PINE PRODUCTIVITY AND ORGANIC MATTER DECOMPOSITION RESPONDS TO NUTRIENT AMENDMENT AND WEED CONTROL TREATMENT HISTORY IN INTENSIVELY MANAGED SECOND ROTATION PINE PLANTATIONS

By

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To my parents
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treatment. In the actively retreate experiment, the F and FW treatments increased litter decay rates compared to the C. Litter P mineralization rates improved with the fertilizer treatment in both experiments. Results from two ex-situ incubation experiments showed that Mn addition accelerated microbial respiration for the Cc, C, Cw, and W treatments. The silvicultural treatment history also affected nutrient responses; N+P suppressed respiration in the CF and F treatments but stimulated it in the Cc and C treatments. Mn addition generally increased soil Mn peroxidase activity for the Cc, C, Cw, and W treatments. Mn peroxidase activity was strongly correlated with microbial respiration (r=0.63, p<0.01), suggesting a potential Mn limitation to enzyme production and OM decomposition. Overall, results demonstrate that long-term site management treatments affected aboveground productivity, organic matter decomposition, and nutrient cycling. These results have also identified a possible mechanism of Mn limitation for OM decomposition in nutrient limited Spodosols in north central Florida.
CHAPTER 1
INTRODUCTION

Background and Problem

Southern pine plantations cover almost 15 million ha area in the southeastern United States (Hartsell and Conner, 2013). Loblolly pine (*Pinus taeda* (L.)) is the predominant species planted in the Gulf and Atlantic Coastal Plains (Schmidtling, 2001). Although loblolly pine outgrows other pines on nutrient rich, well-drained soils, many loblolly pine plantations in the South, i.e., on N and P limited Spodosols of the lower Coastal Plain, are growing below their maximum growth potential. On such sites, forest management systems that ameliorate site conditions and improve pine yields are critical to the success of these plantations.

Nutrient additions and herbicide applications are common silvicultural practices used in intensively managed pine plantations (Fox et al., 2007). With site preparation and use of genetically superior planting stock, these treatments can improve yields by about two- to four-fold (Colbert et al., 1990; Jokela and Martin, 2000; Jokela et al., 2010). These techniques, on the other hand, have dramatically reduced the rotation length of southern pine stands to almost half compared to that in the 1960s (Prestemon and Abt, 2002; Fox et al., 2007). As a result, the spatial extent of pine fertilization in the southern U.S. has increased by about five-fold in the past two decades (Allen et al., 2005), despite volatility of fertilizer prices due to fluctuations in supply and demand (Elser et al., 2014).

Sustaining the productivity of intensively managed pine plantations across rotations has become a critical need in meeting future global wood demands as the world population continues to increase and per-capita forest holdings decrease (Sedjo and Botkin, 1997; FAO, 2010). Although challenges to assess sustained productivity in these plantations exist because of the dynamic shifts in management practices, improved germplasm, and changing climate across
rotations (Nambiar, 1996), understanding how processes such as decomposition and nutrient mineralization change with varying silvicultural practices will offer valuable insights into factors controlling site productivity.

Litter decomposition and nutrient mineralization are important nutrient cycling processes in forest ecosystems. Leaf litter and the forest floor constitute important carbon and nutrient pools. Upon microbial oxidation of complex organic matter to simple organic compounds and mineralization of growth limiting nutrients, these pools are available to plants. In nutrient limited systems, slower decomposition rates may represent a major nutrient limitation for primary producers as inaccessible nutrient pools build up above the mineral soil. This is of concern in southern pine plantations where nutrient availability limits productivity (Colbert et al., 1990; Carter and Foster, 2006). In that context, finding ways to improve litter decomposition and accelerate nutrient recycling may be beneficial in meeting the growth demands of southern pines.

On nutrient poor sites, leaf litter returned to the forest floor is primarily of poor quality due to high nutrient resorption prior to leaf abscission. In intensively managed southern pine stands, litter accumulation in the forest floor represents an important pool for the nutrients like nitrogen (N) and phosphorus (P) (Vogel et al., 2011). Its decomposition is an important process influencing nutrient recycling in forest soils (Gholz et al., 1985b; Vitousek and Sanford, 1986; Freschet et al., 2013). Especially in fertilized stands, nutrient concentrations of pine litter are generally higher compared to unfertilized stands (Dalla-Tea and Jokela, 1994). Upon senescence and decomposition, this pool has the potential to meet inter-rotational nutritional demands for juvenile pines growing on sandy Spodosols (Subedi et al., 2014). However, fertilization or weed control effects on litter decomposition are inconclusive for southern pines (Polglase et al., 1992b; Sanchez, 2001; Gurlevik et al., 2003), and mechanisms for such differences are not clear.
Modification of litter chemistry and subsequent influence on decomposition dynamics may be possible with fertilization or weed control treatments (Polglase et al., 1992a, c; Gurlevik et al., 2004). For instance, Polglase et al. (1992c) observed higher phenolic content in the litterfall from plots receiving weed control treatments. Likewise, fertilization has been shown to increase P mineralization in pine plantations (Polglase et al., 1992a). In contrast, the combination of fertilization and weed control treatments inhibited specific P mineralization (Polglase et al., 1992a). The effect of fertilization on N mineralization was inconsistent and depended on the time of sampling (Polglase et al., 1992a). In contrast, Gurlevik et al. (2004) documented an increase in net N mineralization with the combination of fertilization and weed control in a 14-year-old loblolly pine plantation. The extrapolation of these results to sites with a long history of nutrient amendment or competing vegetation control, however, may not be accurate given the short treatment histories in these studies.

Litter decomposition and nutrient mineralization involves a sequential breakdown of a variety of substrates like celluloses, phenolics, and lignins via extracellular enzymes produced by soil microbes and fungi. Nutrient availability to microbes and fungi may influence production of a variety of extracellular metallomic enzymes associated with the breakdown of these substrates (Allison et al., 2014). Microbial degradation of simple carbohydrates during the initial stages of litter decomposition is often correlated with N availability (Berg and Ekbohm, 1991; Kang et al., 2010). Lignin decomposition, however, may be suppressed with N availability as it suppresses phenol oxidase activity and reduces relative abundances of soil microbes (Frey et al., 2004; Waldrop and Zak, 2006; Hobbie et al., 2012).

Unlike N, manganese (Mn) has been shown to accelerate lignin degradation by serving as a co-factor in Mn peroxidase, a lignolytic extracellular enzyme produced by basidomycetes.
(Perez and Jeffries, 1992). Long-term litter decomposition is also strongly coupled with Mn redox cycling in forest ecosystems (Keiluweit et al., 2015). For instance, production of Mn$^{3+}$ at the site of litter decay was coincident with the degradation of litter. In addition, the production of Mn$^{3+}$ was primarily driven by the ability of fungi to accumulate and redistribute Mn$^{2+}$ at the site of litter decay. Although optimum levels of some micronutrients like copper (Cu) and zinc (Zn), that act as co-factors in microbial enzymes (Wackett et al., 1989), may aid microbial decomposition of litter substrate; higher concentrations of these nutrients may lead to suppressed decomposition via inhibition of soil organisms (Duarte et al., 2004). While the majority of studies on litter decomposition dynamics following nutrient amendments focus on N, P, and potassium (K) (Hobbie, 2005; Knorr et al., 2005; Hobbie et al., 2012), only a few studies have investigated the effects of micro-nutrients on litter decomposition (Kaspari et al., 2008; Powers and Salute, 2011; Whalen et al., 2018; Sun et al., 2019). Nevertheless, these studies report Mn limitations to organic matter decomposition in temperate forest stands with varying N availability (Whalen et al., 2018; Sun et al., 2019).

In the context of southern pines, N and P fertilization may induce micronutrient deficiencies, and reduce pine growth. For example, copper limitation to growth has been documented on sandy Spodosols in Florida (Vogel and Jokela, 2011). Interesting relationships between macro and micronutrients have also been observed in a long-term fertilizer and weed control trial near Gainesville, FL (IMPAC II). For example, soil respiration was higher on sites with higher Cu or Mn availability in the second rotation (Subedi, 2013). Compared to sites with historical weed control treatments (Mn availability: 2.4 $\mu$grams/10cm$^2$/8 weeks), organic matter decomposition was also higher in historically fertilized sites with higher Mn availability (11.3 $\mu$grams/10cm$^2$/8 weeks) (Subedi, 2013). These observations, along with those made in other
forested regions of the world, highlight the importance that micronutrients play in regulating litter decomposition in plant-soil systems. However, a mechanistic understanding of these relationships is still lacking in southern pine plantation ecosystems.

**Review of Literature**

Litter decomposition is a major component of nutrient cycling in forested ecosystems. On nutrient limited sites, slow rates of litter decomposition may exacerbate nutrient limitations because the nutrient pool accumulated as litterfall in the upper soil horizon is slowly available (Gholz and Fisher, 1982). Although fine root turnover plays an important role in nutrient cycling, there is no general consensus on the relationship between fine root turnover rate and nutrient availability (Haynes and Gower, 1995; Lee and Jose, 2003). In general, litter decomposition can be affected by factors such as climate (e.g. temperature, precipitation, evapotranspiration)(Aerts, 1997; Berg et al., 2000; O’Neill et al., 2003), litter chemistry (e.g. N, C:N, lignin content, lignin:N) (Gholz et al., 1985b; Berg and Ekbohm, 1991; Austin and Ballaré, 2010; Manzoni et al., 2010; Prescott, 2010), litter types (Prescott, 2010; Makkonen et al., 2013), geographical variables (Aerts, 1997), decomposer communities (Makkonen et al., 2013), and site specific factors (Berg and McClaugherty, 2014). On a global scale, climate and litter chemistry represent the dominant factors influencing litter decomposition. Zhang et al. (2008), in a meta-analysis of 110 litter decomposition studies, observed that latitude, mean annual temperature, C:N ratio and total N accounted for almost 88% of the variation in litter decomposition rates.

For southern pine plantations growing on nutrient poor soils, nutrient resorption and internal nutrient recycling is an important process to meet nutritional demands. Nutrient resorption (in litterfall) and soil nutrient availability are generally negatively related (Dalla-Tea and Jokela, 1994). For example, Dalla-Tea and Jokela (1994) documented an 8% lower N resorption rate for fertilized vs. unfertilized plots in a 6-year old loblolly pine plantation in north
Florida. As a result, nutrient return from litterfall was significantly higher in fertilized compared to unfertilized plots. For instance, an almost 13-fold N return response was observed for fertilization and weed control plots compared to unfertilized plots (Dalla-Tea and Jokela, 1994). When nutrients are not resorbed, litter must be decomposed for the nutrients to be available for plant uptake. As decomposition may take several years (Berg and Ekbohm, 1991), litterfall may be considered as nutrient sink in the short term. For southern pine stands, although the immediate effects of nutrient amendments on nutrient recycling are documented, the effects of long-term historical nutrient amendments are not well understood. On nutrient limited sites, enhanced nutrient recycling via promotion of litter decomposition and mineralization is critical for maintaining nutrient supply to the site.

Past studies examining the effects of nutrient addition on litter decomposition have focused mainly on N and P because most terrestrial ecosystems are limited by these elements. Previous studies have shown that increases in N, P, and S, that are typically limiting microbial growth, stimulates microbial degradation of soluble compounds and hollocellulose (Berg and McClaugherty, 2014). During the secondary stage of litter decomposition (lignin dominated litter), N additions generally have negative effects on decomposition, as it may bind with lignin to form more complex organic compounds or limit phenol oxidase activity (Frey et al., 2004). In intensively managed southern pine plantations, routine silvicultural practices such as N and P additions are likely to influence litter decomposition dynamics through such mechanisms. Compared to the untreated control plots, Polglase et al. (1992b) reported an almost 45% higher decomposition rate for loblolly pine growing on plots that received fertilizer and weed control treatments. In addition, Polglase et al. (1992c) reported that fertilization and weed control affected N, P, and C fractions in the litter. While fertilization increased the concentration of total
labile P in the O\textsubscript{i} horizon litter, the weed control treatment increased total labile C and phenol concentration in the same litter horizon. Kiser et al. (2013) also reported no differences in total soluble C for fertilized and unfertilized loblolly pine stands in North Carolina. For that same study, soluble phenols and tannins were higher in the unfertilized stands compared to plots receiving irrigation and fertilization treatments. Because higher phenolic concentrations in litter may hinder litter decomposition, additional knowledge regarding the persistence of these treatment effects on litter decomposition in successive rotations is warranted.

Frequent N and P additions have the potential to induce micronutrient limitations in southern pine stands (Jokela et al. 1991; Allen et al., 2005; Vogel and Jokela, 2011). As micronutrients (e.g. Mo, Mn, Fe, Cu, and Zn) serve as enzyme co-factors for most soil microbes (Wackett et al., 1989), their limitation may also impact litter decomposition. In tropical forests, Kaspari et al. (2008) documented an increase in leaf litter decomposition when P was combined with one or more micronutrients (e.g. B, Cu, Fe, Mn, Mo, and Zn). In boreal and temperate forest ecosystems, Mn has been positively related with lignin decomposition (Berg et al., 2013) and negatively related with soil carbon (Stendahl et al., 2017). Recently, Mo limitation to microbes (asymbiotic N\textsubscript{2} fixers) has also been documented in P rich sites in tropical forests of Panama (Wurzburger et al., 2012). These studies in tropical and temperate forests provide some insights on the importance of micronutrients on litter decomposition. In a loblolly pine litter decomposition study, Sadowski (2010) reported that foliar B, Zn, Mn, Fe, and Cu concentrations were strong covariates for litter decomposition in north Florida. These findings highlight the need to investigate the role of micronutrient amendments on litter decomposition in intensively managed southern pine plantations where inter-rotational application of N and P may increase stand growth and induce micronutrient deficiencies.
Objectives

This study examines the long-term sustained productivity of intensively managed loblolly pine plantations using two replicated field experiments that had a history of fertilizer additions and understory competition control using herbicides in the previous rotation. While one experiment was left untreated to determine their carry-over effects into the subsequent rotation, the other experiment continued to receive the same amount and timing of fertilization and competition control treatments as in the prior rotation. This study also examined the carryover effects of the prior rotation’s fertilization and weed control history on proxies of soil carbon dynamics by measuring soil respiration and litter decomposition. Further, it investigated a possible mechanism for micronutrient limitations to organic matter decomposition for intensively managed sites receiving fertilizer additions and weed control treatments. Specifically, the following questions were answered:

• Do past silvicultural treatments have an ameliorative carryover effect on stand growth and development in the following rotation? How does retreatment alter growth response? (Chapter 2)

• Do prior rotation nutrient additions and understory competition control treatments affect soil respiration and organic matter decomposition early in a second rotation loblolly pine stand? (Chapter 3)

• Does the fertilization and weed control treatment history in the prior rotation affect pine litter chemistry, decomposition and nutrient release patterns in the subsequent rotation? Is understory vegetation an important driver of pine litter decomposition in these stands? (Chapter 4)

• Do nutrients limit pine litter and organic matter decomposition if environmental drivers (e.g. temperature and moisture) are not limiting? What mechanism drives this limitation, if any, among treatments? (Chapter 5)
CHAPTER 2
SUSTAINED PRODUCTIVITY OF INTENSIVELY MANAGED LOBLOLLY PINE
PLANTATIONS: PERSISTENCE OF FERTILIZATION AND WEED CONTROL EFFECTS
ACROSS ROTATIONS

Background

The productivity of loblolly pine (*Pinus taeda* L.) stands in the lower Coastal Plain of the
US is often nutrient limited (Allen, 1987; Fox et al., 2007). Forest management strategies in the
last few decades have been continually evolving to both alleviate site limitations and improve the
growth potential of forest plantations (Jokela et al., 2010; D’Amato et al., 2017). Many stands,
especially those growing on Spodosols, are now being managed with fertilizer application and
competing vegetation control treatments that often increase yields and financial returns
(Bengtson, 1979; Colbert et al., 1990; Neary et al., 1990a; Jokela and Stearns-Smith, 1993;
Borders and Bailey, 2001). Albaugh et al. (2018) estimated that almost 316,000 hectares of
loblolly pine stands were fertilized annually between 1996 and 2016 with either phosphorus (P)
or nitrogen (N). Similarly, McCullough et al. (2005) reported that almost 960,000 hectares of
southern pine plantations were managed annually using competing vegetation control in 2001
and 2002. As these practices become more common (Fox et al., 2007) and fertilizer prices
become more volatile due to fluctuations in supply and demand (Elser et al., 2014), a significant
management challenge with the southern pines will be to sustain productivity across rotations
with the efficient use of external nutrient inputs.

Maintaining productivity across multiple rotations is one of the central tenets of long-
term sustainability in managed plantations (Fox, 2000). Previous long-term studies in other
conifer stands in Australia, Europe, and elsewhere provide some insights on how productivity
changes with management practices across multiple rotations (Keeves, 1966; Evans, 1996; Bi et
al., 2007; O’Hehir and Nambiar, 2010; Egnell, 2011; Harwood and Nambiar, 2014). These
studies generally show that better soil nutrient management and silvicultural practices including nutrient amendments and understory competition control, improves stand productivity in the subsequent rotation. There is, however, a paucity of such information for intensively-managed southern pine plantations in the US (Haywood, 1994; Powers, 1999), mainly because of the difficulty of duplicating silvicultural practices across rotations in a region that has been undergoing dynamic changes in management systems. However, end-of-rotation studies have documented nutrient pool size and extractable nutrient changes, suggesting that silvicultural practices could alter nutrient availability after the harvest of the first rotation (Vogel et al., 2011; Zerpa et al., 2014; Tumushime et al., 2019). Inter-rotational studies that duplicate as closely as possible the first-rotation silvicultural practices are fundamental to understanding sustained productivity in these continually evolving plantations.

