass. However in a field study, the contribution of EM hyphae to total soil $R_s$ increased relative to its contribution before or during a drought (Heinemeyer et al. 2007), suggesting that mycorrhizal respiration may contribute non-negligibly to CO$_2$ pulses after rewetting events.
In this study, we quantify the effects of drought on C dynamics as mediated by the belowground community. In mesocosms planted with AM (*Acer saccharum*) or EM tree seedlings (*Quercus alba*), we examine the sensitivity of plant roots, mycorrhizal hyphae, and free-living microbes to an extreme dry-rewetting event, which mimics events projected under climate change. We ask: (1) How is the production of OM-degrading enzymes affected by drought and rewetting? (2) Do belowground biota differ in their R_s sensitivity to (here defined as the rate of respiration decline) and recovery from drought? To examine these processes within an ecological framework, we also measured changes in photosynthesis and fungal community composition in response to a dry-rewetting event. Since AM hyphae have been shown to enhance rhizosphere water retention (Augé et al. 2004), we hypothesized that that AM systems would be less susceptible to drought than EM systems and, consequently, that AM chambers would maintain higher R_s and enzyme activity during drought than EM. In the absence of mycorrhizal hyphae, we expected that AM and EM systems would respond similarly to drought.

### 5.2 Methods

#### 5.2.1 Experimental design

In May 2016, we collected seedlings of an AM species (sugar maple, *Acer saccharum*) and an EM species (white oak, *Quercus alba*) from a mixed hardwood stand in
Oak Ridge, TN, USA. To minimize potential genetic variability, seedlings were collected from a small area (~5 m radius) where both species co-occurred. Initial heights were approximately 15 cm for both species and seedlings were all estimated to be less than 1 year old. To preserve the native microbial community, seedlings were not washed prior to being transplanted into mesocosms.

We constructed mesocosms (38x23.5x18 cm LxWxH or 4-gallon capacity) with mesh partitions that divided each mesocosm into three chambers (Appendix B; Figure 23). One seedling (40 A. saccharum and 20 Q. Alba) was planted into the center chamber of each mesocosm to establish the full chamber. One mesh partition (35 µm) excluded fine root growth, allowing only mycorrhizal hyphae and free-living microbes to colonize (root-exclusion chamber). The second mesh partition (1 µm) excluded fine root and mycorrhizal hyphae growth, allowing only free-living microbes to colonize (microbial chamber). Small holes were drilled through the bottom of each mesocosm to avoid water logging. Each chamber had a schedule 40 PVC collar (10 cm height, 5 cm diameter) installed for gas sampling (see below). Twelve windows (each 3.7 cm diameter) were drilled into each PVC collar to allow biotic and hydrologic connectivity between the bulk soil in each chamber and the soil inside the collar (Appendix B; Figure 23). Mesocosms were filled with Fafard 52 mixed with slow-release Osmocote Plus Fertilizer (15-9-12, NPK).
Seedlings in mesocosms grew and acclimated for four months prior to the start of the experiment. During this time, mesocosms were watered as needed. Mesocosms were divided evenly between treatment (drought) and control (well-watered) conditions (N=20 A. saccharum; N=10 Q. alba per treatment) and randomized onto a bench in the greenhouse. The greenhouse was maintained between 18-21°C throughout the duration of the experiment; 1000 watt HPS lights supplemented ambient sunlight to maintain the light intensity in the greenhouse above 200 µmol m⁻² s⁻¹ during the day (06:00 – 22:00).

The experiment ran for eleven weeks from September – November 2016 (Figure 25; Appendix B). The pre-drought period (week 1) was followed by seven weeks of drought (weeks 2-8), and a three-week recovery period (week 9-11). The duration of this drought corresponds to a 69-year drought in Oak Ridge, TN, with the longest drought (53 days with ≤1mm precipitation) since 1948 occurring in fall 2016 (Menne et al. 2012a, Menne et al. 2012b). During the pre-drought period, all mesocosms were maintained under well-watered conditions. During the drought period, control mesocosms were watered bi-weekly while treatment mesocosms were left un-watered and soils progressively dried down. Control mesocosms were initially maintained between -0.90 and -0.75 MPa (65-75% of field capacity, which was -0.45 MPa) during the drought period. To increase the difference in water availability between drought and control mesocosms, however, we increased water availability of control mesocosms and
maintained them at -0.50 MPa for the remaining five weeks of the drought period.

During the recovery period, we watered control and treatment mesocosms to saturation, and allowed them to drain to field capacity. Because the drought increased the hydrophobicity of treatment soils, treatment mesocosm retained less water than control mesocosms after the first watering during the Recovery period. Consequently, we re-watered all mesocosms to saturation for two consecutive days. For the first re-watering, we watered mesocosms in the evening and allowed them to drain overnight for 12 hours prior to measuring soil $R_s$ (see below) the following morning.