Our understanding of growth response in intensively managed southern pine stands and associated changes in leaf area development, aboveground and belowground biomass allocation, growth efficiency, and nutrient cycling processes (Jokela and Martin, 2000; Martin and Jokela, 2004; Samuelson et al., 2004) are based on a relatively short treatment history that spans a single rotation. However, determining whether fertilization and weed control treatment effects extend beyond a single rotation is important for developing efficient management systems over successive rotations. Some studies have shown that previous-rotation fertilizer application, especially P, enhances productivity in subsequent rotations (Subedi et al., 2014; Everett and Palm-Leis, 2009). However, the legacy effects of past rotation silvicultural practices that also include weed control on second-rotation yields has rarely been investigated in southern pines. Results from Subedi et al. (2014) suggest that fertilization combined with a weed control treatment history may not result in measurable growth benefits for loblolly pines early in the
second rotation, but changes in understory vegetation composition and soil nutrient availability within the soil profile could influence site resource availability later in the rotation. In that context, understanding whether the effects of past silvicultural practices (e.g. on residual soil nutrient supply) on the growth of newly planted pine stands are transient or more long-lasting remains an important question when formulating future silvicultural prescriptions.

Weed control treatments are routinely used to establish southern pine plantations, where competition from understory vegetation negatively influences pine productivity (Colbert et al., 1990; Neary et al., 1990a; Minogue et al., 1991). Increased pine growth associated with weed control treatments likely result from reduced competition for soil nutrients and water with the pines (Neary et al., 1990a). However, reductions in soil and forest floor C may occur following sustained control of competing vegetation in pine stands (Laiho et al., 2003; Sartori et al., 2007; Vogel et al., 2011). In addition, sustained weed control treatments may influence the understory community recovery and re-initiation on intensively managed sites (Miller et al., 1999; Jones et al., 2009; Subedi et al., 2017). While these changes in understory community dynamics were thought to be transient (Brockway et al., 1998), recent evidence shows that intensive weed control treatments can change the understory vegetation functional group composition in the subsequent rotation (Subedi et al., 2017). For example, some understory species having strong nutrient accumulation potential may be eliminated on sites with a history of sustained weed control treatments (Jokela et al., 1991; Subedi et al., 2017). Loss of functional understory vegetation groups may affect nutrient recycling in the subsequent rotation (Nilsson and Wardle, 2005). In that context, it is critical to understand the role that weed control treatments play in the maintenance of long-term site productivity on sandy soils where soil and litter C decomposition and subsequent mineralization of N, P, and other nutrients support pine growth.
In this study, the long-term sustained productivity of intensively managed loblolly pine plantations was examined using two replicated field experiments that had a history of fertilizer additions and competition control in the previous rotation. While one experiment was left untreated to determine carry-over effects into the subsequent rotation, the other experiment continued to receive the same amount and timing of fertilization and competition treatments as in the prior rotation. Specifically, the following questions were addressed:

- Do past silvicultural treatments have an ameliorative carryover effect on stand growth and development in the following rotation?
- Does the continuation of intensive treatments across two rotations alter growth response patterns to silvicultural treatments?

**Materials and Methods**

**Study Area**

The IMPAC (Intensive Management Practices Assessment Center) experiment was established in 1983 to evaluate factors limiting the productive potential of southern pines (Swindel et al., 1988). The study is located approximately 10 km north of Gainesville, Florida (29°30’N latitude and 82°20’W longitude) at an elevation of 45 m from the mean sea level. The climate is warm and humid with a long-term (1984-2017) mean annual temperature of 20.7°C and total annual precipitation of 1207 mm (National Oceanic and Atmospheric Administration, 2018). Pomona fine sands (sandy siliceous hyperthermic Ultic Alaquods) are the predominant soil types at this site. The understory vegetation community was typical of Florida flatwood sites. Woody components of the understory consisted mainly of gallberry (*Ilex glabra* (L.)), saw palmetto (*Serenoa repens* (Bartr.)), fetterbush (*Lyonia ferruginea* (Walt.)), blueberries (*Vaccinium* spp.), and wax myrtle (*Myrica cerifera* (L.)). Chalky bluestem (*Andropogon* spp.), tapered witchgrass (*Dichanthelium* spp.), and nutrushes (*Scleria* spp.) were the graminoid components of the understory (Neary et al., 1990b; Subedi et al., 2017).
Study Design

First rotation study: The first-rotation IMPAC experiment was a $2 \times 2 \times 2$ factorial consisting of two species (loblolly and slash pine ($Pinus elliottii$ var. $elliottii$ (Engelm.))), complete and sustained weed control, and annual fertilization arranged in a randomized split-plot design. This resulted in four treatments within each species (species as whole plots): control (C), weed control only (W), fertilizer only (F), and both fertilizer and weed control (FW). The entire experimental area was site prepared using a single-pass bedding treatment. In January 1983, genetically improved (first generation, open pollinated) 1-0 bareroot stock (grown for 1 year in the seedbed) of both loblolly and slash pine were hand planted (Swindel et al., 1988; Colbert et al., 1990). The F and FW treatments received a balanced fertilizer regime including macro- and micronutrients for the first ten years annually. In May 1993, fertilization was stopped and then resumed from 1998 - 2000 (Jokela and Martin, 2000). Competing vegetation in the W and FW treatments was eliminated using a combination of chemical and mechanical methods for the first ten years until canopy closure impeded further understory development (Colbert et al., 1990; Dalla-Tea and Jokela, 1994). Rotation long production and stand dynamics of the IMPAC experiment were documented by Jokela et al. (2010).

Second rotation study (IMPAC II experiment): In May 2009, the first-rotation IMPAC study was harvested and a second-rotation experiment was overlaid using the same treatment plots. Prior to the harvest of the first-rotation study, the understory vegetation within the C and the F treatments was mulched in place in April 2009 to retain this nutrient pool within the plot boundaries. Mulching was not possible for the W and FW treatments because of the sustained weed control treatment history during the previous rotation. To minimize inputs of tree stem and crown nutrients via harvest residues, each plot was whole-tree harvested and processed outside.
of the treatment plots. Following harvest, the entire study area was later bedded in June, with a second bedding pass conducted in August of the same year.

Original plots in the first rotation were re-established, and those plots were used to examine both the “untreated carryover” and “actively managed retreatment” effects on the pine growth dynamics in the second-rotation experiment. The IMPAC II experiment consisted of two randomized complete block designs, with 3 replications each, having four treatments ($C_C$, $C_F$, $C_{FW}$, $C_W$) for the untreated carryover experiment and four treatments ($C$, $F$, $FW$, and $W$) for the actively managed retreated experiment (Table 2-1). While the untreated carryover experiment, established on the previous slash pine plots, received no treatments in the second rotation, the actively managed retreatment experiment, established on the previous loblolly pine plots, continued to receive similar treatments as in the first rotation.

In October 2009, the W and FW treatments were treated using a broadcast application of 0.84 kg a.e. ha$^{-1}$ imazapyr in the form of Chopper (BASF Corp., Research Triangle Park, NC), 1.12 kg a.e. ha$^{-1}$ triclopyr in the form of Garlon 4 (Dow AgroSciences LLC, Indianapolis, IN), and 0.14 kg ha$^{-1}$ of metsulfuron methyl in the form of Escort® (E.I. du Pont de Nemours and Company, Inc., Wilmington, DE).

In December 2009, the entire study was regenerated using containerized seedlings from a second generation single, full-sib loblolly pine family. Similar to the first rotation, seedlings were planted in each plot at a 1.8 m by 3.6 m spacing, with measurement plots consisting of forty trees per plot (arranged as 8 trees each in 5 beds). Each plot also included a treated buffer consisting of three trees and two beds surrounding each measurement plot ($\sim$268 m$^2$). Six tree spaces of untreated buffer were provided between adjacent treatment plots. Across the treatment plots, an untreated buffer of four beds was maintained (Figure 2-1).
In March 2010, all treatments (actively managed retreated and untreated carryover) received a single application of Fipronil (9.1%) in the form of PTM (BASF Corp., Research Triangle Park, NC) to control Nantucket pine tip moth (Rhyacionia frustrana (Comstock)). Later in May 2010, a banded 0.2 kg a.e. ha\(^{-1}\) imazapyr was applied on the beds of all treatment plots (both untreated carryover and actively managed retreated) to control panicgrass (Panicum L.) and to aid seedling survival. In October 2010, the W and FW treatments received a directed spray application of triclopyr (3%) and imazapyr (1%) to control Ilex glabra (L.) and other understory competitors. Later in September 2011, a directed spray of glyphosate (3%) was applied to the actively managed W and FW treatments to maintain a weed-free environment.

The actively managed retreated (F and FW) experiment received fertilizer annually. Consistent with the last rotation treatments, the total nutrient additions over the first six growing seasons for the F and FW treatments were (kg ha\(^{-1}\)): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B. As done in the first-rotation experiment, the fertilizer was applied in narrow bands (30 cm semicircle) around the base of each tree or planting location. In April 2016, the FW and F treatments were thinned in the actively managed retreated experiment. Thinning was initiated in the FW treatment once it reached full site occupancy (55% of maximum stand density index for loblolly pine; Reineke, 1933). The F treatment was also thinned at the same time with the anticipation that it would reach full site occupancy by the following year, if left un-thinned. Individual trees were selected from the plots using a combination removal of trees with nursery rust and thinning from below. The residual stand density index was brought down to approximately 30% of the maximum stand density index. The thinning operation removed almost 37.2 Mg ha\(^{-1}\) and 21.4 Mg ha\(^{-1}\) of aboveground biomass from the FW and F treatments, respectively. Early second-rotation pine growth and understory
vegetation responses to silvicultural treatments were reported by Subedi et al. (2014) and Subedi et al. (2017).

**Data Preparation**

**Stand measurements**

Annual winter measurements of diameter at breast height (DBH) and height for all trees were made for the first four years beginning in 2010. For the fifth, sixth, and seventh years, DBH was recorded for all trees in the measurement plot; however, heights were recorded on every third tree. Individual tree heights for those years were estimated using log-transformed linear regression equations \((\ln (y) = \beta_0 + \beta_1 \ln(x))\) developed for each experiment using DBH as a dependent variable. Linear equations for each treatment were first developed separately. The test for differences in slopes among the treatments revealed no significant differences (t-test for all treatment pairs; data not shown) for both the actively managed retreated and untreated carryover experiments. As a result, tree height inventory data were pooled together to develop a height equation for both experiments (Table A-1). All assumptions for linear regressions were met prior to conducting the analyses. Logarithmic corrections were made on all estimates of height (Sprugel, 1983).

Total aboveground biomass at ages 3, 4, 5, 6, and 7 years was estimated using the equation developed by Gonzalez-Benecke et al. (2014) for loblolly pine;

\[
Y=0.026256 \times \text{DBH}^{2.014144} \times \text{Ht}^{0.864052}
\]

where, \(Y\) is the oven-dry above-ground biomass in kilograms, DBH is the diameter at breast height expressed in cm, and Ht is the total height of the tree in meters.

Current annual increments from ages 4 through 7 years were estimated as the difference in total aboveground biomass accumulation between the current year and the previous year. For the thinned FW and F plots, 7th year CAIs were estimated as the difference between age 7 year
residual aboveground biomass and age 6 year residual aboveground biomass after the thinning operation. Reineke’s stand density index (SDI; Reineke, 1933) was estimated as:

$$SDI = 0.404686 \times tpha \left( \frac{Dq}{25.4} \right)^{1.605}$$

where tpha is trees per hectare, and Dq is the quadratic mean diameter in centimeters.

Quadratic mean diameter (cm) was estimated as: $$Dq = \sqrt{\left( \frac{BA}{tpha \times 0.00007854} \right)}$$ where BA is basal area in m² ha⁻¹.

**Nutrient use efficiency index**

Nutrient use efficiency (NUE) index for this study at age 4-year was estimated as the reciprocal of the weighted concentration of all aboveground tree components [kg (dry weight) kg⁻¹ (nutrient)] (Santana et al., 2000); i.e.,

$$\text{NUE index} = \frac{1}{\sum_{i=0}^{n} C_i \times A_i}$$

where i denotes the aboveground component of loblolly pine (e.g. foliage, stem, bark, or branch), Cᵢ denotes nutrient concentration of the iᵗʰ component, and Aᵢ denotes percentage allocation of total aboveground biomass to the iᵗʰ component.

For estimation of component biomass, highly significant allometric equations were developed using the destructive harvest data generated from the first rotation 4-yr-old loblolly pines growing on the same site as the current study (Colbert, 1988) (Table A-2). All estimates of biomass were corrected for logarithmic bias (Sprugel, 1983).

Nutrient analyses of pine foliage for ages 1, 2, 4, and 5 years were conducted by collecting fully elongated needles (approximately 25 fascicles) from five random trees in each measurement plot. For age 2 (Subedi et al., 2014) and 4 years, stem wood, bark, and branch tissues were also collected from five randomly selected pine trees in each measurement plot.
These tissues were oven-dried at 65°C to a constant weight and then ground in a Wiley mill to pass through a 1-mm sieve. These samples were then analyzed for macro- and micronutrients at the Micro-Macro International Laboratory in Athens, GA. About 0.5 g of ground tissue was first dry-ashed in a muffle furnace, and then the samples were brought up to volume with aqua regia (3:1 HNO₃/HCl). The extracts were analyzed using inductively coupled plasma atomic emission spectroscopy (ICP–AES). Total N was analyzed in a CNS analyzer (Leco Corp.) using the Dumas method (Campbell, 1992).

**Soil nutrient availability**

Subedi et al. (2014) also documented soil nutrient concentrations in the untreated carryover experiment. Briefly, nutrient concentrations were estimated by collecting soil samples from all plots at depth intervals of 0 to 10, 10 to 20, 20 to 50, and 50 to 100 cm in November 2012 using a 7.6-cm-diameter auger. Eight samples were collected from each treatment plot. The samples from the same depth intervals were thoroughly mixed and weighed. Approximately 15% of the mixed sample was then subsampled. Roots were removed from the subsamples. About 100 g of subsample was then air-dried and ground in a mortar and pestle to pass thorough a 2-mm sieve. Soil macro- and micronutrients were extracted using the Mehlich III procedure (Mehlich, 1984).

**Leaf area index**

Monthly needlefall from March 2015 to March 2017 was collected, dried and used to estimate all-sided leaf area index (LAI; Martin and Jokela 2004). Needlefall data were corrected for senescence-related biomass reductions (Dalla-Tea and Jokela, 1994). For the actively retreated experiment, the FW and F treatments were thinned in the middle of a needlefall collection period in April 2016. As a result, all-sided LAI was estimated only for the untreated carryover experiment.
Second-rotation fertilization efficacy on height growth

Fertilization efficacy on height growth (FEH) was calculated as the difference in height gains (>75 percentile) between the fertilized (HF, HCF) and control treatments (HC, HCC). For the first rotation, FEH was calculated as: HF-HC. For the second rotation, FEH was calculated as: (HF-HC)-(HCF-HCC), where the carry-over influence of the first-rotation fertilization was estimated with HCF-HCC. FEH was calculated annually. An FEH greater than zero indicated a positive fertilizer effect on height gain. One sample t-tests were used to investigate whether the FEHs for each rotation were different from zero as stand development progressed.

Data Analysis

Analysis of variance (ANOVA) for a randomized complete block design was used to test the effects of fertilizer and weed control on total aboveground loblolly pine biomass, current annual increment (CAI), basal area (BA), stand density index (SDI) (Reineke’s SDI; Reineke, 1933), and nutrient use efficiency index for both the actively managed retreated and the untreated carryover experiments. Kolmogorov Smirnov and equal variance tests were used to ensure that the data met assumptions of normality and homoscedasticity, respectively (Massey, 1951). For data not meeting the assumptions of normality and homoscedasticity, log transformations were made prior to ANOVA in SAS (SAS Institute, 2007). Significant difference among treatment means were separated using Tukey’s studentized range (Honestly significant difference) test at a significance level of 0.05, unless noted otherwise.

Results

Height and Diameter Distribution

From ages 1 through 7 years, all treatments in the second rotation outperformed all treatments in the first rotation, except for the C treatment which had lower mean tree height than the first rotation FW treatment in the seventh year (e.g. 9.6 m in the C vs. 10.4 m in the first
rotation FW) (Figure 2-2). For the untreated carryover experiment, seventh-year height gains compared to the associated first-rotation treatments, were almost 2.3- fold for the CC (10.4 m/4.5 m), 1.4- fold for the CF (11.8 m/8.4 m), 1.1- fold for the CFW (11.4 m/10.4 m), and 1.3- fold for the CW (10.9 m/8.4 m) treatments. Likewise, for the actively managed retreated experiment, second rotation seventh year heights were almost 2.1-, 1.4-, 1.3-, and 1.3- fold higher, respectively, for the C (9.6 m), F (12.3 m), FW (13.2 m), and W (11.1 m) treatments compared to the corresponding first-rotation treatments.

Silvicultural treatment intensity affected the age 6 diameter distribution in both experiments. Mean tree DBH in the second rotation exceeded the first rotation for all treated plots, and the actively retreated plots exceeded the untreated carryover for all treatments except for the controls (C vs CC). The CF treatment had the highest sixth year mean tree DBH (9.9 cm) among treatments in the untreated carryover experiment (Figure 2-3). In addition, all carryover treatments in the second rotation had higher mean tree DBH than the first rotation except for the CFW treatment (Figure 2-3). In the actively managed retreated experiment, sixth year mean tree DBH was highest for FW (14.6 cm) compared to all other treatments. Sixth year mean tree DBH were 2, 1.2, 1.1, 1.1- fold higher for the C, F, FW, and W treatments than the respective first-rotation estimates (Figure 2-3).