**5.2.2 Soil water content**

To maintain mesocosms at the target water contents, we weighed mesocosms, calculated the volumetric water content of each mesocosm (based on known mesocosm soil weight), and added water by hand at least weekly to bring water volume up to the target level. We constructed water release curves using soil subsamples that we progressively dried and re-measured in a WPC4 Water Potential Meter (Decagon Devices; Pullman, USA). We used these curves to estimate the water potential of each mesocosm based on its volumetric water content and known bulk density. Although water release curves constructed by drying wet soils may differ from those constructed by wetting dry soils, our rewetting event during the Recovery period aimed to bring soils to full saturation rather than slowly increase soil water availability. For this reason,
we did not construct separate water release curves to estimate soil water content during the recovery period. The presence of root and fungal biomass, as well as likely differences in soil structure (e.g. bulk density, water content) among the three chambers of each mesocosm increased uncertainty of the actual water potentials in each chamber. However, the integrated whole mesocosm estimate still provided a good guide to relative water stress between mesocosms and should be considered in that context. We report water availability as soil water potential ($\Psi_{\text{soil}}$), as calculated from abovementioned water release curves.

### 5.2.3 CO$_2$ measurements

To assess soil $R_s$, we measured CO$_2$ efflux at least weekly by sampling the capped PVC collar headspaces (196 cm$^3$) in each chamber. PVC collars were capped with PVC caps fitted with butane septa. Caps were installed with needle vents to avoid headspace pressure changes during capping, and the headspace was mixed by pumping 3x35 mL with a syringe. The needle vent was removed from the septum and a 1-mL sample was drawn from the headspace and immediately injected into a Li-Cor 6252 infrared gas analyzer (Li-COR Inc., Lincoln, Nebraska, USA), modified to detect small volume CO$_2$ sample injections carried with an N$_2$ carrier gas. PVC collars were left capped for ~1.5 hours, and resampled after this incubation. To minimize diel variation in $R_s$ rates, we sampled headspaces only in the morning (08:00 – 11:00), and sampled mesocosms and
chambers in the same order every sampling date. At each sampling time, we used standard CO₂ gases to create a standard curve with which we calculated the CO₂ concentration in each sample’s headspace. During the drought period, watering events of control mesocosms occurred no closer than two days prior to Rs measures to minimize any short term increases in Rs due to the watering event.

5.2.4 Enzyme activity assays

To assess the nutrient acquisition functioning of the microbial and mycorrhizal communities, we measured potential soil enzyme activity in each chamber from a subset of mesocosms (N=5 from each treatment) at the end of each period (pre-drought, drought, recovery). Soil samples were stored at 4°C and analyzed within one week of collection for enzyme activity according to Bell et al. (2013). We targeted seven enzymes involved in organic matter degradation: α-glucosidase, β-glucosidase, cellobiohydrolase, xylanase, N-acetyl glucosaminidase, leucine-amino peptidase, and acid phosphomonoesterase. Briefly, we homogenized 1 g field moist soil in 70 mL of 50 mM sodium acetate buffer (pH 4) with an immersion blender. We incubated 800 µL soil slurry with 200 µL fluorescent substrate in 96-well deep-well plates for two hours at 25°C. We prepared separate standard curves for each soil sample by incubating 800 µL soil slurry with 200 µL standard (4-Methylumbelliferone, MUB; or 7-Amino-4-methylcoumarin, MUC, reference for leucine-amino peptidase only) in deep-well plates.
with sample plates. After incubating, we inverted and centrifuged deep-well plates, and transferred 250 µL to black-bottom 96-well plates and read them on a fluorescence microplate reader (365 nm excitation, 450 nm emission). We report enzyme activity as the sum of N (N-acetyl glucosaminidase and leucine-amino peptidase), P (acid phosphomonoesterase), and C (all others) degrading enzymes. See Table 3 in Appendix B for mean enzyme activity of individual enzymes.

5.2.5 Photosynthesis and leaf water potential

To assess belowground C dynamics in context of potential availability to roots or mycorrhiza, we measured photosynthesis on a subset of plants (N=5 of each species in each treatment) with a Li-COR 6400 (Li-COR Inc., Lincoln, Nebraska, USA) at six time points throughout the experiment. One leaf was chosen per plant and measured at each time point. On one plant, the sample leaf abscised during the drought treatment; for all other samples, we measured the same leaf regardless of its wilting status.

To quantify the degree of plant water stress during the drought, we measured pre-dawn leaf water potential from all plants on the last day of the Drought period. One leaf was collected from each plant before dawn, placed into a plastic bag with a small piece of slightly moistened paper towel and stored at 4 °C until analysis could be completed over the next several hours using a PMS Pressure Chamber Model 610 (PMS Instrument Co., USA).
5.2.6 Biomass

At the conclusion of the experiment, we harvested above- and belowground plant biomass from each mesocosm. Root growth had filled full chambers, including inside the PVC collars. We excavated the remaining root biomass from the bulk chamber soil and rinsed it in water to remove excess soil. Aboveground and belowground biomass was dried at 70 °C until a constant mass, then weighed. Root:shoot ratios (R:S) were calculated as the root biomass divided by the shoot biomass.

5.2.7 Statistical analyses

We used the lme() function in the nlme package (version 3.1) in R (3.3.2 Team 2011) to build linear mixed effects models to test for differences in response variables across experimental treatments and through time. In all models, we controlled for unmeasured differences among plants (e.g. initial biomass) by including mesocosm as a random effect. Fixed effects included species (maple, oak), treatment (drought, control), or chamber (microbial, root-exclusion, full). At each experimental period (pre-drought, drought, recovery), we examined how photosynthetic rate, soil water potential ($\Psi_{\text{soil}}$) and leaf water potential ($\Psi_{\text{leaf}}$) varied among species and treatments.

To examine how enzyme production was affected by drought and rewetting, we tested for the effects of treatment (control vs drought), chamber (full, root-exclusion, or
microbial), and their interaction on enzyme activity at each time point. We report ANOVA results of linear mixed effects models.

To assess how $R_s$ rates were affected by drought and re-watering, we examined $R_s$ in two ways. First, we used mixed effects models to compare $R_s$ (relative to mean control $R_s$) during the final week of each experimental period between chambers; comparisons between chamber means were analyzed with Tukey post-hoc tests. For the recovery period, we also compared relative $R_s$ between chambers at each time point following rewetting. We calculated Holm-corrected p-values with the p.adjust() function in the stats package in R to test for differences in relative $R_s$ between chambers across weeks within the recovery experimental period. Second, we examined the drought-sensitivity of $R_s$ across species and chambers. To do so, we tested for significant effects of final biomass (aboveground and belowground), species, $Ψ_{soil}$, and the species*Ψ_{soil} interaction in predicting log-transformed $R_s$ rates. These models, too, included mesocosm identification as a random effect to control for unmeasured differences among plants. Because we tested for $Ψ_{soil}$ as a continuous predictor of $R_s$, we did not test for treatment differences. Significant main effects of species indicated a significant intercept difference; significant interaction terms indicated that species differed in their sensitivity to drought (i.e. the rate at which their respiration rates declined with drought). Best models were chosen to minimize the Akaike Information Criterion (AIC).
5.3 Results

5.3.1 Plant biomass, water availability, and photosynthesis

Oaks had significantly higher root:shoot ratios (R:S) than Maples (R:S\text{Oak} = 2.05 ± 0.04; R:S\text{Maple} = 0.86 ± 0.02; F=35.0, p<0.001), but R:S did not differ between drought treatments. Oak mesocosms (Ψ\text{soil} -0.85 ± 0.01 MPa) and Maple mesocosms (-0.87 ± 0.01 MPa) had similar water availability in the pre-drought period, and there were no pretreatment differences in Ψ\text{soil} of mesocosms assigned to drought or control treatments. (Appendix B; Figure 24). Water availability in drought mesocosms declined substantially throughout the seven-week drought. By the end of the drought, Ψ\text{soil} in drought Maple and Oak mesocosms was -1.74 ± 0.02 and -1.73 ± 0.03 MPa respectively (Ψ\text{soil} of control Maple and Oak mesocosms was -0.64 ± 0.01 and -0.60 ± 0.01 MPa). After two weeks of well-watered conditions during the recovery period, differences in water availability between drought and control mesocosms had disappeared for both species.

Differences in soil water availability at the end of the drought corresponded to differences in the pre-dawn leaf water potential (Ψ\text{leaf}) between control and drought plants (t=4.06, p<0.001). Species did not differ in Ψ\text{leaf} despite Oak plants having significantly higher root:shoot ratios than Maple plants (F=35.0, p<0.001). For maple, Ψ\text{leaf} of control and drought plants was -0.42 ± 0.04 MPa and -1.24 ± 0.24 MPa, respectively. Oak Ψ\text{leaf} of control and drought plants was -0.59 ± 0.09 and -1.34 ± 0.26 MPa.
Despite strong differences in water availability, \( \Psi_{\text{leaf}} \), and root:shoot ratio, photosynthesis was highly variable throughout the experiment and there were no significant differences between control and drought Maple or Oak plants at any date. Throughout the drought, mean photosynthetic rate of Maple and Oak control plants was 1.20 ±0.08 µmol CO\(_2\) m\(^{-2}\) sec\(^{-1}\) and 5.00 ±0.15 µmol CO\(_2\) m\(^{-2}\) sec\(^{-1}\). Mean photosynthetic rate of Maple and Oak drought plants was 0.43 ±0.02 µmol CO\(_2\) m\(^{-2}\) sec\(^{-1}\) and 3.92 ±0.15 µmol CO\(_2\) m\(^{-2}\) sec\(^{-1}\). Across all experimental periods, photosynthetic rates differed significantly between species (t=5.58, p<0.001) but not between control and drought treatments (p=0.140).