**Aboveground Biomass Accumulation**

Aboveground biomass accumulation in the second-rotation stands was increased by the historical fertilization only (CF) treatment, but when combined with past weed control the fertilizer effect was muted early in stand development (Figure 2-4a). At age 3 years, only the CF treatment accumulated significantly more biomass compared to all other treatments. Despite having the same historical nutrient additions as the CF treatment, biomass accumulation for the CFW treatment did not differ from the CC treatment at this early age. However, as stand
development progressed, the biomass accumulation trends for the \( C_{FW} \) treatment started to separate from the \( C_C \) treatment and were similar to that observed for the \( C_F \) treatment by age 5 years. By the seventh year, the \( C_F \) (63 Mg ha\(^{-1}\)) and \( C_{FW} \) (60 Mg ha\(^{-1}\)) treatments accumulated about 1.6- and 1.5- fold more biomass compared to the \( C_C \) (40 Mg ha\(^{-1}\)) treatment, respectively. Biomass accumulation in the \( C_W \) (48.3 Mg ha\(^{-1}\)) treatment was not different from the \( C_C \) treatment.

In the actively retreated experiment, the trends were different than the untreated carryover experiment (Figure 2-4b). From early in the stand rotation, continuation of the fertilization and weed control treatments in the second rotation significantly increased loblolly pine aboveground biomass accumulation compared to the control. By age 7 years, the \( FW \) treatment accumulated 2.8-fold more biomass (standing crop aboveground biomass + thinning removal at age 6 year) compared to the \( C \) treatment. Aboveground biomass accumulation in the actively managed retreated experiment followed the trend: \( FW \) (90.6 Mg ha\(^{-1}\)) > \( F \) (71.8 Mg ha\(^{-1}\)) > \( W \) (55.1 Mg ha\(^{-1}\)) > \( C \) (31.8 Mg ha\(^{-1}\)).

**Stand Density Index (SDI) and Current Annual Increment (CAI)**

The prior rotation’s fertilization and weed control treatment histories affected the SDI (Reineke, 1933) in the second rotation. In general, SDI estimates for the untreated carryover experiments were significantly higher for the \( C_F \) and \( C_{FW} \) treatments compared to the \( C_C \) treatment from ages 3 through 7 years (Figure 2-5a). For example, seventh-year SDIs were almost 1.3- fold higher for the \( C_{FW} \) and \( C_F \) treatments compared to the \( C_C \) (159) treatment. Seventh-year SDI was not different for the \( C_W \) treatment (183) compared to the \( C_C \) treatment. In the actively managed retreated experiment, continuation of the fertilization and weed control treatments resulted in significantly higher SDI values for the \( FW \) (260) compared to the \( F \) (213), \( W \) (177), and \( C \) (119) treatments at age 6 years (Figure 2-5b). For the \( FW \) treatment, full site
occupancy (defined as 55% of maximum SDI of 450) was reached at age 6 years. These results suggest that historical fertilization and weed control treatments accelerated stand development in the second rotation.

In the untreated carryover experiment, the CAI for the C_F treatment peaked at about 15.1 Mg ha\(^{-1}\) yr\(^{-1}\) at age 6 years, with a corresponding basal area of 18.6 m\(^2\) ha\(^{-1}\) (Figure 2-5c). For the actively managed retreated experiment, the CAI for the FW treatment peaked at 23.5 Mg ha\(^{-1}\) yr\(^{-1}\) and 21.4 m\(^2\) ha\(^{-1}\) of basal area at age 5 years and then started to decline thereafter (Figure 2-5d). When pooled and compared across rotations, CAI for the second rotation was significantly higher than that in the first rotation for ages 5 and 6 years, suggesting increased second-rotation productivity for all treatments (Figure 2-6). For instance, the fifth year CAIs for the second rotation were respectively almost 11.7, 2.0, 1.2, 1.7- fold higher for the C_C, C_F, C_FW, and C_W (Figure 2-6a), and 8.8, 2.3, 1.9, and 1.7- fold higher for the C, F, FW, and FW treatments (Figure 2-6b) compared to respective first-rotation estimates (C=0.9, F=7.4, FW=12.1, and W=7.6 Mg ha\(^{-1}\)yr\(^{-1}\)).

**Nutrient Use Efficiency Index**

Except for Mg, Mn, and Zn, NUE index did not differ among treatments in the untreated carryover plots (Figure 2-7, Table B-1). Magnesium NUE indices were 1.15- and 1.24- fold higher in the C_F and C_FW treatments compared to the C_C treatment (2066 kg (dry wt.) kg\(^{-1}\)(nutrient)). However, Mn NUE index was 37% lower for the C_F and 42% lower for the C_FW treatments compared to the C_C treatment (17.2 kg (dry wt.) g\(^{-1}\)(nutrient)). Zinc NUE index was lower for the C_FW treatment when compared to the C_C treatment (C_FW: 46.9 kg (dry wt.) g\(^{-1}\)(nutrient) vs. C_C: 56.5 kg (dry wt.) g\(^{-1}\)(nutrient)).

In the actively managed experiment, the NUE indices for P and K were significantly lower in the fertilized plots than the control. When compared with the C treatment, the NUE
index for P and K were almost 26% and 36%, and 21% and 29% lower for the F and FW treatments respectively. Magnesium NUE index was 1.3-fold higher for the F treatment compared to the C treatment. For other nutrients like N, Ca, S, B, Cu, Mn, and Zn, NUE index in the actively managed treatments did not significantly differ in the F, FW, and W treatments when compared with the C treatment (Table B-1).

**Leaf Area Index**

Maximum all-sided LAI at age 5 year for loblolly pine was significantly higher in the C_F (9.78 m² leaf area. m⁻²) compared to the C_C (5.96 m² leaf area. m⁻²) treatment. Maximum all-sided LAI followed the trend: C_F > C_FW (8.80 m² leaf area. m⁻²) > C_W (6.97 m² leaf area. m⁻²) > C_C. This trend suggests that LAI was responsive to the historical treatments that increased soil nutrient availability (Subedi et al., 2014). In addition, a significant positive linear relationship (R²=0.902; p<0.0001) between maximum all-sided LAI and stemwood increment suggested that a unit increase in LAI would translate into about 0.99 Mg ha⁻¹ yr⁻¹ of stemwood production (Figure 2-8).

**Fertilization Efficacy on Height Growth**

Comparison of fertilizer efficacy on height growth between the first and second rotations showed that the second-rotation fertilizer treatments for the actively managed experiment did not initially (age 1–4 years) have a measurable effect on height gain compared to the first rotation (Figure 2-9). Notably, there was a significant gain in height due to fertilization in the first rotation (FEH >0 from age 1 through age 6 years). However, after the fourth year in the second rotation fertilizer additions in the actively managed experiment contributed to significant height gains (FEH>0 for age 5 and 6 years).
Foliar Nutrient Status

Foliar N concentrations for all treatments in the untreated carryover experiments were higher than the critical level (12 g kg\(^{-1}\); Allen, 1987; Jokela 2004) from ages 1 to 4 years (Figure 2-10 a,b). In the fifth year, foliar N concentrations were well below the critical level suggesting N deficiency for the untreated experiment. Foliar P concentrations, however, were generally lower than the critical level of 1.2 g kg\(^{-1}\) beginning age 2 years in the untreated carryover experiment. Foliar N and P trends in the actively managed retreated experiment were different than the untreated carryover experiment; both foliar N and P concentrations for the F and FW treatments were at or above the critical level even after the fourth year (Figure 2-10 c, d).

Discussion

Sustaining productivity across rotations is one of the key principles underpinning sustainable forest management and represents an important area of scientific inquiry in intensively managed plantations (Nambiar, 1996; Fox, 2000; Vance et al., 2010). Direct assessments of sustained productivity are often limited by changes in bio-physical attributes, evolution of management regimes, and differences in planting stock genetics between rotations (O’Hehir and Nambiar, 2010). In intensively managed southern pine stands, a multi-rotational experimental design, similar cultural practices, and a common genetic source, were used to compare and contrast loblolly pine growth responses to both the legacy effects of the previous rotation’s treatments and to those reapplied in the subsequent rotation.

Large gains in yield have traditionally followed fertilizer inputs and weed control treatments for loblolly pine growing on nutrient-limited sites (Colbert et al., 1990; Borders et al., 2004; Samuelson et al., 2004; Jokela et al., 2010); benefits that were repeated in the second rotation with continued nutrient additions. Unexpected, however, were the very large increases in growth in the control treatments across rotations. Genetic improvement of planting stock (a
second-generation full sib pine family in second rotation vs. multiple first-generation open pollinated pine families in the first) likely increased growth in the current rotation. For example, Li et al. (1999) found that first-generation open-pollinated pine families increased volume by only 7 to 8% compared to unimproved genetic material, whereas a second-generation full-sib-family resulted in volume gains of about 35 to 40% (Whetten and Kellison, 2010). In addition, more effective site management practices like double pass bedding (Lauer and Zutter, 2001; Zhao et al., 2008), herbaceous weed control to enhance seedling survival (Morris et al., 1993), and Nantucket pine tip moth control (King et al., 2014) all likely contributed to the second-rotation growth increase. Enrichment of atmospheric CO₂ by almost 60 ppm between rotations (Keeling and Keeling, 2017) was estimated to increase the growth potential by about ~ 7% (McCarthy et al., 2010; Kirschbaum, 2011); a small increase relative to the 9-12 fold increase in annual productivity observed in the control treatments.

During the early stages of stand development, the second-rotation treatment responses were generally higher for the C_F plots that also had an intact understory vegetation present from the previous rotation (Subedi et al., 2014). Interestingly, those treatment plots where understory vegetation was absent, e.g. C_FW and C_W, had no early growth benefits compared to the C_C treatment until year 4. One possible reason for these response differences could relate to a larger “assart effect” and flush of labile nutrients associated with the mulched understory vegetation on the C_F and C_C plots (Burger and Pritchett, 1984; Vitousek et al., 1992; Zerpa et al., 2014). The N found in the understory vegetation and mulched at the beginning of the second rotation was 37 kg ha⁻¹ for the C_C and 48 kg ha⁻¹ for the C_F plots (Vogel et al., 2011). In addition, soil nutrient pools associated with the C_FW treatments suggested a downward movement of nutrients within the solum, especially P, in the absence of understory vegetation (Subedi et al.,
2014). However, a non-significant difference between growth responses in the CF and CFW treatment after age 4 years suggests that early growth limitation associated with historical P movement from the E to Bh and Bt horizons in the CFW treatment likely diminished as root development increased and nutrient pools in the deeper horizons were accessed (Adegbidi et al., 2004). As a result, an increase in P uptake of about 87% was observed for the CFW compared to only 20% for the CF treatments from ages 2- to age 4-years (Figure C-1). By the seventh year, growth differences between the CF and CFW treatment had diminished, perhaps due to lower competition levels from the graminoid-dominated understory in the CFW treatment compared to shrub-dominated understory in the CF treatment (Miller et al., 2003; Subedi et al., 2017). In addition, higher mortality associated with density-related competition and fusiform rust in the CF treatment likely contributed to the sudden decline in CAI in the seventh year [Mortality: CF (14%) > CC (6%) = CW (3%) = CFW (2%), p = 0.007]. Nevertheless, higher leaf area development and associated allocation to stemwood production in the CF and CFW treatments, when compared to the CC, suggested that the carryover effects from the first-rotation fertilization and weed control treatments had a positive influence on second-rotation yields.

Continuation of the first-rotation treatments into the second rotation resulted in larger growth responses than the first rotation. For example, sixth year total aboveground biomass accumulation for the FW treatments (50 Mg ha\(^{-1}\)) in the first rotation (Jokela and Martin, 2000) were almost 86% lower compared to these second-rotation estimates. Though the individual treatment trends were similar to those observed in the first rotation (Colbert et al., 1990; Jokela et al., 2010), there were changes in relative effectiveness between the W and F treatments across the two rotations. For example, the F and W treatments had nearly identical effects on biomass accumulation in the first rotation at age 6-year (Jokela and Martin, 2000), but in the second
rotation the F treatment had almost 30% higher biomass accumulation than the W treatment. Increased biomass accumulation associated with the F treatment highlights the role that nutrient availability plays on loblolly pine growth. However, fertilization also benefitted understory vegetation growth and potentially increased nutrient immobilization in the understory biomass. For example, about 68 kg ha\(^{-1}\) of N was immobilized in the understory biomass in the F plots at age 2 years compared to 47 kg N ha\(^{-1}\) in the C plots (Subedi et al., 2014). The W plots gained about 76% higher biomass compared to the C plots by the sixth growing season, suggesting that the competition for soil nutrients by the understory vegetation still impacted pine growth in the second rotation (Neary et al., 1990a). When understory competition control was combined with fertilization, maximum growth response was observed for pines in the FW plots. Our sixth year biomass estimates for the most productive FW plots were similar to the value of 72 Mg ha\(^{-1}\) reported for six-year-old pine plantations managed with irrigation, fertilization, and competition control on Ultisols in southern Georgia (Samuelson et al., 2004).

On nutrient-rich sites, the NUE of plants tends to be lower when compared to nutrient-limited sites (Vitousek, 1982; Li et al., 1991). Strong negative relationships were observed between Mehlich III extractable Mn and Zn in the 0-50 cm soil depth and corresponding NUE index for the untreated carryover experiment (Mn: \(r = -0.74, p < 0.01\); Zn: \(r = -0.86, p < 0.01\); Figure C-2). For example, while Mehlich III extractable Mn in the C\(_{FW}\) treatment (1.13 ppm in 0-50 cm) was 4.3-fold higher compared to the C\(_{C}\) treatment (0.26 ppm in 0-50 cm), Mn use efficiency index for the C\(_{FW}\) was 0.6-fold lower compared to the C\(_{C}\) treatment. Our estimates for N, P, and K use efficiency index were similar to the values of 177, 2203, and 491, respectively, reported by Gholz et al. (1985) for 5-year old slash pine plantations growing on Spodosols in north Florida. However, estimated Ca and Mg use efficiencies in our study were
4.5- and 2.7- fold higher, respectively, than reported by Gholz et al. (1985). Higher Ca and Mg efficiencies are presumably an artifact of nutrient dilution associated with higher pine growth rates in our study. Adegbidi et al. (2005), for example, observed dilution of foliar Ca and Mg at ages 3 and 4 years for fertilized loblolly pine stands growing on Spodosols. In addition, dilutions in stemwood Ca concentrations ranging from 31 to 48% were observed between ages 2 and 4 years (Subedi, 2013). Greater stemwood production per unit nutrient uptake as trees mature reflects faster growth rate and stand development during this rotation.

Except for N and S, the second-rotation NUE indices were higher than the first-rotation estimates reported by Colbert (1988) for this site (Table B-2). Reduced N and S use efficiencies between the rotations, regardless of treatments, could be partly associated with atmospheric N and S depositions during these periods. Atmospheric deposition between 1988 and 2014 was calculated to contribute about 189 kg ha\(^{-1}\) of N and 153 kg ha\(^{-1}\) of S for this site (McDonnell and Sullivan, 2014). In addition, genetic differences in planting stock between rotations could likely affect NUE indices. Li et al. (1991), for instance, observed strong genetic control on nitrogen use efficiency (narrow-sense heritability: 0.69 to 0.84) in loblolly pines. However, the higher second-rotation P, K, Ca, and Mg use efficiency indices suggests that the second-rotation loblolly pines accumulated more aboveground biomass for a given amount of nutrient uptake compared to the first rotation. These inter-rotational differences in NUEs, irrespective of treatments, may be partly associated with nutrient dilutions associated with this fast-growing and genetically superior second rotation germplasm (Adegbidi et al., 2005; Whetten and Kellisson, 2010). From a forest management perspective, increased NUEs would suggest the potential for lower fertilizer needs to maintain higher yields when deploying superior genotypes; research examining the
performance of many genotypes, along with their interactions with silvicultural treatments and varying site types would be required to fully address this possibility.

On sites having high residual fertility, additional nutrient additions may not yield the same levels of response as previously unfertilized sites (Jokela et al., 2004; Zhao et al., 2016). The relatively higher height gains associated with fertilization in the first rotation were expected because these plots presumably had lower initial soil nutrient availability (e.g., site index: 19.5 m for the control treatment; Jokela et al., 2010) and loblolly pine, a nutrient-demanding species, responded well to nutrient additions (Jokela and Martin, 2000). In the second rotation, however, the F plots were inherently more productive (site index: 26.2 m, Jokela et al., 2010) and height responses to second-rotation fertilizer additions were small relative to the first-rotation responses. Results from this study suggest that nutrient inputs received during the first rotation adequately supported pine growth for the first four years during the second rotation (e.g., fertilizer carryover effect). Therefore, evidence of an apparent fertilizer height response beginning at age 5 likely reflected increased stand nutrient demands relative to soil supply once the canopy was fully developed. Observed trends in foliar nutrient levels also showed that, after the fourth year, the concentrations of N and P fell below the critical level for all treatments in the untreated carryover experiment (Allen, 1987; Jokela, 2004). In contrast, the F and FW plots from the actively managed retreated experiment had foliar N and P levels that were at or above the critical levels. These results suggest synchronizing fertilizer application to plant demand in the successive rotation will increase both the economic and biological efficiencies of previously fertilized stands. Other factors to improve the efficacy of external nutrient inputs in these plantations would include distributing nutrient additions over the life of the stand (Albaugh et al.,
2015), using enhanced efficiency fertilizer blends (Raymond et al., 2016), and developing fertilization regimes that consider maximum attainable responses (Zhao et al., 2016).