### 5.3.2 Enzyme activity

Prior to drought, there were no differences in potential enzyme activity (EA) between plant species, between chambers within a mesocosm, or between control versus drought mesocosms. EA decreased during the drought (Figure 17), and recovered after two weeks of well-watered conditions. In Oak mesocosms, drought mesocosms had significantly lower activity than control mesocosms of C (F=12.1, p=0.008), N (F=60.8, p<0.001), and P (F=195.7, p<0.001) degrading enzymes (Figure 17). This corresponds to a more than 60% reduction in potential C, N, and P degrading EA in drought mesocosms relative to control. In Maple mesocosms, drought mesocosms had significantly lower activity than control mesocosms of N (F=14.0, p=0.006) and P degrading enzymes.
(F=35.5, p<0.001), and no statistical difference in C degrading EA (F=3.8, p=0.088; Figure 17). This corresponds to a ~50% decline in C and N degrading potentials, and a 60% reduction in P degrading potential. By the end of the recovery period, treatment differences in C, N, and P degrading EA had largely disappeared for both Maple (C: p=0.127; N: p=0.226; P: p=0.151) and Oak mesocosms (C: p=0.099, N: p=0.459, P: p=0.064).
Figure 19: Enzyme activity declined during the drought for Maple and Oak mesocosms and recovered during the recovery. Boxes span the interquartile range, and whiskers extend this 1.5x; the median value is denoted with a black line; outliers are represented with open circles. Asterisks indicate significance main treatment effects within each period (p<0.100 = •, p<0.050 = *, p<0.010 = **, and p<0.001 = ***).

5.3.3 Respiration sensitivity to drought

Respiration (Rₛ) varied between species, among chambers, and across time. Pre-drought, there was no difference in Rₛ between drought and control mesocosms for either species, so we summarize pre-drought Rₛ patterns across both treatments. Pre-drought in Maple mesocosms, Rₛ was 23% higher in the full than the microbial chamber
(t=3.99, p<0.001), and did not differ between the full and root-exclusion chambers. Pre-
drought in Oak mesocosms, Rs was 31% higher in the full chamber than the microbial
chamber (t=3.49, p=0.001) and 23% higher in the full than in the root-exclusion chamber
(t=2.83, p=0.007).

Drought dramatically reduced Rs across all chambers for both species (p<0.001
for both species). Mesocosm chambers within each species varied in their sensitivity to
drought, and mean Rs was reduced to 5-10% of that of control chambers (Figure 18). In
Maple mesocosms, the root-exclusion chamber had significantly higher relative Rs
during drought (11.2 ±0.3% of control chambers) than the microbial chamber (7.1 ±0.3%;
p=0.023) but not than the full chamber (7.9 ±0.3%; p=0.089; Figure 18). In Oak
mesocosms, the full chamber had significantly higher relative Rs during the drought
(11.2 ±0.7%) than the microbial chamber (4.8 ±0.5%; p=0.016), but not compared to the
root-exclusion chamber (6.9 ±0.7%; p=0.144; Figure 18)
Figure 20: Relative soil respiration was similar across chambers during the Drought, but remained reduced in full Maple chambers during Recovery. Boxes span the interquartile range, and whiskers extend this 1.5 times; the median value is denoted with a black line; outliers are represented with open circles. Different letters indicate significant differences within a species.

To examine the drought-sensitivity of $R_s$, we first compared the correlation between $\Psi_{soil}$ and $R_s$ between chambers within a species. In Maple mesocosms, $R_s$ from the root-exclusion chamber was the most resistant to drought and was less sensitive (i.e. lowest slope) than both full ($t=3.90$, $p<0.001$) and microbial chambers ($t=3.43$, $p<0.001$).

In Oak mesocosms, $R_s$ in the microbial chamber was particularly sensitive to drought, more so than the root-exclusion ($t=2.78$, $p=0.006$) and the full chambers ($t=3.90$, $p<0.001$).

We also compared the drought-sensitivity of $R_s$ between species within each chamber (Figure 19). Maple and Oak mesocosms also differed in their drought-
sensitivity, but only in the absence of plant roots. In full chambers (i.e. including plant roots), Rs of both species declined with decreasing water availability (t=18.71, p<0.001; Figure 19). However, their drought-sensitivity did not differ in the presence of plant roots, as indicated by the lack of Species*Ψsoil interaction term included in final best models in the full chamber (Figure 19). In contrast, in the absence of roots, Maple mesocosms were less sensitive to drought than Oak mesocosms: Rs declined more gradually in Maple mesocosms than Oak in both the root-exclusion (Species*Ψsoil: t=2.12, p=0.035) and microbial chambers (Species*Ψsoil: t=1.97, p=0.049).

Figure 21: Oak mesocosms were significantly more sensitive to drought than Maple mesocosms in the absence of plant roots. See Table 4 in Appendix B for full model output.
5.3.4 Respiration recovery from drought

To control for the effects of increasing water availability following the drought treatment, we saturated the soils in both control and drought mesocosms during the recovery period. Despite being maintained under the same conditions as control mesocosms, $R_s$ from drought mesocosms remained reduced relative to control mesocosms in all chambers in both species ($p<0.05$ for all drought vs control for all chambers of both species). Even after two weeks of well-watered conditions, $R_s$ from drought mesocosms was ~50-75% of control $R_s$ in Maple mesocosms, and 50-60% of control $R_s$ in Oak mesocosms (Figure 18). In Maple mesocosms, the full chamber had lower relative $R_s$ recovery (46.5 ±1.8%) than both the microbial (52.6 ±2.3%; $p=0.020$) and root-exclusion chambers (64.3 ±2.8%; $p<0.001$). All chambers of Oak mesocosms recovered similarly ($p>0.05$ for all Tukey post-hoc pairwise tests).