Productivity declines in successive rotations of managed plantations are unlikely to occur unless site management practices are poor (Keeves, 1966; Fox, 2000; Scott and Dean, 2006; Eisenbies et al., 2009; O’Hehir and Nambiar, 2010; Egnell, 2011; Vangansbeke et al., 2015). Particularly in nutrient-limited sandy soils such as ours, silvicultural practices that minimize nutrient losses, esp. N and P after harvest, coupled with the maintenance of soil nutrient supply, are critical strategies for ensuring long-term site productivity (Smith et al., 2000; Jerabkova et al., 2011; Maier et al., 2012; Achat et al., 2015). Higher second-rotation CAIs, even for the untreated second rotation, compared to the first rotation, suggest that intensive silvicultural practices and deployment of improved genotypes in the US South have maintained site productivity beyond a single rotation. Foliar and soil nutrient levels reported for our study provides evidence that the first-rotation fertilization and understory vegetation retention history increased both soil nutrient availability and pine productivity in the successive rotation (Subedi et al., 2014). In our study, correction of nutrient deficiencies, esp. P, via first-rotation fertilizer additions improved inherent site productivity and contributed to a sustained changes in peak volume yields for pines in the second rotation (Type-2 growth response; Snowdon, 2002). In contrast, the weed control only treatments did not change the inherent site productivity levels for this experiment, so their impacts on growth rates advanced the stage of stand development (Type-1 growth response; Snowdon, 2002) in the second rotation. However, “inherent site productivity” is actually a complex interplay of abiotic, biotic and cultural factors like changing climate, atmospheric nutrient deposition and CO2 levels, invasive species, planting stock genetics, and silvicultural practices that can all affect future productivity in managed pine.
plantations (Fox, 2000; McMahon et al., 2010; Whetten and Kellison, 2010; Gonzalez-Benecke et al., 2017). Decoupling these factors and their interactions requires multi-rotational studies that use contemporary silvicultural practices and tree genetics.

Summary

This study was designed to investigate both the legacy effects of the previous rotation’s intensive silvicultural treatments and the continued application of prior silvicultural practices on inter-rotational yields of loblolly pine. The novel results from this study provide direct evidence that common but intensive fertilization and sustained weed control practices applied in the previous rotation improved pine growth in the subsequent rotation. Gradual reductions in early growth differences observed over time between the CF and CFW treatments in the untreated carryover experiment (Subedi et al. 2014) suggest a diminishing “assart” effect associated with the CF pre-harvest understory mulching treatment. Other factors like increased understory competition levels in the CF treatment, and improved access to soil nutrients, esp. P, present in deeper soil horizons of the CFW treatment may also have been responsible for these dynamic treatment response differences. Fertilizer carryover effects were also evident between rotations. Significantly higher growth responses, leaf area index, and soil nutrient availability, especially for the CF treatment, suggests that this historically fertilized site was more productive early in the second rotation. Thus, managers may consider delaying fertilizer additions until after establishment in the second rotation. Because the silvicultural treatments used in this study were more intensive than typically used in many southern pine plantations, fertilizer carryover effects would likely be less pronounced in operationally treated stands. Higher second rotation levels of biomass accumulation associated with the actively managed FW treatment suggests that these treatments helped support more rapid pine growth through continued nutrient availability, and reductions in site resource availability associated with understory competitors. Formulation of
future silvicultural practices in southern pine plantations should consider site management history, timing of nutrient amendments, and the role that understory vegetation plays in site nutrient retention and/or as competitors affecting long-term productivity and site sustainability. This study also suggested that improved cultural practices (e.g., advanced genetics, seedling stock, site preparation), improved nutrient use efficiency, enhanced soil nutrient availability, and environmental factors (e.g., elevated atmospheric CO$_2$) have important influence on inter-rotational differences in site productivity. Additional multi-rotational studies that are designed to better understand site × silviculture and silviculture × genetic interactions will clarify whether silvicultural practices and genetics continue to increase productivity in future rotations.
Table 2-1. Treatments applied to a loblolly pine plantation growing on a Spodosols in North Florida at the Intensive Management Practices Assessment Center (IMPAC) II study.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>First-rotation treatment</th>
<th>Second-rotation treatment</th>
<th>Treatment abbreviation</th>
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<tbody>
<tr>
<td>Untreated carryover</td>
<td>Control</td>
<td>Untreated</td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
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<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>Untreated</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Fertilizer (Cumulative nutrient addition for the rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B) + weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>Untreated</td>
<td>C&lt;sub&gt;FW&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>Untreated</td>
<td>C&lt;sub&gt;W&lt;/sub&gt;</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>Control</td>
<td>Control</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
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<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>Fertilizer (Cumulative nutrient additions for the first six years (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B)</td>
<td>FW</td>
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<td></td>
<td>Fertilizer (Cumulative nutrient addition for the rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B) + weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>Fertilizer (Cumulative nutrient additions for the first six years (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B) + weed control (Chemical methods)</td>
<td>FW</td>
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<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>Weed control only (Chemical methods)</td>
<td>W</td>
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</tbody>
</table>
Figure 2-1. Experimental layout of the IMPAC II study near Gainesville, Florida.
Figure 2-2. Heights of loblolly pine trees from age 1 through age 7 for the a. first rotation, b. second rotation untreated carryover, and c. second rotation actively managed retreated experiments at the IMPAC studies. Error bars represent standard errors.
Figure 2-3. Sixth year diameter distribution for the first and second rotation experiments at the IMPAC studies. Double-dashed, dot-dashed, and dashed vertical lines represent mean tree DBH (cm) for the first rotation, second rotation actively retreated, and second rotation untreated carryover experiments, respectively.
Figure 2-4. Total aboveground loblolly pine biomass accumulation for second rotation loblolly pine stands growing in the a. untreated carryover, and b. actively managed retreated experiments on Spodosols in north central Florida. Error bars represent standard errors. Within a given age, treatments followed by same letters were not significantly different at $\alpha = 0.05$ (Tukey’s HSD). The seventh-year biomass for the FW and F treatments include biomass removed via thinning.
Figure 2-5. Current annual increment and stand density index for second rotation loblolly pine stands growing in the untreated carryover (a, c), and actively managed retreated (b, d) experiments on Spodosols of north Florida. Horizontal dashed line in the SDI vs. Stand age panel marks the beginning of self-thinning for loblolly pines (55% of maximum SDI). Error bars represent standard errors.
Figure 2-6. Current annual increments in the first and second rotation a. untreated carryover, and b. actively managed retreated experiments at the IMPAC studies. Solid line is the 1:1 line. All treatment means were pooled together. The dashed lines show the linear relationships between the second (y) and first rotation (x) CAIs (a. $y=9.869+0.395x$, $R^2=0.78$; b. $y=5.945+1.174x$, $R^2=0.88$).
Figure 2-7. Nutrient use efficiency index for 4-year-old loblolly pines growing in the untreated carryover and actively managed retreated experiments at the IMPAC II study. Within a given experiment, treatments followed by same letter were not significantly different at $\alpha = 0.05$ (Tukey’s HSD). Mean separations with uppercase letter represent the actively managed retreated experiment, whereas lowercase letters represent the untreated carryover experiment.
Figure 2-8. Relationship between sixth year stemwood growth and fifth year maximum leaf area (all-sided) index for loblolly pines growing in the untreated carryover experiment on Spodosols of north Florida. Solid line shows a linear relationship between the fifth year maximum all-sided LAI and the sixth year stemwood growth (Stemwood growth (Mg ha\(^{-1}\) yr\(^{-1}\)) = 1.23+0.9934\times\)Maximum all-sided LAI (m\(^2\) leaf area m\(^{-2}\))

\[ R^2 = 0.902 \]

\[ p < 0.0001 \]
Figure 2-9. Fertilization Efficacy on Height growth measured as mean height gain (m) of trees (>75 percentile) at the IMPAC studies for the first six years. First rotation response (F-C) was estimated as difference between the F and C treatments for loblolly pine in the first rotation. Second rotation response (F-C)-(C_F-C_C) was estimated as the difference between the net response in the F treatment vs. C and the response in the C_F vs. C_C treatment. Asterisks denote that the mean height gain is different from zero at $\alpha = 0.05$. Error bars represent standard errors.
Figure 2-10. Foliar N and P concentrations for loblolly pines in relation to stand age for the treatments in the untreated carryover (a, b) and actively managed retreated (c, d) experiments at the IMPAC II experiment. Horizontal dashed lines represent foliar critical levels of 12 g kg\(^{-1}\) for N and 1.2 g kg\(^{-1}\) for P (Allen, 1987; Jokela, 2004). Error bars represent standard errors.
CHAPTER 3
SOIL RESPIRATION AND ORGANIC MATTER DECOMPOSITION DYNAMICS
RESPOND TO FERTILIZER AND COMPETITION CONTROL ACROSS ROTATIONS IN
LOBLOLLY PINE STANDS

Background

In nutrient limited forests, nutrient additions often increase net ecosystem productivity (NEP) or terrestrial C sequestration (LeBauer and Treseder, 2008; Reed et al., 2011; Yuan and Chen, 2012; Fernández-Martínez et al., 2014; Fay et al., 2015; Bracho et al., 2018). Changes in forest NEP could affect the global terrestrial C cycle because of the nearly 861 Pg of C stored in forests (Pan et al., 2011). When NEP has been increased with nutrient additions, an increase in net primary production (NPP) has often been reported, but also a decrease in heterotrophic respiration (Rh) (Bowden et al., 2004; Janssens et al., 2010; Ramirez et al., 2010; Bracho et al., 2018). Of these two processes, relatively less is understood how Rh responds to nutrient additions (Cleveland and Townsend, 2006; Sun et al., 2014; Zhong et al., 2016). When examining nutrient limitation theory and NPP or Rh, most experiments have focused on nitrogen (N) and phosphorus (P) limitations to these processes (Vitousek and Farrington, 1997; Vitousek et al., 2010) Reed et al., 2011). However, multiple nutrient elements may affect NPP and Rh (Kaspari et al., 2008; Billings et al., 2010; Powers and Salute, 2011; Reed et al., 2011; Camenzind et al., 2018) and land managers often manipulate a suite of element cycles to increase primary productivity. In managed forests, the effect of altered nutrient cycles on microbial decomposition processes is particularly important, as changes in decomposition processes drive long-term ecosystem C storage and nutrient availability.

Managed pine plantations in the southeastern United States cover more than 16 million ha (Hartsell and Conner, 2013) and sequester more than 0.21 Pg C per year (Johnsen et al., 2001). As many of these plantations grow on soils recognized as nutrient limited (Fox et al.,
silvicultural treatments that include fertilization and the use of herbicides to control competing vegetation have been regularly applied to alleviate nutrient deficiencies and improve yields (Jokela et al., 2010). Previous mid- and end of rotation studies have suggested that these treatments also cause changes in the C storage in the trees, forest floor and soil (Harding and Jokela, 1994; Shan et al., 2001; Vogel et al., 2011; Bracho et al., 2018). For example, Bracho et al. (2018) reported that mid-rotation fertilizer additions increased net ecosystem C storage by about 3 Mg C ha⁻¹ yr⁻¹ compared to an unfertilized loblolly pine (Pinus taeda L.) plantation in north Florida. Using intensive fertilization treatments, Vogel et al. (2011) reported an increase of about 51 Mg C ha⁻¹ in the forest floor and soil C pools at the end of a 26-year rotation in a loblolly pine plantation. While such expedited gains in C pools may increase the carbon sink potential of these plantations (Maier and Kress, 2000), it remains unclear whether legacy effects of fertilization practices alter processes like microbial respiration and organic matter decomposition that, in turn, regulate these C pools following a harvest.

Decomposition of organic matter in forests are mainly regulated by heterotrophs via processes such as Rh (microbial respiration during decomposition of soil organic matter) that respond to nutrient availability (Fernández-Martínez et al., 2014). With N fertilization, soil organic matter decomposition has declined in many temperate forest ecosystems (Janssens et al., 2010), primarily due to changes in microbial communities and processes (Ramirez et al., 2012; Vicca et al., 2012). Nitrogen fertilization often inhibits the activity of extracellular enzymes produced by soil microbes (e.g., phenol oxidase production by basidiomycetes), resulting in less organic matter decomposition (Saiya-Cork et al., 2002; Allison et al., 2008). However, the low availability of N, P, potassium (K), and other nutrients has also corresponded to lower organic matter decomposition rates in nutrient-poor forests (Knorr et al., 2005; Kaspari et al., 2008;
Hobbie et al., 2012; Trum et al., 2015; Camenzind et al., 2018; Whalen et al., 2018). In the pine plantations of the southeastern United States, forest growth responses from fertilization with N and P and other macronutrients are often accentuated by including micronutrients (Jokela et al., 1991; Vogel and Jokela, 2011; Carlson et al., 2014), suggesting that Rh could also be limited by multiple elements.

The potential of forests to store C also depends on litter deposition and, in particular for soils, the C deposition by roots. Belowground litter deposition from fine root growth and turnover is difficult to measure but often correlates with soil respiration (Rs) (Lee and Jose, 2003; Wang et al., 2017; Drum et al., 2019); Rs is, therefore, commonly used as a general index of belowground C cycling (Davidson et al., 2002; Litton et al., 2007; Fernández-Martínez et al., 2014). However, the Rs responses to nutrient additions are variable (Ryan et al., 1996; Maier and Kress, 2000; Olsson et al., 2005; Janssens et al., 2010; Hasselquist et al., 2012), often because plant belowground allocation and Rh move in opposing directions with fertilizer additions. By measuring Rh and Rs at the same time, insights can be gained into how fertilization influences soil microbial and root processes.

The effects of controlling competing vegetation using herbicides in managed southern pine forests has caused C pools in the forest floor and soil to increase and/or decrease (Shan et al., 2001; Vogel et al., 2011). These variabilities in C cycling responses likely reflect the multiple ways understory species may affect nutrient and other processes controlling C cycling. Understory plants disproportionately affect the cycling of multiple nutrients through biomass accrual; however, for young plantations in the southeastern United States, nutrient accumulation varies among species (Subedi et al., 2014) and different species gain dominance with past silvicultural treatments (Subedi et al., 2017). Removal of competing vegetation also affects Rh
by influencing the availability of soil organic substrates for microbial activity (Blazier et al., 2005), the chemical structure of organic matter (Polglase et al., 1992c; Ibell et al., 2010), soil microbial biomass and composition (Busse et al., 1996; Ratcliff et al., 2006), and the soil environment (Devine and Harrington, 2007; Parker et al., 2009). Each of these factors influence soil organic matter decomposition (Curiel Yuste et al., 2007) through the alteration of soil microbial processes (Wardle et al., 1999; Wu et al., 2011; Strickland et al., 2017). In plantations where previous weed control treatments modify understory vegetation initiation, growth, and composition (Subedi et al., 2017), the legacy effects of these treatments on soil respiration and organic matter decomposition could continue into the next rotation.

On a sandy, nutrient poor site in North Florida, long-term fertilizer additions resulted in increases, while the removal of competing vegetation slightly decreased forest floor and soil C pools at the end of a rotation in a 26-year-old loblolly pine stand (Vogel et al., 2011). Using the same experiment, this study evaluated if the legacy effects of the prior rotation’s treatments altered Rh and organic matter decomposition patterns early into the next rotation. A long-term replicated field experiment was used to address the following questions:

- Do first rotation nutrient additions and understory competition control treatments affect Rs and Rh patterns among treatments in a second rotation loblolly pine stand?
- How does silvicultural treatment history (e.g., nutrient addition and understory competition control) affect the drivers of decomposition (temperature, moisture, nutrient availability) and the mass loss of a common organic matter substrate?

Results from this study will provide insights into understanding the legacy effects of the prior rotation’s silvicultural management history on the primary drivers (e.g., Rh, organic matter (OM) decomposition) of NEP in stands in the subsequent rotation. Depending on the magnitude of changes in these belowground processes, NEP may substantially increase or decrease with management history. In the context of rising atmospheric CO₂ and climate change (Bonan,
2008), such information from managed forest plantations will be valuable in developing regional and global carbon management strategies.

Methods

Study Area

A replicated field experiment established in 1983 by the Intensive Management Practices Assessment Center (IMPAC) at the University of Florida (Swindel et al., 1988) was utilized for this study. The main intent of the original IMPAC experiment, located at 29°30’N latitude and 82°20’W longitude, was to evaluate the factors that limit the biological growth potential of southern pines. The study area lies at an elevation of 45 m from the mean sea level. The climate of the study area is warm and humid with the long-term mean annual temperature (1984-2012) of 20.6°C annual precipitation of 1178 mm (National Oceanic and Atmospheric Administration, 2012). The predominant soil type is a poorly drained Pomona fine sand (sandy siliceous hyperthermic Ultic Alaquods). Understory vegetation at the IMPAC study was typical of Coastal Plain flatwoods sites (Neary et al., 1990; Subedi et al. 2017).

Study Design

Originally, the IMPAC experiment was designed as a 2×2×2 factorial consisting of species (loblolly and slash pine (Pinus elliottii Engelm. var. elliottii)), complete and sustained weed control, and annual fertilization arranged in a randomized split-plot (species as whole plots) design with three replications. This resulted in four treatments within each species: control (C), weed control only (W), fertilizer only (F), and both fertilizer and weed control (FW). The entire experimental area was site prepared using a single-pass bedding treatment. Genetically improved (first generation, open pollinated) 1-0 bareroot stock (grown for 1 year in the seedbed) of both loblolly and slash pine were hand planted in January 1983 (Swindel et al., 1988). The F and FW treatments received balanced levels of macro- and micronutrients annually for the first
ten years. In addition, the F and FW treatments received fertilizers during the 16 through 18th growing seasons (1998–2000; Martin and Jokela, 2004). Fertilizers were applied in narrow bands (30 cm semicircle) around the base of each tree or planting location. Total nutrient additions over the life of the original study for the F and FW treatments for both species were (kg.ha⁻¹): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B (Jokela et al., 2010).

Competing understory vegetation was controlled in the W and FW treatments annually for the first ten years (1983 - 1993) using a combination of chemical and mechanical methods (Neary et al., 1990b; Dalla-Tea and Jokela, 1994). The weed control treatment was discontinued after canopy closure. The total C and N pools at the end of the rotation for the original experiment were reported by Vogel et al. (2011).

Before whole tree harvesting in May 2009, all treatment plot corners were re-monumented, and the understory vegetation in the C and F treatments was mulched in place (April 2009) to retain the nutrient pool within the plot boundaries. Mulching was not necessary for the W and FW treatments because of the sustained weed control treatment history from the previous rotation. In addition, harvested trees were processed off the treatment plots to ensure that nutrient inputs into the soil did not occur via the harvested residues from the pine trees or adjacent treatment plots. Following harvest, the entire study area was later bedded in June, with a second bedding pass conducted in August of the same year.

The IMPAC II study was established in the original IMPAC study plots with the intent of understanding inter-rotational effects of intensive silvicultural treatments on the second rotation growth and soil nutrient availability. Subedi et al. (2014) presented the second rotation IMPAC II study design in detail. Briefly, IMPAC II study consists of two randomized complete block design experiments (n=3 replications each) to investigate the carryover and the continued
management effects of fertilizer inputs and weed control treatments on second rotation loblolly pine plantations (Subedi et al., 2017). The second rotation “untreated carryover” experiment was used for this study. The untreated carryover experiment received no treatment in the second rotation and are now referenced as Control (C_C), Fertilizer only (C_F), Fertilizer and Weed control (C_FW), and Weed control only (C_W) treatments (Table 3-1).

Like in the previous rotation, loblolly pines were planted in each plot at a 1.8 m by 3.6 m spacing, with measurement plots (0.02 ha) consisting of forty trees per plot (8 trees each in 5 beds). Each of the measurement plots was provided with a treated buffer of three trees and two beds, resulting in a 0.08 ha treatment plot. An untreated buffer of six tree spaces was provided between two adjacent treatment plots. Across the treatment plots, an untreated buffer of four beds was maintained. A single, full-sib loblolly family was used to regenerate the entire study area in December 2009 using containerized stock.

All treatments in the untreated carryover experiment received a single application of Fipronil (9.1%) in the form of PTM™ (BASF Corp., Research Triangle Park, NC, USA) in March 2010 to control Nantucket pine tip moth (Rhyacionia frustrana (Comstock)). No herbicide or fertilizer was applied on the untreated carryover experiment, with the exception of a banded 0.2 kg a.e. ha⁻¹ imazapyr application in May 2010 to control Dichanthelium spp. and to aid seedling survival.

During September and October of 2009, two small trenched plots (~250 cm²) were established on the bed and inter-bed position in each measurement plot randomly. Trenches were dug manually to a depth of 50 cm to sever roots, and 2 mm thick plastic barriers installed to prevent lateral root growth inside the trenched plots. A prior study by Ewel et al. (1987) used similar plastic barriers (two layers of 0.25 mm thick plastic) to effectively prevent lateral root
growth into the trenched sites in 9- and 29-year-old Florida slash pine plantations. In these young stands, it was assumed that roots did not infiltrate into the trenched plots from below, as Adegbidi et al. (2004) reported that root development of young loblolly pine stands did not exceed beyond a 50 cm soil depth over the first few years of stand development. In a 20-year-old slash pine plantation, Van Rees and Comerford (1986) reported that almost 84% of root mass of understory vegetation was present in the 0-50 cm soil depth. For the duration of this study, trenched plots were kept free of live vegetation to prevent development of new root systems. One PVC collar [“root exclusion” (RE) collar] was placed in the middle of each trenched plot to measure Rs. Two PVC collars (one in the bed and one in the inter-bed) were placed above the mineral soil outside the trenched plots to measure the Rs (plots without RE). For this study, a randomized complete block experimental design with three replicates was used.

**Soil Respiration**

Monthly measures of soil respiration rates were conducted from March 2011 through April 2012, except for the growing season (from May 2011 to September 2011) when soil respiration measurements were made twice per month. During the growing season, instantaneous soil respiration was measured using a 6400-09 Soil CO₂ Flux Chamber [a portable infrared-gas analyzer (IRGA)] attached to the LI-6400 Portable Photosynthesis System (Li-Cor, Inc., Lincoln, NE). During each measurement, the soil CO₂ flux chamber was placed onto the soil collars that were permanently established within the measurement plots. A total of four soil collars per treatment plot, made from PVC pipe (diameter 10.16 cm, height 8 cm), were inserted about 3 cm into the soil surface. Of the four collars, two each were randomly placed on the bed and inter-bed positions (with and without RE). To minimize the effects of disturbance, the soil collars were installed at least one year prior to the initiation of soil respiration measurements in March 2011. The temperature of the soil surface (~0 to 15 cm) was measured at each time period with a soil
probe thermocouple inserted within 5 cm of the measurement collar. Similarly, three measurements of soil moisture (top 12 cm of soil) were made within 10 cm of the collar immediately following each soil respiration measurement using time domain reflectometry (Hydrosense Soil Water Measurement System (CS 620), Campbell Scientific, Inc., Logan, UT). Soil respiration, soil temperature, and soil moisture were averaged for each treatment plot prior to analysis.

Total annual Rs and Rh were calculated by adding all estimated mean daily Rs and Rh for each plot for a year, respectively. Exponential functions \( \text{Mean Daily Soil Respiration} = a \times e^{b \times \text{Mean Daily Soil Temperature}} \), where \( a \) and \( b \) are parameters; \( p < 0.01, R^2 \) range: 0.4-0.7) were used to estimate mean daily soil respiration for each plot. Mean daily soil temperature for each treatment was estimated from exponential equations \( \text{Mean Daily Soil Temperature} = a \times e^{b \times \text{Mean Daily Air Temperature}} \), \( p < 0.01; R^2 \) range: 0.6-0.7) developed using mean daily air temperature from the weather station (Gainesville Regional Airport) nearest the study site.

Net ecosystem productivity was estimated using a biometric approach (Clark et al., 2001; Bracho et al., 2012), as the difference between net primary productivity (NPP) and Rh. Net primary productivity for the study was estimated using the understory and pine biomass growth data (Subedi, 2013; Subedi et al., 2014). Understory aboveground biomass growth was estimated by using clip-plot surveys (Subedi, 2013). Understory root biomass growth was estimated using root:shoot ratios and allometric equations (Mokany et al., 2006; Hagan et al., 2009; Subedi, 2013). Pine root biomass was calculated using root:shoot ratios reported by Adegbidi et al. (2002). Biomass was converted to carbon using a conversion factor of 0.5 (Bracho et al., 2012).

**Soil Nutrient Supply**

Soil nutrient concentrations were estimated by collecting soil samples from all plots at the same depth intervals (0-10, 10-20, 20-50, and 50-100 cm) in November 2012 using a soil
auger (7.6 cm diameter). Eight samples were collected from each treatment plot using a stratified random method. The samples from the same depth intervals were thoroughly mixed and weighed. Approximately 15% of the mixed sample was then subsampled. Live roots were removed prior to subsampling. Almost 100 g of subsample was then air dried and ground in a mortar and pestle to pass through a 2-mm sieve. Soil macro- and micronutrients were extracted using the Mehlich III procedure (Mehlich, 1984). All samples were analyzed in the Micro-Macro International Laboratory in Athens, GA, USA.

**Decomposition of Organic Matter**

In March 2011, sixty *Betula papyrifera* (Marsh.) tongue depressors (15 × 1.9 cm) were buried in the beds of every treatment plot to understand the effects of silvicultural treatments on the decomposition of a homogenous source of organic matter. To precisely re-locate these depressors, six arrays of 10 depressors each were assembled and connected using monofilament line, and randomly buried in each measurement plot. Both in July and in November 2011, 10 tongue depressors in each measurement plot were removed from the soil to determine the amount of mass loss due to decomposition. The remaining 40 tongue depressors from each measurement plot were removed in March 2012. The tongue depressors were oven dried at 65°C to a constant weight and then gently brushed to remove any adhering soil particles before weighing. Careful attention was made to ensure that no decayed materials were lost during sample preparation.

**Data Analysis**

A repeated measures analysis of variance method was used to test the effects of past treatments on soil respiration (Littell et al., 2006). The following general repeated measures model, with covariates, was used:

\[ Y = \mu + b + D + T + DT + Ct + Cm + e \]
where, $\mu$ = overall mean; $b$ = random effect of the block with \( b \sim N(0, \sigma^2_b) \); $D$ = fixed effect of the date of measurement; $T$ = fixed effect of the treatments; $DT$ = fixed interactive effects between treatments and date; $Ct$ = effects of soil temperature (covariate); $Cm$ = effects of soil moisture (covariate); and $e$ = error term (random error with \( e \sim N(0, \sigma^2_e) \)).

Treatments, dates, and their interactions, along with covariates, were considered fixed effects. The PROC MIXED procedure in SAS was used for the statistical analyses. When a covariate was not significant in the model, it was removed from the final model. The selection of the autoregressive (1) covariance structure was based on the corrected Akaike information criteria (AIC\(_C\)) (Burnham and Anderson, 2002). When assumptions of normality and homoscedasticity were not met, data were square-root transformed prior to analysis. In addition, the effects of treatments and date of measurement on soil temperature and soil moisture were also investigated using repeated measures ANOVA. Multiple linear regression was used to investigate the relationships between soil respiration, soil temperature, and soil moisture.

Correlation analyses on soil respiration and soil nutrient availability were also done. When correlation analysis revealed significant relationships, a generalized regression with Adaptive Lasso evaluation (Zou, 2006) and AIC\(_C\) validation was used to isolate factors (e.g. soil temperature, soil moisture, and nutrients correlated with mean soil respiration) that explained variation in soil respiration.

Decomposition mass loss of the *Betula papyrifera* (Marsh.) tongue depressors was analyzed using the PROC MIXED procedure, with months after burial and treatments being fixed effects. Treatment effects were declared significant when $p$ values were $< 0.05$, unless otherwise stated.
Results

Soil Respiration

Both Rs and Rh rates were affected by measurement date (Rs: $F_{(15,110)} = 7.71, p < 0.001$; Rh: $F_{(15,110)} = 8.20, p < 0.001$) and past silvicultural treatments (Rs: $F_{(3,22.9)} = 4.40, p = 0.014$; Rh: $F_{(3,16.1)} = 5.18, p = 0.011$). Interaction between measurement dates and silvicultural treatments were not significant (Rs: $F_{(45,101)} = 0.57, p = 0.982$; Rh: $F_{(45,99.2)} = 0.82, p = 0.765$). Mean Rs rates were lower for all treatments during the winter season and higher during the growing season, with values ranging from 1.55 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ in January 2011 to 6.28 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ in June 2011 (Figure 3-1). The treatment differences in mean Rs rates were also significant for this study (Figure 3-2). For instance, mean Rs rates in the CF (4.56 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and CFW (4.49 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) treatments were 1.29- and 1.27- fold higher than in the CC (3.53 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) treatment. Mean Rs in the CW (3.82 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) was not significantly different from all treatments. Mean Rh rates, however, showed a different trend than the Rs rates: the CW (2.97 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) had a significantly lower Rh rate than the CF (4.02 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) treatment. Mean Rh rates in the CFW (3.66 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) and CC (3.36 $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) treatments were not different from the CW treatment (Figure 3-2). Estimates of annual soil C flux to the atmosphere via Rs ($p = 0.15$) and Rh ($p = 0.16$) (Mar 2011 – Apr 2012) were not significantly different among treatments. However, annual trends closely followed the mean for Rs i.e. CF (16.0 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CFW (15.3 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CC (12.4 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CW (12.0 Mg C ha$^{-1}$ yr$^{-1}$) and Rh rates, i.e. CF (13.7 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CFW (12.8 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CC (11.4 Mg C ha$^{-1}$ yr$^{-1}$) ≥ CW (9.8 Mg C ha$^{-1}$ yr$^{-1}$) (Figure 3-2).

Soil Temperature and Soil Moisture

When averaged across treatments, soil temperature in the upper 15 cm differed significantly among measurement dates and ranged between ~40°C in May 2011 and ~15°C in
January 2012 for both the Rh and Rs collars (Figure 3-1). When averaged across the study period, the soil temperature differed significantly among treatments for the Rh collars (Figure 3-3). For example, the CFW (29.2°C) and CW (28.4°C) treatments had almost 2°C higher soil temperatures compared to the CC (26.8°C) treatment. In addition, the CF treatment (27.4°C) had significantly lower soil temperature compared to the CFW treatment. Similar trends were also observed for the Rs collars: CFW (29.5°C) ≥ CW (28.3°C) > CF (27.0°C) = CC (26.4°C).

The site was dry over the period of study, with average soil volumetric moisture contents ranging from <1 to 6% for both the Rs and Rh collars (Figure 3-1). When averaged across the study period, significant treatment effects were observed in volumetric soil moisture content for the Rh collars ($F_{(3,27.8)} = 3.15, p = 0.04$). Moisture content was higher in the CFW (3.34%) treatment compared to the CF (2.42%) and CC (2.39%) treatments (Figure 3-3). Treatments, however, had no such influence on mean volumetric soil moisture content in the Rs collars when pooled across the measurement period ($F_{(3,30.4)} = 1.29, p = 0.29$; CFW (2.3%), CW (1.9%), CC (1.9%), and CF (1.7%)). However, relatively lower soil moisture content was expected for the Rs collars given the higher aboveground biomass (Subedi et al., 2014), and presumably greater moisture loss via transpiration in the CF and CC treatments.

Temporal changes in the Rs and Rh responded to soil temperature, but not to soil moisture. Soil temperature explained about 33% of the variation in Rs and 41% in Rh (Table 3-2). Volumetric soil moisture content explained only about 2% of the total variation in Rh. There were no treatment differences in the Rs and Rh responses to soil temperature (Exponential response modeled as: $\text{Soil Respiration} = a \times e^{b \times \text{soil temperature}}$, where $a$ and $b$ were scale and growth parameters respectively) in this study (ANOVA on parameters not presented; Figure 3-
4). When pooled across treatments, the $Q_{10}$ (calculated as $Q_{10} = e^{10 \times b}$; Davidson et al., 1998) values for Rs and Rh were 1.23 and 1.25, respectively.

**Soil Nutrient Availability in the Upper Soil Surface and Their Relationship to Rs and Rh**

The first rotation fertilizer additions increased the Mehlich-III extractable soil nutrient concentrations in the upper soil horizons. Calcium and Mg concentrations were slightly higher in the 0-10 cm depth for the CFW treatment compared to the CW treatment ($p = 0.08$). Treatments did not significantly affect N, P, and K concentrations in the second rotation (data not shown). However, treatment effects were more evident for micronutrients in the surface horizon. For example, soil Mn concentrations were almost 3.2-fold higher in the CF treatment compared to the CW treatment (Figure 3-5). Likewise, Cu concentrations in the CF treatment were 4- and 4.5-fold higher compared to the CC ($p = 0.08$) and CW treatments ($p = 0.07$), respectively.

Significant correlations between Rh and Mehlich III extractable soil micronutrient concentration in the 0-10 cm depth were observed in this study. Manganese ($r = 0.66$, $p = 0.019$), Zn ($r = 0.63$, $p = 0.028$), and Cu ($r = 0.62$, $p = 0.033$) were highly correlated with mean Rh (Table 3-3). Further evaluation of the effect of Mn, Cu, Zn, soil temperature, and soil moisture on Rh using a generalized regression with Adaptive Lasso estimation and AICc validation suggested that Mn concentration and soil temperature were the significant variable remaining in the model (final model: $Rh = 0.4559 + 1.0066 \times$Mn concentration + 0.08249$ \times$Soil temperature; $R^2 = 0.49$; RMSE = 0.881; Figure 3-6a). Only Mn ($r = 0.62$, $p < 0.05$) and Zn ($r = 0.69$, $p < 0.05$) concentrations showed positive relationships with Rs. Soil macronutrient concentrations were not correlated with either Rh or Rs (Table 3-3). When examining the relationships between Rs and variables like Mn, Zn, soil temperature, and soil moisture, only Zn and soil temperature remained in the model (final generalized regression model with Adaptive Lasso and minimum
AICc validation; Mean Rs = 0.9251×Zn concentration + 0.08749×Soil temperature; $R^2 = 0.42$; RMSE = 1.12; Figure 3-6b).

**Decomposition of a Common Substrate Among Treatments**

Silvicultural treatment history and time since burial had significant effects on the mass loss of the *Betula papyrifera* (Marsh.) tongue depressors (Table 3-4, Figure 3-7). Average mass loss over the 12-month period for the CF treatment was significantly higher than the CW treatment ($p = 0.027$). For example, the mass loss associated with the CF treatment was approximately 93% compared to 85% for the CW treatment (Figure 3-7). In addition, the decomposition was almost 8% slower in the CW treatment compared to the CC treatment ($p = 0.06$).

**Discussion**

Rapid gains in timber yields from southern pine plantations due to intensive management practices have increased the C sequestration potential in the US South (Han et al., 2007). The need to better understand the long-term implications of these forest management practices on soil respiration and organic matter decomposition is important relative to the current issues of global climate change (Rustad et al., 2000; Woodbury et al., 2007). The study described here was the first conducted in intensively managed southern pine plantations to examine how silvicultural treatments in the first rotation affected belowground processes important for regulating C dynamics in the second rotation. Rotation-long application of fertilizers and sustained control of competing vegetation applied in the prior rotation, followed by no additional treatment, provided a unique opportunity to investigate silviculture legacy effects on soil respiration and belowground processes in the subsequent rotation.