We also examined the relative $R_s$ recovery at all individual recovery time points. Averaged over species and chambers, drought mesocosms exhibited significantly lower respiration than control mesocosms at all time points within the recovery period ($p<0.001$ for all weeks; Figure 20A). During the recovery period, whole mesocosm water content was initially lower in drought versus control mesocosms (i.e. 12 hours and 36 hours post-rewatering; $p<0.001$ for both time points), but not thereafter (i.e. 1 week, $p>0.99$; and 2 weeks post-rewatering, $p>0.66$). For control and drought mesocosms
respectively, $\Psi_{\text{soil}}$ 12 hours after re-watering was estimated to be $-0.54 \pm 0.00$ and $-0.65 \pm 0.00$ MPa, and 36 hours after re-watering was $-0.54 \pm 0.00$ and $-0.59 \pm 0.00$ MPa. Both control and drought mesocosms experienced a spike in $R_s$ rates 36 hours after re-watering (Figure 20A). The slope (Treatment* $\Psi_{\text{soil}}$) of the correlation between $R_s$ and $\Psi_{\text{soil}}$ did not differ between treatment groups (control vs drought) either in the pre-drought (F=0.2, p=0.704) or recovery periods (F=0.2, p=0.656) indicating no legacy effects on the overall effect of water availability on respiration (Figure 20B). However, the intercept of the regression line differed significantly between treatment groups only in the recovery period (Fig 4B; $F_{\text{treatment}}=45.1$, p<0.001), suggesting that the drought resulted in a sustained $R_s$ deficit.
Figure 22: Legacy of drought after re-watering. (a) Both control and drought mesocosms of both species experienced spike in Rs rates 36 hours after re-watering, but Rs remained significantly reduced in drought mesocosms relative to control (p<0.001 for each time point). (b) The relationship between Rs and Ψ\textsubscript{soil} did not differ between control and drought mesocosms prior to drought (solid lines), but after re-watering, Rs from drought mesocosms remained lower than control mesocosms over the same Ψ\textsubscript{soil} range (dashed lines). See text for statistical details. In panel (a), the black line indicates the time of re-watering, 12 hours prior to the Week 9 measurement.

After re-watering, Rs relative to control mesocosms differed between chambers (Figure 21). Across all recovery time points and both species, the root-exclusion chamber had the highest Rs recovery (59 ±2% of control Rs). Both the microbial and full chamber recovered to ~50% of well-watered control Rs. These patterns differed slightly between species. In Maple mesocosms, the root-exclusion chamber had significantly higher recovery than the full chamber at all time points (Holm-corrected p<0.001 for each time point; Figure 21). The root-exclusion chamber also initially had higher recovery than the
microbial chamber at 12 (p=0.054) and 36 hours post re-wetting (p=0.005; Figure 21). In contrast, in Oak mesocosms the full chamber showed greater $R_s$ recovery than root-exclusion (p=0.047) and microbial chambers (p=0.036) 12 hours after rewetting, after which there was no difference in $R_s$ recovery between full, root-exclusion, or microbial chambers (Figure 21).

Figure 23: Relative recovery of $R_s$ in Maple and Oak mesocosms after rewaterning. The black line separates the Drought and Recovery experimental periods and indicates the time of rewaterning, 12 hours prior to the Week 9 measurement. See text for statistical details among chambers and weeks.

5.4 Discussion

In this study, we found that drought-sensitivity of respiration differed both between mesocosms planted with an arbuscular (Maple) and an ectomycorrhizal (Oak) species, and among belowground biotic groups within each species. In contrast to our expectations, microbial-only chambers showed the highest drought-sensitivity, exhibiting the steepest decline in $R_s$ as soils dried. In accordance with our expectations,
however, Maple mesocosms were more resistant to drought than Oak mesocosms, although only in the absence of plant roots. This suggests that differences in the drought responses of Oak and Maple mesocosms are not directly due to tree physiology, but instead may be due to their associated mycorrhizal or free-living microbial communities. To confirm that these results are due to mycorrhizae or free-living microbes, and not due to species-specific tree physiology, additional research with other tree-mycorrhizal associations is needed.

5.4.1 Drought sensitivity of carbon dynamics across biotic groups

Our results consistently show a decline of belowground carbon cycling in response to a severe, seven-week drought. Respiration rates decreased drastically in all chambers of both mesocosms types (colonized by arbuscular mycorrhizae (AM) on Maple seedlings, or colonized by ectomycorrhizae (EM) on Oak seedlings). Interestingly, however, in the full chambers, which included plant roots, mycorrhizal hyphae, and free-living microbes, Maple and Oak mesocosms did not differ in their sensitivity to drought. This was surprising and contradicts well-established findings on drought responses of tree species with hydraulic trait differences (McDowell 2011, Skelton et al. 2015, Anderegg et al. 2016) as well as the traits of maples and oaks specifically (Roman et al. 2015). However, Brzostek et al. (2014) found similar growth responses of *A. saccharum* and *Q. alba* in mixed hardwood forests during prolonged drought.
Nevertheless, isohydric species (maples) are thought to close their stomata to reduce water losses during dry conditions, sacrificing carbon acquisition to maintain water potential, while anisohydric species (oaks) are thought to keep their stomata open, maintaining photosynthesis even under drier conditions. These hydraulic differences identified in adult trees may not correspond to those of seedlings (Pratt et al. 2014), which we used in our study. Alternatively, the severity of the drought imposed in this experiment may have overwhelmed any physiological differences between tree saplings in the full chambers.