Mean soil respiration rates in this study were comparable to the reported ranges for loblolly pine plantations growing under varying management regimes (Gough et al., 2005;
Samuelson et al., 2009; Templeton et al., 2015). For instance, in a one-year-old loblolly pine plantation in the Coastal Plain of South Carolina, soil respiration rates ranged from $1.1 \, \mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1}$ to $8.5 \, \mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1}$ (Gough et al., 2005). Samuelson et al. (2009) reported soil respiration rates of $1.9$ to $6.3 \, \mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1}$ in seven-year-old loblolly pine stands in South Carolina growing under irrigation and fertilization treatments. In an 11-year-old loblolly pine plantation growing on excessively well-drained, sandy Ultisols in North Carolina, soil respiration ranged from a low of $1 \, \mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1}$ in January to greater than $5 \, \mu\text{mol CO}_2\,\text{m}^{-2}\,\text{s}^{-1}$ in June (Maier and Kress, 2000). Estimates of annual soil C loss via Rs from 2-year-old pine stands in this study were within the ranges of $7$ to $22 \, \text{Mg C ha}^{-1}\text{yr}^{-1}$ reported for early- and mid-rotation southern pine stands (Maier and Kress, 2000; Lee and Jose, 2003; Gough et al., 2005; Ewel et al., 2008; Samuelson et al., 2009). However, these estimates of Rs were higher than reported by Pangle and Seiler (2002) for a 2-year-old loblolly pine stand growing in the Virginia Piedmont where the site was prepared with a chop and burn treatment.

On nutrient-limited sandy Spodosols, as characterized in this study, the previous rotation’s fertilization treatments increased both Rs and Rh in the subsequent rotation compared to the unfertilized treatments. Similar responses in Rs have been observed in early- to mid-rotation pine stands subjected to nutrient additions in the same rotation (Pangle and Seiler, 2002; Lee and Jose, 2003; Samuelson et al., 2009). These results, however, were not consistent with the findings from other studies in mid-rotation loblolly pine stands (Maier and Kress, 2000; Butnor et al., 2003; Bracho et al., 2018), where reported reductions in Rs followed fertilizer additions. In a meta-analysis of N manipulation studies in forest ecosystems, Janssens et al. (2010) also reported a general decline in Rs following N additions; however, in stands younger than 4 years, such declines were not evident. It has often been hypothesized that, in aggrading
stands, complementary effects of fertilization on the root (positive effect) and microbial respiration (Rh) (negative effect) resulted in no net change in total Rs (Lee and Jose, 2003; Gough and Seiler, 2004). In young harvested stands like ours, the suppression effects of fertilization on Rs and Rh may seldom be observed, possibly because fertilization did not change belowground biomass allocation patterns alone (Retzlaff et al. 2001), but accelerated both root development and microbial activity (Janssens et al., 2010). These results also suggest that forest floor and soil C pools at the end of rotation in fertilized plantations were more likely to be released to the atmosphere via Rh increase early in the subsequent rotation.

On N and P limited sites, decomposition rates of organic matter often depend on nutrient availability and the presence of labile C for soil microbes (Blazier et al., 2005; Fontaine et al., 2007; Cotrufo et al., 2013). Fresh organic matter inputs could ‘prime’ soil microbes, resulting in enhanced decomposition processes (Bingeman et al., 1953; Kuzyakov, 2010). Decomposition of understory vegetation components following harvest (e.g. Ilex glabra (L.), a dominant Mn accumulator; Subedi et al., 2014) in the CF and CC treatments likely increased labile C and nutrient availability early in the second rotation. This positive feedback mechanism could have enhanced the organic matter decomposition processes and contributed to the higher amounts of mass loss in the Betula papyrifera (Marsh.) tongue depressors for these treatments relative to the CW treatment (Blazier et al., 2005; Dashtban et al., 2010; Trum et al., 2011; Keiluweit et al., 2015; Camenzind et al., 2018). Also, in the first rotation, the weed control treatment had higher phenolic concentrations in the litter and surface soil compared to the fertilizer treatments (Polglase et al., 1992c). Such differences, due to treatment, could have affected organic matter decomposition responses early into the next rotation (Schimel et al., 1996; Kraus et al., 2003; Coq et al., 2010).
Previous research has documented aboveground growth responses of slash pine to Mn additions on poorly drained Spodosols (Jokela et al., 1991); however, organic matter decomposition responses to micronutrient additions were not measured. The strong positive correlations observed between mean Rh and surface soil Mn availability in this study suggests that organic matter decomposition on recently established sites may be inherently nutrient limited. Though there were almost 32% lower soil C pools in the forest floor, roots, and understory vegetation that were incorporated in the C<sub>W</sub> (66.5 Mg C ha<sup>-1</sup>) than the C<sub>F</sub> treatments (97.5 Mg C ha<sup>-1</sup>) at the end of the first rotation, these pools did not correspond with differences in Rh responses. For example, no correlation was observed between forest floor C or N pools at the end of the first rotation and the mean Rh rates early in the second rotation (C: \( r = 0.39, p = 0.2; \) N: \( r = 0.41, p = 0.18; \) Vogel et al. 2011). In addition, soil Mn concentration and soil temperature were the only variables that explained Rh variation in this study. Some evidence from temperate forests and continental forests in China showed that increased Mn<sup>2+</sup> availability accelerated organic matter decomposition in N enriched sites (Trum et al., 2011; Whalen et al., 2018; Sun et al., 2019) by facilitating the production of lignin oxidizing extracellular enzymes (e.g. Mn peroxidase, laccase) (Dashtban et al., 2010; Sun et al., 2019). This seems important because Rh measurements were made almost 22 months after the harvest of the first rotation and organic matter substrate remaining from this harvest (severed roots) and forest floor were likely more recalcitrant (lignin rich). Significant Mn relationship with Rh along with Mn relationships with decomposition observed in other studies lead us to hypothesize that soil micro-nutrient availability, esp. Mn, also exerts substantial control over heterotrophic responses on this nutrient-limited site. Additional replicated decomposition experiments with Mn additions, alone or in
combination with other nutrients, may be necessary to explore this mechanism in nutrient limited Spodosols.

Recently established forests are generally a significant source of C inputs to the atmosphere (Bracho et al., 2012). Our estimates agree with this observation, with NEP values of -6.2, -6.3, -8.1, and -6.5 Mg C ha\(^{-1}\) yr\(^{-1}\) for the C\(_C\), C\(_F\), C\(_{FW}\), and C\(_W\) treatments, respectively. In general, ecosystem C losses for this study were like those reported by Bracho et al. (2012; -5.3 Mg C ha\(^{-1}\) yr\(^{-1}\)) for two-year-old slash pine plantations growing on similar soils. For the C\(_F\) treatment, greater NPP (7.4 Mg C ha\(^{-1}\) yr\(^{-1}\) in C\(_F\) vs 4.7 Mg C ha\(^{-1}\) yr\(^{-1}\) in C\(_{FW}\)) resulted in relatively smaller ecosystem C loss compared to the C\(_{FW}\) treatment, despite having higher decomposition rates and Rh values. In addition, subsequent nutrient mineralization following OM decomposition may have partly resulted in higher nutrient supply for the C\(_F\) treatment (P: 2.5-fold; Mn: 2.6-fold higher; Subedi et al., 2014) compared to the C\(_{FW}\) treatment, and supported early growth demands of the understory vegetation and pine trees (Subedi et al., 2014). From the perspective of C management, intensive fertilization and understory competition removal resulted in more ecosystem C loss, suggesting that fertilization alone (with understory retention) could be a desirable silvicultural practice to reduce ecosystem C loss by improving biomass yields early in the next rotation.

Strong relationships between soil temperature, soil moisture, and soil respiration exist across different terrestrial ecosystems (Lloyd and Taylor, 1994; Davidson et al., 1998; Fang and Moncrieff, 2001; Lee and Jose, 2003; Gough et al., 2005; Jassal et al., 2008; McElligott et al., 2017). However, for young and recently established sites, these relationships tend to be weaker as observed in our study and others (Gough et al., 2005; Tyree et al., 2008). For a young, 2-year-old stand growing on Ultisols in Virginia, Tyree et al. (2008) reported that soil temperature
accounted for only 30% of the total variation in soil respiration. Larger microsite variation due to localized accumulation of organic matter in recently harvested sites likely contributed to the weak relationship between soil temperature and Rs or Rh in our study. In addition, large soil temperature ranges over the course of our study (range: ~15 to 40°C during measurement) decreased the sensitivity of soil respiration at higher temperatures (Luo et al., 2001; Peng et al., 2009), and the decoupling of soil respiration from soil temperature at low soil moisture levels (Jassal et al., 2008) could result in a weak soil temperature-respiration relationship. Low $Q_{10}$ values ($< 2$) for the Rs and Rh were likely due to reduced Rs or Rh sensitivity to temperature at low soil moisture contents (Kirschbaum, 1995; Curiel Yuste et al., 2007; Jassal et al., 2008). Similarly, the weak relationship observed between volumetric soil moisture content and Rh suggests that in sandy Spodosols soil moisture in the surface horizon may not have a strong control on soil respiration during the early stages of stand development. Like in this study, others have documented relatively weak relationships between soil respiration and soil moisture compared to soil temperature in early- to mid-rotation loblolly pine stands (Gough et al., 2005; Tyree et al., 2008; Samuelson et al., 2009). Unlike stands having closed canopies, where microsite variation may be lower due to “steady state” conditions (e.g., wind, insolation, soil organic matter), our results suggest that soil temperature and soil moisture alone may not accurately predict variation in Rs and Rh in open canopy or young forest systems.

**Summary**

This study, conducted on nutrient-limited Spodosols in north Florida, documented the legacy effects of the previous rotation’s fertilization (macro and micronutrients) and competing vegetation control treatments on soil respiration and organic matter decomposition processes in the subsequent rotation and identified important drivers of these processes. Our results indicated that historical nutrient amendments ($C_F$ and $C_{FW}$) had a positive effect on total soil respiration, at
least during these early stages of stand development. In contrast, lower heterotrophic soil respiration was observed under the sustained understory competition control (C_W) treatment compared to fertilization (C_F) in the previous rotation. Consistent with the heterotrophic soil respiration response, the competing vegetation control treatment history resulted in lower decomposition of a common organic substrate than did the fertilization treatment history. In addition, facilitation of organic matter decomposition in the understory retention treatments (C_F and C_C) reinforces the potential role that understory plants may have on nutrient cycling and mineralization processes. Both soil temperature and soil Mn availability tracked variation in heterotrophic respiration in young stands, but soil moisture did not. Instead, a strong correlation between soil Mn availability and mean heterotrophic soil respiration suggests that organic matter pools accumulated in the previous rotation due to fertilization may be decomposed more easily in the subsequent rotation because of a micronutrient dynamic. Because nutrient limited Spodosols cover almost 5.7 million ha of land in the southeastern United States (Adegbidi et al., 2002), further studies examining the effects of Mn supply on specific microbial processes (e.g., specific enzyme production, substrate use efficiency) will be important in understanding the effects of silvicultural practices on organic matter decomposition and nutrient cycling.
Table 3-1. Treatments applied to a loblolly pine plantation growing on a North Florida Spodosol for the IMPAC II untreated carryover experiment

<table>
<thead>
<tr>
<th>Treatment abbreviations</th>
<th>First-rotation treatment</th>
<th>Second-rotation treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_C</td>
<td>Control</td>
<td>untreated</td>
</tr>
<tr>
<td>C_F</td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation (kg ha(^{-1})): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>untreated</td>
</tr>
<tr>
<td>C_FW</td>
<td>Fertilizer (same as the C_F treatment) + weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>untreated</td>
</tr>
<tr>
<td>C_W</td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>untreated</td>
</tr>
</tbody>
</table>
Table 3-2. Stepwise regressions of factors influencing soil respiration rates for juvenile loblolly pine stands growing on Spodosols in north Florida for the IMPAC II untreated carryover experiment.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Parameter estimate</th>
<th>Partial $R^2$</th>
<th>F Ratio</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rs</td>
<td>0.0277</td>
<td>0.329</td>
<td>93.323</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rh</td>
<td>0.0276</td>
<td>0.406</td>
<td>141.666</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.0329</td>
<td>0.023</td>
<td>7.853</td>
<td>0.005</td>
</tr>
</tbody>
</table>

Note: Soil respiration data were log transformed prior to stepwise regression. Data were pooled across treatments.
Table 3-3. Pearson correlation coefficients ($r$) between soil respiration rates and Mehlich III extractable soil nutrient concentrations (0-10cm) for juvenile loblolly pine stands growing on Spodosols in north Florida for the IMPAC II untreated carryover experiment (n=12).

<table>
<thead>
<tr>
<th>Soil nutrients</th>
<th>Rs</th>
<th></th>
<th></th>
<th>Rh</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$p$Value</td>
<td>$r$</td>
<td>$p$Value</td>
<td></td>
</tr>
<tr>
<td>N concentration</td>
<td>-0.272</td>
<td>0.392</td>
<td>0.080</td>
<td>0.805</td>
<td></td>
</tr>
<tr>
<td>P concentration</td>
<td>-0.224</td>
<td>0.484</td>
<td>-0.136</td>
<td>0.673</td>
<td></td>
</tr>
<tr>
<td>K concentration</td>
<td>-0.176</td>
<td>0.585</td>
<td>-0.136</td>
<td>0.674</td>
<td></td>
</tr>
<tr>
<td>Ca concentration</td>
<td>0.183</td>
<td>0.569</td>
<td>0.306</td>
<td>0.333</td>
<td></td>
</tr>
<tr>
<td>Mg concentration</td>
<td>0.262</td>
<td>0.411</td>
<td>0.096</td>
<td>0.768</td>
<td></td>
</tr>
<tr>
<td>B concentration</td>
<td>-0.430</td>
<td>0.164</td>
<td>-0.098</td>
<td>0.763</td>
<td></td>
</tr>
<tr>
<td>Cu concentration</td>
<td>0.557</td>
<td>0.060</td>
<td>0.615</td>
<td>0.033</td>
<td></td>
</tr>
<tr>
<td>Mn concentration</td>
<td>0.616</td>
<td>0.033</td>
<td>0.659</td>
<td>0.020</td>
<td></td>
</tr>
<tr>
<td>Zn concentration</td>
<td>0.698</td>
<td>0.012</td>
<td>0.628</td>
<td>0.029</td>
<td></td>
</tr>
<tr>
<td>Mo concentration</td>
<td>-0.432</td>
<td>0.161</td>
<td>-0.091</td>
<td>0.779</td>
<td></td>
</tr>
<tr>
<td>Fe concentration</td>
<td>0.009</td>
<td>0.978</td>
<td>0.261</td>
<td>0.414</td>
<td></td>
</tr>
<tr>
<td>Al concentration</td>
<td>0.320</td>
<td>0.311</td>
<td>0.463</td>
<td>0.129</td>
<td></td>
</tr>
</tbody>
</table>
Table 3-4. Effects of fertilization and weed control on the 12-month mass loss of a common organic substrate (*Betula papyrifera* (Marsh.) tongue depressors) for the IMPAC II actively managed retreated and untreated carryover experiments in north Florida.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean % original mass remaining (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C&lt;sub&gt;C&lt;/sub&gt;)</td>
<td>6.82 (± 4.45) a</td>
</tr>
<tr>
<td>Fertilizer only (C&lt;sub&gt;F&lt;/sub&gt;)</td>
<td>6.80 (± 1.43) a</td>
</tr>
<tr>
<td>Fertilizer+ weed control (C&lt;sub&gt;FW&lt;/sub&gt;)</td>
<td>12.69 (± 1.77) ab</td>
</tr>
<tr>
<td>Weed control only (C&lt;sub&gt;W&lt;/sub&gt;)</td>
<td>14.71 (± 4.01) b</td>
</tr>
</tbody>
</table>

Note: Means followed by the same letter are not significantly different (α = 0.1)
Figure 3-1. Temporal variation in Rh and Rs rates, soil temperature, and mean volumetric soil moisture content among treatments for juvenile loblolly pine growing on Spodosols in north Florida for the IMPAC II untreated carryover experiment. The notations: C_C, C_F, C_FW, and C_W, respectively, represent the plots that received the control, fertilization only, fertilization + weed control, and weed control only treatments in the previous rotation. Error bars represent standard error of the mean.
Figure 3-2. Mean soil respiration rates and annual C flux (Mar 2011- Mar 2012) for juvenile loblolly pine growth on Spodosols in north Florida for the IMPAC II untreated carryover experiment. The notations: $C_C$, $C_F$, $C_{FW}$, and $C_W$, respectively, represent the plots that received the control, fertilization only, fertilization + weed control, and weed control only treatments in the previous rotation. Treatments with similar letters were not significantly different at $\alpha = 0.05$. Error bars represent the standard error of the mean.
Figure 3-3. Mean soil temperature and volumetric soil moisture content near the Rh and Rs soil collars for juvenile loblolly pine growing in Spodosols in north Florida for the IMPAC II untreated carryover experiment. Within the soil respiration component, treatments with similar letters were not significantly different at $\alpha = 0.05$. Error bars represent the standard error of the mean.
Figure 3-4. Soil temperature response curves for the a. Rs and b. Rh rates in a 2-year-old loblolly pine plantation growing on Spodosols in north Florida for the IMPAC II experiment. Exponential functions of the form $y = ae^{bx}$ were fitted for each treatment. ANOVA on parameters ‘a’ and ‘b’ were not different among treatments for both Rs and Rh at $\alpha = 0.05$. When treatments were pooled, $R^2$ values were 0.30 for the Rs ($R^2$: $C_C = 0.23$, $C_{CF} = 0.35$, $C_{FW} = 0.33$, and $C_W = 0.33$) and 0.37 for the Rh ($R^2$: $C_C = 0.44$, $C_{CF} = 0.47$, $C_{FW} = 0.38$, and $C_W = 0.39$).
Figure 3-5. Mehlich III extractable soil nutrient concentration across different soil depths in juvenile loblolly pine stands growing on Spodosols in north Florida for the IMPAC II untreated carryover experiment. Within a soil depth, treatments followed by the same letter are not significantly different at $\alpha = 0.1$. Error bars represent standard error of the mean.
Figure 3-6. Plot of actual vs. predicted (a) Rh and (b) Rs based on the generalized linear regression model (Rh=0.4559+1.0066×Mn concentration+0.08249×Soil temperature; Rs=0.7848+0.9251×Zn concentration+0.0875×Soil temperature). Solid circle, triangle, inverted triangle, and square, respectively denote C_C, C_F, C_FW, and C_W treatments.
Figure 3-7. Effects of fertilization and weed control treatments on the decomposition of *Betula papyrifera* (Marsh.) tongue depressors in juvenile loblolly pine stands growing on Spodosols in north Florida for the IMPAC II untreated carryover experiment. Error bars represent the standard deviation.
Background

In terrestrial ecosystem, litter decomposition is an important nutrient cycling process that returns more than half of the net primary productivity to the soil (Vitousek, 1984; Wardle et al., 2004) and contributes to terrestrial CO$_2$ flux to the atmosphere (Raich and Schlesinger, 1992; Houghton, 2007; Sayer et al., 2007). During litter decomposition, complex organic matter present in the leaf litter and forest floor pools are reduced to simple organic compounds, and nutrients are mineralized to readily available forms for plant uptake. A complex interplay of abiotic and biotic factors such as the physicochemical environment (Aerts, 1997; Zhang et al., 2008; Brandt et al., 2009), resource quality or relative decomposability (Berg et al., 2000; Zhang et al., 2008; Austin and Ballaré, 2010; Zhu et al., 2016), and decomposer organisms (Wall et al., 2008; García-Palacios et al., 2013; Bradford et al., 2017) influences the rate of litter decomposition in these ecosystems. While litter decomposition is generally rapid in nutrient-rich ecosystems (Zhang et al., 2008), slower litter decomposition rates common in nutrient limited ecosystems may represent a major nutrient limitation for net primary production as inaccessible nutrient pools build up above the soil surface in these systems.

Fertilization with N and P generally results in nutrient-rich litter, but its effect on litter decomposition is not consistent across ecosystems (Knorr et al., 2005). Particularly in forest ecosystems, there is a large degree of variability in decomposition response to N additions with reports of lower (Carreiro et al., 2000; Chen et al., 2013), higher (Polglase et al., 1992b; Sanchez, 2001) or no net change (Prescott et al., 1999; Gurlevik et al., 2003; Kiser et al., 2013). Studies also suggest that these responses are tied to the C chemistry of the litter. For example,
Carreiro et al. (2000) documented that litter decomposition due to N addition increased in low lignin litter (low lignocellulose index), but decreased in high lignin litter (high lignocellulose index) due to potential effects in cellulase or phenol oxidase activity. While historical focus on N and P limitations to aboveground productivity (LeBauer and Treseder, 2008; Vitousek et al., 2010) may partly be the reason for N and P amendment effects on litter decomposition being the focus of many these studies (Hobbie, 2005; Knorr et al., 2005), the effects of nutrients other than N and P remain largely unknown for temperate ecosystems (Kaspari et al., 2008; Powers and Salute, 2011; Keiluweit et al., 2015; Trum et al., 2015). In that context, understanding how macro- and micro-nutrient amendment practices influence litter decomposition and subsequent nutrient release is important for quantifying nutrient availability and modeling ecosystem functions in temperate ecosystems.

On nutrient poor sites, leaf litter returned to the forest floor is primarily of poor quality due to high nutrient resorption prior to leaf abscission (Dalla-Tea and Jokela, 1994). Because the return of nutrient poor, low-quality litter may retard both microbial decomposition and nutrient mineralization (Cornwell et al., 2008; Hoorens et al., 2010), long-term retention of nutrients in the soil as leaf litter may be observed on these sites. Most pine plantations in the lower Coastal Plain of the US grow on N and P- limited sites where nutrient amendments and competition control are common but important silvicultural practices for alleviating nutrient limitations to stand growth (Colbert et al., 1990; Fox et al., 2007; Jokela et al., 2010) These silvicultural practices improve foliage production and litter inputs to the soil (Martin and Jokela, 2004). For example, fertilization increased forest floor C pools by about 2.3- fold when compared to unfertilized plots in a 26-year-old loblolly pine plantation growing on a nutrient limited Florida Spodosol (Vogel et al., 2011). Liu and Greaver (2010) reported that N fertilization increased
aboveground litter inputs to the soil by about 20% on average globally. Besides its direct influence on the amount of litter inputs to the soil, nutrient additions and competing vegetation control treatments have been shown to influence litter nutrient release, and litter chemistry, including phenolics (Polglase et al., 1992c; Gurlevik et al., 2003; Kiser et al., 2013) that can have potential ramifications on litter decomposition and nutrient cycling in these stands. Given the short treatment histories of these studies, understanding whether their effects on litter quality, and subsequent litter decomposition are temporary or more long-lasting remains an important but rarely examined question in plantations managed beyond a single rotation.

In southern pine plantations, understory vegetation control is a prevalent management strategy to improve pine productivity by eliminating competition for nutrients and other site resources (Neary et al., 1990a; Jokela et al., 2010). Studies investigating the effects of herbicide treatments report either transient or permanent changes in understory vegetation composition (Miller et al., 1999; Jones et al., 2009; Subedi et al., 2017). Changes in community composition may result in changes in litter composition and quality of the forest floor. For example, hardwoods present in the understory vegetation in southern pine plantations result in litterfall with high decomposability due to higher nutrient concentrations and lower lignin content (Piatek and Allen, 2001; Subedi et al., 2014). In addition, Polglase et al. (1992b) reported changes in pine litter chemistry (increase in total labile C and phenol concentration) for sites that received weed control treatments in north Florida. Changes in micro-environment (e.g. temperature, moisture) and associated changes in microbial activity may be observed where competing understory vegetation has been removed in plantations (Ohtonen et al., 1992). Potential effects of competing vegetation control on root inputs to the soil may alter decomposition patterns by affecting labile C sources necessary for microbial activity (Subke et al., 2004; Wu et al., 2011).
Modification of the light environment when shrubby understory vegetation is removed may also result in changes in phenolics of other herbaceous understory components (Mole et al., 1988). Though earlier efforts by Piatek and Allen (2001) provide some insights on the effect of understory vegetation on litter decomposition, such effects have not been investigated in stands managed intensively over multiple rotations. The paucity of long-term multi-rotation studies limits our understanding of whether lasting effects of sustained weed control treatments on understory vegetation composition alter pine litter decomposition patterns and affect nutrient cycling in stands managed beyond a single rotation.

Long-term fertilization and weed control treatments effects on pine litter decomposition were investigated in an intensively managed pine plantation. It was hypothesized that fertilization increases litter decomposition primarily by influencing litter quality (nutrient ratios) and the litter mix with understory vegetation. It was also hypothesized that the weed control treatment reduces litter decomposition due to changes in litter quality and the lack of a litter mix with understory vegetation. Specifically, two second rotation experiments with a treatment history of fertilization and weed control treatments in the prior rotation was used to address two major questions:

- Does the fertilization and weed control treatment history in the prior rotation affect litter decomposition and nutrient release patterns in the subsequent rotation?
- Are the trends of litter decomposition similar to that observed in the untreated rotation if the prior rotation’s treatments are continued into the subsequent rotation? Is understory vegetation an important driver of litter decomposition in these stands?

**Methods**

**Site Description**

The IMPAC (Intensive Management Practices Center) experiment was established in 1983 to evaluate factors limiting the productive potential of southern pines (Swindel et al.,
The study is located approximately 10 km north of Gainesville, Florida (29°30’N latitude and 82°20’W longitude) at an elevation of 45 m from the mean sea level. The climate is warm and humid with a long-term (1984-2017) mean annual temperature of 20.7°C and total annual precipitation of 1207 mm (National Oceanic and Atmospheric Administration, 2018). Pomona fine sands (sandy siliceous hyperthermic Ultic Alaquods) are the predominant soil types at this site. The understory vegetation community was typical of Florida flatwood sites. Woody component of the understory consisted mainly of gallberry (*Ilex glabra* (L.)), saw palmetto (*Serenoa repens* (Bartr.)), fetterbush (*Lyonia ferruginea* (Walt.)), blueberries (*Vaccinium* spp.), and wax myrtle (*Myrica cerifera* (L.)). Chalky bluestem (*Andropogon* spp.), tapered witchgrass (*Dichanthelium* spp.), and nutrushes (*Scleria* spp.) were the graminoid components of the understory (Neary et al., 1990b; Subedi et al., 2017).

**Study Design**

The first rotation IMPAC experiment was designed as a 2×2×2 factorial consisting of species (loblolly and slash pine (*Pinus elliottii* var. *elliottii* (Engelm.))), complete and sustained weed control, and annual fertilization arranged in a randomized split-plot design. This resulted in four treatments within each species (species as whole plots): control (C), weed control only (W), fertilizer only (F), and both fertilizer and weed control (FW). The entire experimental area was site prepared using a single-pass bedding treatment. In January 1983, genetically improved (first generation, open pollinated) 1-0 bareroot stock (grown for 1 year in the seedbed) of both loblolly and slash pine were hand planted (Swindel et al., 1988; Colbert et al., 1990). The F and FW treatments received a balanced fertilizer regime including macro- and micronutrients for the first ten years annually. In May 1993, fertilization was stopped and then resumed from 1998 - 2000. Competing vegetation in the W and FW treatments was eliminated using a combination of
chemical and mechanical methods for the first ten years until canopy closure impeded further understory development (Colbert et al., 1990; Dalla-Tea and Jokela, 1994).

In May 2009, the first rotation IMPAC study was harvested and a second rotation experiment was overlaid using the same treatment plots. Prior to the harvest of the first rotation study, the understory vegetation within the C and the F treatments was mulched in place in April 2009 to retain this nutrient pool within the plot boundaries. Mulching was not necessary for the W and FW treatments because of the sustained weed control treatment history from the previous rotation. To ensure no inputs of nutrients via harvest residues, each plot was whole-tree harvested and processed off the treatment plots. Following harvest, the entire study area was later bedded in June, with a second bedding pass conducted in August of the same year.

Original plots in the first rotation were re-established and those plots were used to examine both the “untreated carryover” and “actively managed retreatment” effects on the pine growth dynamics in the second-rotation experiment. The IMPAC II experiment now consists of two randomized complete block designs, with 3 replications each, having four treatments (C_C, C_F, C_FW, C_W) for the untreated carryover experiment and four treatments (C, F, FW, and W) for the actively managed retreated experiment (Table 4-1). While the untreated carryover experiment, established on the previous slash pine plots, received no treatments in the second rotation, the actively managed retreatment experiment, established on the previous loblolly pine plots, continued to receive similar treatments as in the first rotation (Subedi et al. 2014).

In October 2009, the W and FW treatments were treated using a broadcast application of 0.84 kg a.e. ha⁻¹ imazapyr in the form of Chopper (BASF Corp., Research Triangle Park, NC), 1.12 kg a.e. ha⁻¹ triclopyr in the form of Garlon 4 (Dow AgroSciences LLC, Indianapolis, IN),
and 0.14 kg ha\(^{-1}\) of metsulfuron methyl in the form of Escort® (E.I. du Pont de Nemours and Company, Inc., Wilmington, DE).

In December 2009, the entire study was regenerated using containerized seedlings from a second generation single, full-sib loblolly family. Like the first rotation, loblolly pines were planted in each plot at a 1.8 m by 3.6 m spacing, with measurement plots consisting of forty trees per plot (arranged as 8 trees each in 5 beds). A treated buffer consisting of three trees and two beds surrounded each measurement plot. Six tree spaces of untreated buffer were provided between two adjacent treatment plots. Across the treatment plots, an untreated buffer of four beds was maintained (Figure 4-1).

In March 2010, all treatments (actively managed retreated and untreated carryover) received a single application of Fipronil (9.1%) in the form of PTM (BASF Corp., Research Triangle Park, NC) to control Nantucket pine tip moth (Rhyacionia frustrana (Comstock)). Later in May 2010, a banded 0.2 kg a.e. ha\(^{-1}\) imazapyr was applied on the beds of in all treatment plots (both untreated carryover and actively managed retreated) to control tapered witchgrass and to aid seedling survival. In October 2010, the W and FW treatments received a directed spray application of triclopyr (3%) and imazapyr (1%) to control gallberry and other understory competitors. Later in September 2011, a directed spray of glyphosate (3%) was applied to the actively managed W and FW treatments to maintain a weed free environment.

The actively managed retreated (F and FW) experiment received fertilizer annually. Consistent with the last rotation treatments, the total nutrient additions over the first six growing seasons for the F and FW treatments were (kg ha\(^{-1}\)): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B. As done in the first rotation experiment, the fertilizer was applied in narrow bands (30 cm semicircle) around the base of each tree or planting
location. In April 2016, the FW and F treatments were thinned in the actively managed retreated experiment to meet other long-term study objectives. The thinning operation removed almost 44.6 Mg ha\(^{-1}\) of aboveground biomass from the FW treatment and 25.7 Mg ha\(^{-1}\) from the F treatment.

**Litterbag Decomposition Study Design and Sampling**

Pine litter collected from the measurement plots across all treatments in 2015 was oven dried at 65\(^{\circ}\)C to a constant weight. A total of 1008 litterbags were prepared for the experiment. Nylon litterbags (12*12cm (1-mm mesh) which excludes meso- and macro-fauna) were filled with 6g of oven dried litter material. These were placed randomly on top of the mineral soils in the corresponding measurement plots of the IMPAC II study in May and June of 2016. Two galvanized ‘U’-shaped pins were used to secure litterbags in place during the study. From all treatments, six bags were retrieved at seven different times over about 13 months. Fresh weight of litterbags was recorded prior to oven drying to a constant weight at 65\(^{\circ}\)C. Differences in these weights were used to measure moisture content of the litterbags after each retrieval. Oven dried samples were gently brushed to remove any adhering soil particles before weighing. Careful attention was made to ensure that no decayed materials were lost during sample preparation. One temperature sensor (iButton\(^{\text{®}}\); Maxim Integrated, San Jose, CA) was placed on top of the mineral soil below the litter layer in all treatment plots to measure soil surface temperature during the decomposition study. In addition, annual litterfall for each treatment was estimated by using litter traps. Six 0.6 m\(^2\) litter traps were randomly placed in each measurement plot in the study area. Litterfall (pine needles) was collected monthly for a one-year period and oven-dried to a constant weight at 65\(^{\circ}\)C.
Gallberry, a common understory shrub, was chosen to investigate its priming influence on pine litter decomposition across the different silvicultural treatments. Preliminary analyses by Subedi (2013) showed that gallberry was both the dominant understory shrub (C: 49%, F:42%, Cc: 49%, and Cf: 46% of the total understory biomass at age 2 years) and micronutrient accumulator (e.g. Mn and Zn; Subedi, 2013) at the IMPAC II study. For this study, a total of 15 [3 litter-types: i) pine (50%) + gallberry (50%) from control treatment, ii) pine (50%) + gallberry (50%) from fertilizer treatment, and iii) pine only (100%)] litterbags (5 from each litter-types) were randomly placed above the mineral soil in all measurement plots in August/September 2016. After a year, these litterbags were retrieved, and pine litter was separated carefully from gallberry litter. Pine litter mass loss was estimated and compared among treatments and litter types.

**Chemical Analyses**

Macro- and micro-nutrient concentrations in the litter were estimated at four (0, ~2, ~6, and ~13 months) time intervals. Litter was dried in the oven (65°C) to a constant weight and carefully brushed to remove any adhering soil particles prior to grinding it in a Wiley mill to pass through 1mm sieve. Macro- and micro-nutrients were analyzed at the Micro-Macro International Laboratory in Athens, GA. About 0.5 g of ground litter was dry-ashed in a muffle furnace, and then brought up to volume with aqua-regia (3:1 Nitric/Hydrochloric acid). The extracts were then analyzed using inductively coupled plasma atomic emission spectroscopy (ICP-AES). Total N was analyzed using a CNS analyzer (Leco Corp.) and the Dumas method (Campbell, 1992). Nutrient content was estimated by multiplying nutrient concentrations and mass left for a given sample time interval. Similar analyses were conducted on initial gallberry litter.

Fiber lignin concentration was estimated for initial litter, and litter retrieved after 2, 6 and ~13 months. Briefly, oven dried litter samples were ground in a Wiley mill to pass through a
1 mm screen. Acid detergent fiber determinations were made by placing 0.5 g of sample in ANKOM filter bags (F57) and running the sample bags through a hot acid detergent solution (Catalog No. FAD20CB; ANKOM technology, Macedon, NY) for one hour using an ANKOM A200 Fiber analyzer (ANKOM technology, Macedon, NY). Sample bags were then rinsed, dried, and weighed prior to lignin determination. Sample bags were placed in 72% H₂SO₄ for three hours and agitated periodically. The rinsed sample bags were dried, weighed, and ashed in a muffle furnace to estimate lignin concentration.

**Data Preparation and Statistical Analyses**

Litter decomposition was estimated as the percent of original sample mass remaining at different time intervals. The litter decay rate constant (k) for each retrieval was estimated using an exponential decay model (Olson, 1963):

\[
\frac{X}{X_0} = e^{-kt}
\]

where, \(X\) was the sample mass at a given time, \(X_0\) was the initial sample mass, and \(t\) was the time in years. The final decay rate (k year\(^{-1}\)) for the study was estimated by pooling all retrievals together and using the same exponential model.