In contrast to the full chamber, Maple and Oak mesocosms differed in their drought-sensitivity in absence of plant roots. This suggests that differences observed in the root-exclusion and microbial chambers may be attributable to the biota inhabiting those chambers, rather than directly to the hydraulic traits of the plant host itself. Maple mesocosms showed enhanced drought-resistance relative to Oak mesocosms both in the absence of plant roots (i.e. root-exclusion chamber), and in absence of roots and mycorrhizal hyphae (i.e. microbial chamber). Maple showed enhanced drought resistance despite having lower photosynthetic rates than Oaks during the drought, and thus presumably less C to allocate belowground to mycorrhizae and as root exudates. However, without knowing the size of belowground biotic pool, we cannot assess the carbon flux relative to biotic demand. Microbe-only chambers showed the greatest $R_e$. 

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sensitivity overall, suggesting that $R_s$ declines observed in root-exclusion chambers may also be driven by the free-living microbial community of both Oak and Maple mesocosms. However, this fails to explain the lack of difference between Oak and Maple mesocosms in the full chambers, as we would expect patterns driven entirely by free-living microbes to be consistent across all chambers. Resource competition between microbes, mycorrhizae, and plants makes it difficult to partition bulk respiration into its constituent biotic sources, such that the response of microbes to drought-stress may differ in the presence of a competitor (such as mycorrhizae). As such, we discuss below physiological differences between both AM and EM fungi, and free-living microbes and mycorrhizae that may account for our observations. We speculate that such differences between belowground biota may contribute to $R_s$ drought responses observed in different chambers.

In chambers that include plant roots, we observed no difference in the $R_s$ sensitivity to drought between Maple and Oak mesocosms. In the full chambers, strong competition for water (Kuzyakov 2006) and carbon (Augé 2001) may have enhanced the drought-sensitivity of all respiring biota in Maple mesocosms. In the absence of plant roots, however, glomalin secreted by arbuscular mycorrhizae may have increased soil aggregation (Rillig 2004) and buffered the soil from water losses in the Maple mesocosms. In addition, arbuscular mycorrhizal hyphae are generally finer than
ectomycorrhizal hyphae (Agerer 2001, Egerton-Warburton et al. 2003, Allen 2007), so AM may have access to water stored in smaller soil pores than EM. As a consequence, respiration from AM-dominated systems may be more resistant to drought than EM-dominated systems. Drought has been shown to increase root colonization of AM in field communities, and there may be a tradeoff between the mycorrhizae investing in root-colonizing or soil-exploring biomass (Staddon et al. 2003). Again, however, we encourage the replication of this work with additional tree-mycorrhizal associations to ensure our results are not due to (tree) species-specific physiological differences.

In the absence of both roots and hyphae, soil microbes from Maple mesocosms also showed less drought-sensitivity than in Oak mesocosms. Carbon degrading enzyme activity was significantly lower in drought Oak mesocosms versus control, but only marginally so in droughted Maple mesocosms versus control. This suggests that the free-living microbial community of the Oak mesocosms may have lower overall capacity to acquire carbon than free-living microbes of Maple mesocosm. In addition, despite high variability in our photosynthesis measurements, EM fungi are thought to exert a greater C-demand on their plant hosts than AM fungi (Soudzilovskaia et al. 2015). Reduced photosynthate moving belowground in Oak mesocosms may help explain the higher drought-sensitivity of Oak mesocosms, particularly in root-exclusion chambers. As such, we speculate that the drought-stress in Oak mesocosms may be compounded
by additional carbon stress in Oak mesocosms, which may have contributed to the high drought sensitivity of the Oak mesocosms in root-exclusion and microbe-only chambers. It remains unclear whether additional climate change variables (e.g. elevated CO$_2$) would exacerbate or moderate such differences.

**5.4.2 Recovery capacity of carbon dynamics**

Respiration from both Maple and Oak mesocosms was strongly limited in its ability to recover from an extreme drought. Respiration rates from mesocosms exposed to drought remained lower than control mesocosms despite being maintained at similar, high water availability for two weeks. This finding contradicts a number of studies that have found a rapid return to background respiration rates correlated with recuperating microbial activity (Placella et al. 2012, Barnard et al. 2013, Blazewicz et al. 2014), although Meisner et al. (2013) found that longer droughts exhibited longer lag periods before microbial communities exhibited increased growth. We found a strong drought-legacy that continued to suppress respiration even after two weeks of high water availability. This finding may be explained in different ways. Rewatering may have flushed newly labile C substrates out of our mesocosms, leaving behind a depleted resource supply for belowground biota relative to control mesocosms. Alternatively, drought impact to the plant host may have reduced belowground C allocation due to legacy effects on photosynthesis. Finally, the belowground community composition may
have shifted, resulting in a persistently altered relationship between respiration and water availability.

Although AM are thought to have shorter turnover times than EM (Staddon et al. 2003, Soudzilovskaia et al. 2015), we did not find evidence that AM exhibited more rapid respiration recovery than EM. However, within 12 hours of re-watering, the root-exclusion chamber of Maple mesocosms exhibited significantly higher recovery relative to the other Maple chambers. These findings suggest that, first, AM can play an important role in the CO₂ pulses that occur following dry-rewetting cycles, and second that recovery of AM activity may help to initiate plant recovery following an extreme drought.

An improved understanding of the mechanisms underlying soil respiration is needed to refine predictions of terrestrial CO₂ emissions under future climate scenarios (Friedlingstein et al. 2014, Bradford et al. 2016). In particular, as extreme climatic events increase in severity and frequency, it is important to understand whether our current framework for understanding soil Rₛ can be extended to extreme environmental conditions (Vicca et al. 2014). Our results suggest that ecosystems dominated by different tree species and their associated mycorrhizae may respond to and recovery from drought differently. In addition to physiological differences between tree-hosts, resource use patterns of belowground biota may influence Rₛ responses from
rhizosphere and bulk soil. Incorporating such ecologically-relevant nuances into our understanding of the drought-responses of soil as a whole may improve the spatial accuracy, and thus our ability to scale up, estimates of terrestrial CO$_2$ flux.
6. Conclusions

The goal of my dissertation research was to quantify the importance of plant resource acquisition on community and ecosystem processes via their impacts on plant functioning. I did this in two ways. First, I examined the role of nitrogen availability on plant recovery following fire. Second, I compared the drought-sensitivity of two tree species which differ in their mycorrhizal symbionts. By providing a mechanistic basis to explain plant responses to various disturbances, my work can improve predictions of ecosystem functioning under future environmental conditions.

In accordance with previous studies on biogeochemical responses to fire, I found that inorganic nitrogen availability spiked after a prescribed burn. By measuring nitrogen pool sizes every week over the growing season, I provided a finer resolution of nitrogen dynamics than had been previous reported. I combined this with measures of microbial nitrogen cycling rates and with estimates of ash-derived nitrogen deposition in order to advance our understanding of what was driving this pulse. In contrast to hypotheses from other work, I found that neither ash deposition nor microbial processing could account for the observed increase in nitrogen availability. I proposed a new hypothesis to explain this phenomenon: that nitrogen can build up in the soil when plant demand for nitrogen is reduced following biomass destruction.
I documented substantial differences in nitrogen uptake capacity between co-occurring understory species. Extending concepts from the plant economics spectrum belowground, I used patterns of nitrogen capture and root traits to classify species as resource-acquisitive or resource-conservative. Despite differences in resource capture, however, I found that nitrogen availability played a minimal role in the outcome of pairwise competitive interactions, as measured through biomass regrowth. This is in contrast to what would be expected if the relationships between leaf resource capture and processing and hypothesized in the plant economics spectrum held belowground. Instead, I found that nitrogen uptake and biomass regrowth were inversely related to the same root trait. This suggests that plants may differ in the conditions under which they maximize their growth. Species which are well-adapted to capturing nitrogen may prioritize growth following non-destructive nitrogen pulses (e.g. after rain events); species which are well-adapted to resprouting may resprout quickly after disturbances.

In complex field communities, nitrogen availability played only a minor role in structuring their responses to simulated fire disturbance. Instead, fire history was the main driver shaping recovery patterns of longleaf pine understory species. Individuals exposed to frequent fires initially resprouted quickly, but over the full growing season regrew more slowly and had lower net productivity than individuals exposed to infrequent fires. My findings suggest that interspecific differences in pulsed-nitrogen
capture play only a limited role in influencing community and ecosystem level ecological dynamics. Instead, intraspecific plasticity in response to previous disturbance frequency was responsible for recovery dynamics observed in field communities.

I also compared the response of belowground respiration between oaks, which form symbiotic associations with ectomycorrhizal fungi, and maples, which form associations with arbuscular mycorrhizal fungi. In mesocosm chambers which excluded plant roots, oak mesocosms were more sensitive to drought than maple mesocosms. Oak mesocosms also exhibited lagged reductions in potential enzyme activity whereas potential enzyme activity of maple mesocosms largely recovered after two weeks of well-watered conditions. Arbuscular mycorrhizae have finer hyphae than ectomycorrhizae, which may them to access water stored in smaller soil pores than larger-diameter hyphae. This may allow species which associate with arbuscular mycorrhizae to tolerate more severe or prolonged droughts than species with associate with ectomycorrhizae.

Together, my work provides two examples of how plant resource acquisition strategy (root morphological trait values or mycorrhizal association) may have repercussions for plant responses to disturbance. Root morphology and mycorrhizal association are traits which can be generalized to other species. As such, this work
provides a foundation to understand ecosystem responses to environmental stressors which can be tested in other systems.
Appendix A: Testing for niche differentiation: nitrogen uptake and biomass regrowth have contrasting relationships with root traits: Supplement

Figure 24: Leaf %N was measured pre-fertilization. SA is leaf surface area (cm²); R:S is root:shoot ratio; SRL is specific root length (m g⁻¹).
Appendix B: Soil respiration sensitivity to drought differs between an arbuscular and an ectomycorrhizal system: Supplement

Table 3: Mean potential enzyme activity (EA) and standard error of the mean (SEM) of each enzyme sampled during each period (Pre-drought, Drought, Recovery). We report EA separately for each drought treatment (drought or control) and species treatment (Maple/arbuscular mycorrhizae or Oak/ectomycorrhizae) within each chamber (Full, Root-exclusion, or Microbial). Enzyme substrate codes are as follows: α-glucosidase (AG), β-glucosidase (BG), cellobiohydrolase (CB), xylosidase (XYL), N-acetyl glucosaminidase (NAG), leucine-amino pepidase (LAP), and acid phosphomonoesterase (PHOS). EA is reported in nmol per gram of dry soil per hour.

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Table 4: Treatment effects on respiration from Maple and Oak mesocosms on the last week of sampling of each experimental period. Trt is treatment (Control or Drought), Ch is Chamber (Microbial, Root-ex., or Full), and Ch x Trt is their interaction. We report F and P values; bolded values indicate a significant effect (p<0.050).

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<td>F  P</td>
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Table 5: Treatment effects on respiration from Microbial, Root-exclusion, or Full chambers prior to rewetting. Sp is species (Maple or Oak), Ψsoil is soil water potential, Sp x Ψsoi is the interaction between species and soil water potential, and Biomass refers to aboveground (Full) biomass. We report F and P values; bolded values indicate significant effect or a predictor.

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</table>
Table 6: Chart showing which week (weeks 1-11) each measurement was taken within each Experimental Period (Pre-Drought, Drought, and Recovery). We measured soil respiration (Rs), Enzyme Activity (EA), Soil and Leaf water potential ($\Psi_{soil}$, $\Psi_{leaf}$), Microbial community composition (Micro. Comm.) and Photosynthesis (Photo.). Gravimetric-based $\Psi_{soil}$ was measured at least weekly. Respiration was measured twice in week 9: 12 hrs following rewetting and 2 days following rewetting. For all other measurements, we sampled in each indicated week.

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Figure 25: Schematic of mesocosm design. A seedling is planted in the center chamber (Full), which includes plant roots, mycorrhizal hyphae, and free-living microbes. Root excluding mesh (35 µm) allows only free-living microbes and mycorrhizal hyphae to colonize the Root-exclusion chamber. Hyphal excluding mesh (1 µm) excludes root and hyphal growth, allowing only free-living microbes to colonize the Microbial chamber. PVC collars in each chamber have holes drilled into the shaft below the soil line, allowing biotic and hydrologic connectivity across PVC barrier. The tops of these collars were capped during Rs measurements.
Figure 26: Soil water potential ($\Psi_{\text{soil}}$) was measured biweekly throughout the experiment. Solid lines are Maple mesocosms, dashed lines are Oak mesocosms. See Table 6 in Appendix B for Weeks relative to Experimental Periods. The grey rectangle indicates the Drought period.
Appendix C: Fire history but not nitrogen availability mediates plant recovery from subsequent disturbances: Supplement

The following allometric equation relates the area \( x \) of *Pteridium aquilinum* to its biomass \( y \).

\[
y = (-6 \times 10^{-7})x^2 + 0.0039x
\]
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Science 343:548-552.


Saito, L., W. W. Miller, D. W. Johnson, R. G. Qualls, L. Provencher, E. Carroll, and P. Szameitat. 2007. Fire Effects on Stable Isotopes in a Sierran Forested Watershed. This study was supported by the Nevada Agricultural Experiment Station, College of Agriculture, Biotechnology, and Natural Resources, University of Nevada, Reno, publication number 52055531, and the US Forest Service, Lake Tahoe Basin Management Unit. Journal of Environmental Quality 36:91-100.


Biography

Cari Danon Ficken was born in 1986 in Cincinnati, Ohio and has moved frequently within the United States and internationally throughout her life. She graduated from Kenyon College in 2009 with a Bachelor of Arts degree in Biology. After graduating, she worked with the US Forest Service surveying trees in Appalachia, the US Department of Agriculture Agricultural Research Service measuring greenhouse gas emissions from Prairie Pothole wetlands in North Dakota, and at Archbold Biological Station tracking the demography of rare species endemic to the Lake Wales Ridge in south central Florida.

In 2012, Cari moved to North Carolina to begin her PhD at Duke University. She has received support for her research through a Duke University Biology Department Grant-in-Aid of Research Award, Sigma Xi Grant-in-Aid of Research Award, Bass Summer Research Fellowship, DOE-ORNL Go! Fellowship, American Society of Naturalists Student Research Award, NSF Doctoral Dissertation Improvement Grant and Jo Rae Wright Fellowship for Outstanding Women in Science; she was also awarded the New Phytologist Best Student Presentation Award for her oral research presentation at the Ecological Society of America 2015 Annual Meeting. She has published her research in *Wetlands Ecology and Management*, the *Journal of Environmental Quality*, *Ecosystems*, *Biogeosciences*, and *PLoS ONE*. 