A repeated measures analysis of variance method was used to test the effects of past treatments on pine litter mass loss (Littell et al., 2006). The following general repeated measures model was used:

\[
y = \mu + b + S + D + SD + e
\]

where \(\mu\) = overall mean; \(b\) = random effect of the block with \(b \sim N(0, \sigma^2_b)\); \(S\) = fixed effect of the silvicultural treatment; \(D\) = fixed effect of no. of days; \(SD\) = fixed interactive effects between silvicultural treatment and no. of days; and \(e\) = error term (random error with \(N(0, \sigma^2_e)\)). The PROC MIXED procedure in SAS (SAS Institute, 2007) was used for the statistical analysis. Correlations between periodic mass loss estimates made for each measurement plots were
modeled using error structure (autoregressive (1)) selected using AICc criteria. Effects were declared significant when p values were < 0.05 and means were separated using Tukey’s HSD test at $\alpha=0.05$.

The effects of fertilizer and weed control on the decay rate constant (k), and litter nutrients for both the untreated carryover and the actively managed experiments were analyzed using analysis of variance (ANOVA) for a randomized complete block design. Kolmogorov Smirnov and equal variance tests were used to ensure that the data met assumptions of normality and homoscedasticity, respectively (Massey, 1951). For data not meeting the assumptions of normality and homoscedasticity, square root transformations were made prior to ANOVA in SAS (SAS Institute, 2007). When treatment effects were significant, means were separated using Tukey’s HSD test $\alpha = 0.05$.

Correlation analyses were further conducted to investigate the significant modifiers of litter decomposition (nutrient concentrations, C/nutrient ratios, lignin concentration, lignin/nutrient ratios) across varying ranges of silvicultural treatments in managed pine plantations. Briefly, all treatments and experiments were pooled together. When correlations revealed significant relationships with litter decay rate, a generalized linear regression model was developed with significant modifiers and soil temperature as independent variables and litter decay rate as the dependent variable. From this generalized regression model with Adaptive Lasso estimation (Zou, 2006) and AICc validation, only factors with active effects (coefficient $\neq 0$) were selected to form a new generalized linear regression model. The final model was selected when all factors in the model were significant at a 95% confidence level.

For estimating the effect of gallberry on pine needle decomposition, the following model was used:
\[ y = \mu + b + S + L + SL + e \]

where, \( \mu \) = overall mean; \( b \) = random effect of the block with \( b \sim N(0, \sigma^2_b) \); \( S \) = fixed effect of the silvicultural treatment; \( L \) = fixed effect of the litter type; \( SL \) = fixed interactive effects between silvicultural treatment and litter type; and \( e \) = error term (independent, \( N(0, \sigma^2_e) \)). The PROC MIXED procedure in SAS (SAS Institute, 2007) was used for the statistical analysis.

Effects were declared significant when \( p \) values were < 0.05, unless noted otherwise.

**Results**

**Annual Needlefall and Soil Temperature**

The fertilization treatment history significantly increased annual pine litterfall amounts compared to the control or weed control treatments (Figure 4-2). From March 2016 to March 2017, almost 4.2 Mg ha\(^{-1}\) of litterfall was estimated for the \( C_F \) and 3.7 Mg ha\(^{-1}\) for the \( C_{FW} \) treatments. Litterfall in the \( C_C \) and \( C_W \) treatments were about 37% and 48% lower, respectively, compared to the \( C_F \) treatment. In the actively retreated experiment, the \( FW \) plots had the highest annual litterfall from March 2015 to March 2016 among treatments (\( FW: 5.9 \) Mg ha\(^{-1}\); \( F: 4.5 \) Mg ha\(^{-1}\); \( W: 2.5 \) Mg ha\(^{-1}\); \( C: 1.5 \) Mg ha\(^{-1}\)) (Figure 4-2a). However, a thinning operation in the \( FW \) and \( F \) plots in April 2016 resulted in lower annual litterfall amounts for these treatments in the following year. Nevertheless, annual litterfall during the period of litterbag incubation in the actively retreated experiment was 2.1, 2.9, 2.5, and 1.6 Mg ha\(^{-1}\) for the \( FW, F, W, \) and \( C \) treatments, respectively (Figure 4-2b).

Mean monthly soil temperature below the litter layer ranged from 28.7\(^\circ\)C in the \( C_W \) treatment in July 2016 to 14.6\(^\circ\)C in the \( C_C \) treatment in January 2017 for the untreated carryover experiment. For the actively managed retreated experiment, the mean monthly soil temperature ranged from 30.3\(^\circ\)C in the \( FW \) treatment in July 2016 to 14.3\(^\circ\)C in the \( C \) treatment in January
2017. When averaged over the study period, there were significant differences in soil temperature below the litter layer among treatments only for the actively managed retreated experiment. For the untreated carryover experiment, mean soil temperature was highest for the C_W 23.2°C treatment, followed by C_FW 22.8°C, C_F 22.5°C, and C_C 22.3°C treatments (Figure 4-3). In the actively managed retreated experiment, mean soil temperature followed the trend FW (24°C) > W (23.4°C) > F (22.6°C) = C (22.5°C) treatments. On average soil temperature, was 1.5°C higher in the FW compared to the C treatment.

**Pine Litter Mass Loss and Decay Rate**

Pine litter decomposition was affected by silvicultural treatments for both experiments (Figure 4-4). For the untreated carryover experiment, the C_F treatment accelerated pine litter mass compared to the C_W treatment. For instance, the C_F treatment lost approximately 22% of initial mass whereas the C_W treatment lost about 18% over 13 months. Litter decomposition was highest for the C_F followed by the C_C, C_FW, and C_W treatments. Estimated litter decay rates for each retrieval decreased exponentially as decomposition progressed (Figure 4-5a). When all sampling periods were pooled together, the decay rate (year⁻¹) followed the trend C_F (0.27) > C_C (0.23) = C_FW (0.23) = C_W (0.22) (Tukey’s HSD α = 0.05).

For the actively retreated experiment, mass loss due to decomposition followed the trend: F ≥ FW ≥ W ≥ C (Figure 4-4). Decomposition was almost 42% higher in the F treatment compared to the C treatment. Over 13 months, litter in the F treatment lost about 23.5% of its initial mass, compared to 16.6% for the C treatment. Mean decay rate, pooled over all sampling periods, was highest for the F (0.28 year⁻¹) treatment followed by the FW (0.28 year⁻¹), C (0.23 year⁻¹), and W (0.23 year⁻¹) treatments (p=0.04). As in the untreated carryover experiment, litter
decay rates for each retrieval in the actively retreated experiment decreased exponentially as decomposition progressed (Figure 4-5b).

**Litter Quality and Carbon:Nutrient Ratios**

Lignin concentrations in the initial litter was not significantly affected by the prior rotation’s fertilization or competing vegetation control treatments in the untreated carryover experiment. However, the absolute values were highest for the C_W (29.7%) treatment followed by the C_FW (28.5%), C_F (27.4%), and C_C (27.4%) treatments (Figure 4-6a). The initial litter lignin:N ratios for the untreated carryover experiment were weakly significant (p=0.09) among treatments, and highest for the C_C (90.2) followed by the C_W (84.3), C_FW (78.5), C_F (72.1) treatments. By the end of the study, the lignin:N ratios were significantly different among treatments (Figure 4-6b). Initial lignin:P ratios differed significantly among treatments [C_W (1243) > C_FW (623) = C_F (544); C_C (931) > C_F].

In the actively retreated experiment, the FW treatment had the highest lignin concentrations among treatments (Figure 4-6c). For example, the lignin concentration in the FW treatment was 29% compared to about 26.4% in the C treatment. Lignin concentration in the F treatment was almost 7% lower than the FW treatment. The W treatment (27.6%) did not result in any significant changes in initial lignin concentrations when compared among treatments. The initial lignin:N ratio, however, followed the trend C (93.4) > W (75.2) > F (55.3) = FW (49.6); a pattern similar to the untreated carryover experiment. As decomposition progressed, the lignin:N ratio concentrations for the F and C treatments increased and became higher when compared to the FW and W treatments (Figure 4-6d). The initial lignin:P ratio differed significantly among treatments [C (1154) = W (1060) > FW (383) = F (355)].

Initial litter nutrient concentrations differed among treatments for the untreated carryover experiment. Nitrogen, P, Mg, Mn, and Zn were significantly different among treatments (Figure
4-7). Except for Mg, the prior fertilization treatment history resulted in higher nutrient concentrations in the initial litter. For instance, N and P concentrations in the C_F treatment were almost 1.3- and 1.6-fold higher than the C_C treatment. However, Mg concentrations were significantly higher in the C_C (1300 ppm) and C_W (1281 ppm) treatments compared to the C_FW (1050 ppm) and C_F (975 ppm) treatments. The fertilizer treatment in the previous rotation, alone or in combination with weed control (C_F, or C_FW), resulted in a decrease in the initial litter C:N and C:P ratios for the untreated carryover experiment (Figure 4-8). There was almost a 20% decrease in the initial C:N and 37% decrease in the initial C:P ratios in the C_F treatment compared to the C_C treatment. In general, the C:N and C:Ca ratios declined as decomposition progressed, irrespective of treatments. The C:P and C:K ratios in the untreated carryover experiment decreased after an initial increase. For all treatments, the C:Mg values increased as decomposition progressed (Figure 4-8).

The active treatments in the second rotation resulted in differences in N, P, K, and Mg concentrations in the initial litter (Figure 4-7). For example, the F treatment was 1.7-fold higher for N, 3.2-fold higher for P, and 5.4-fold higher for K concentrations compared to the C treatment. Similarly, the FW treatment resulted in 2.1-, 2.9-, 4.5-fold higher concentrations of N, P, and K, respectively, in the litter compared to the C treatment. Like in the untreated carryover experiment, both the F and FW treatments had lower initial litter Mg concentrations than the C and W treatments. There were no significant differences in micronutrient concentrations in the initial litter among treatments. The F and FW treatments resulted in lower C:N and C:P ratios in the initial litter. The C:N ratio was 40% and the C:P ratio was 68% lower in the F treatment compared to the C treatment. For the FW treatment, initial C:N and C:P ratios were lower by almost 50% and 66%, respectively, when compared to the C treatment. The C:P
and C:K ratios initially increased and then remained stable for the FW and F treatments, but decreased for the C and W treatments during the study period. The C:Mg ratio increased as decomposition progressed. Interestingly, this increase was more pronounced in the FW treatment than in the other treatments (Figure 4-9). Unlike the actively retreated experiment, the C:P and C:K ratios in the untreated carryover experiment decreased after an initial increase. The C:Mg ratio also followed the same pattern as in the actively retreated experiment.

**Nutrient Release**

Nutrient release patterns also differed among treatments in the untreated carryover experiment. Nitrogen was mineralized for the first two months, and thereafter was immobilized until the end of the study (Figure 4-10). Phosphorus was also released in all treatments, but the rate of P mineralization was stabilized after two months. Treatment effects on P dynamics were significant after 6 months; P was mineralized more in the CFW (51% of initial P) and CF (51% of initial P) treatments compared to the CW (31% of initial P) treatment. Potassium release patterns were similar to the overall release patterns of P, but there were no treatment differences in percent of initial K release (Figure 4-10). Calcium mineralization patterns showed a different trend; it was mineralized in the CFW and CW treatments but immobilized in the CF and CC treatments. Magnesium was mineralized in all treatments as decomposition progressed. In contrast, Zn was immobilized in all treatments (Figure 4-10).

Nutrient release patterns during decomposition were also affected by silvicultural treatments in the actively retreated experiment and followed similar trends as that observed in the untreated experiment. For example, N was mineralized for the first two months in all treatments and thereafter that it was immobilized (Figure 4-11). Phosphorus was mineralized in all treatments during the study period. However, there were differences in P mineralization rates among treatments. For example, almost 69% of the initial P in the F and 66% in the FW
treatments were released compared to about 31% in the C treatment by the end of the study. Potassium was also mineralized in all treatments except for the C treatment, where it was mineralized just for the first two months. Calcium was mineralized in the F (16% of initial Ca) and FW (23% of initial Ca) treatments, but was immobilized in the W treatment. Unlike Ca, Mg was mineralized in all treatments during the study period. As in the untreated carryover experiment, Zn was immobilized in all treatments (Figure 4-11).

Effects of Gallberry Leaves on Pine Litter Decomposition

Mixing pine litter with gallberry leaves resulted in increased pine litter mass loss for both experiments (Figure 4-12). When all silvicultural treatments were pooled, pine litter mass loss in the untreated carryover experiment was about 31.6% when mixed with gallberry leaves from the control plot compared to about 26% when pine only litter was used. When all litter types were pooled, silvicultural effects on litter decomposition were significant; litter decomposition in the CF treatment was significantly greater than the CFW treatment, but was not different compared to the C and W treatments (p = 0.008). In addition, there were no significant differences in pine litter mass loss in the pine + gallberry mix (1:1) despite differences in the origin of gallberry leaves (from control plots vs fertilized plots).

Mass loss associated with the pine only litter in the actively managed retreated experiment was about 26.6% per year compared to 32.2% when gallberry leaves from the control plots were combined. Similar to the untreated carryover experiment, pine litter mass loss rates in the pine + gallberry mix (1:1) were the same despite different origins of gallberry leaves (control plot or fertilized plots) (Figure 4-12). Litter decomposition in the F and C treatments were marginally higher than in the FW and W treatments when all litter types were pooled (Table 4-2; Tukey’s HSD, α=0.1). In addition, there were no significant interactions in pine litter mass loss between silviculture and litter mix types for either experiment (Table 4-2).
relationships between pine litter decay rates and litter chemistry

When pooled among treatments and experiments, significant positive correlations with litter decay were observed for N, P, K, Mg, C:N, C:P, C:K, C:Mg, C:Mn, lignin:P, lignin:K, lignin:Mg, lignin:B, lignin: Mn, and lignin:Zn ratios (Table 4-3). A generalized linear regression model with Adaptive Lasso estimation and AICc validation for litter decay rate with these significant variables and soil temperature revealed that only the lignin:P ratio was significant \( (r^2 = 0.525, \text{RMSE} = 0.021, \lambda \text{ penalty} = 0.070) \) (Figure 4-13). The resulting model was: litter decay rate \( (k \text{ year}^{-1}) = 0.29004 - 5.6695 \times 10^{-5} \times \text{lignin:P ratio}. \)

Discussion

For pine plantations growing on nutrient limited lower Coastal Plain soils in the southeastern US, nutrient cycling, via litter decomposition, remains an important yet relatively less studied process in the context of multi-rotational management. This study utilized two novel long-term replicated experiments to examine the legacy effects of the prior rotation’s fertilization and weed control treatment histories on pine litter decomposition dynamics in the subsequent rotation.

Effects of Intensive Silvicultural Practices across Rotations on Pine Litter Decomposition

For six-year-old loblolly pine growing on N and P limited Spodosols, residual effects of the first rotation’s fertilization treatments on litter decomposition were evident in the untreated second rotation. When treatments were continued into the second rotation, both fertilization and the combination of fertilization and weed control treatments accelerated pine litter mass loss. Our estimates of litter mass loss \( (\text{year}^{-1}) \) in the fertilized plots were lower than the range of 30-35% mass loss observed by Sanchez (2001) and Gurlevik et al. (2003) in mid-rotation loblolly pine stands. Likewise, annual mass loss estimates for this study were also lower than the range of 29 to 37% reported for six-year-old loblolly pines from the same site in the first rotation (Polglase et
al., 1992b). Differences in the placement of litterbags could have contributed to lower litter decomposition rates in current study compared to others. All litterbags were placed on top of the mineral soil, whereas Sanchez (2001) and Polglase et al. (1992a) placed litterbags on top of the Oe horizon. Studies suggest that microbial activity in the Oe and Oa layers is higher than in the surface mineral soils (Andersson et al., 2004; Frey et al., 2014). Nevertheless, our estimates of litter mass loss for the control treatments were similar to the value of 15% year\(^{-1}\) reported for unfertilized slash pine plantations near our study site (Gholz et al., 1985b). Based on the litter decay constants estimated in this study, it would take about 21.7 years in the C\(_C\) vs 18.5 years in the C\(_F\) treatment in the untreated carryover experiment and 22.1 years in the C vs 17.6 years in the F treatment in the actively retreated experiment to decompose 99% of the original litter mass (Mean residence time\(_{99}\) = 5/k year; Olson, 1963).

Contrasting differences in litter mass loss patterns in the untreated carryover and the actively retreated experiments mainly for the plots having a history of the combined fertilizer and weed control treatments was interesting. For example, the FW treatment had significantly higher decomposition rates compared to the C treatment, whereas the C\(_{FW}\) treatment had similar decomposition rates compared to the C\(_C\) treatment. These contrasting trends may be due to active differences in the biotic and abiotic factors affecting litter decomposition. For example, thinning in the FW treatment, when combined with understory removal, was associated with a soil temperature increase (FW: 1.5°C higher than the C treatment; C\(_{FW}\): 0.4°C higher than the C\(_C\) treatment) compared to the unthinned C\(_{FW}\) treatment, and could have contributed to the differing litter decay patterns. This interpretation is further supported by a significant positive relationship between litter decay rate (e.g. FW-C and C\(_{FW}-C_C\)) and mean soil temperature in the FW and C\(_{FW}\) treatments (\(r^2 = 0.69, p = 0.04\)). In addition, increased solar radiation in the thinned FW
treatment compared to the C treatment may have potentially increased photodegradation of pine litter. Such an effect may have been small in the unthinned CFW treatment compared to the CC treatment. In lignin rich litter, photo degradative effects are particularly important because lignin effectively absorbs light in the ultraviolet to visible spectrum and gets preferentially disintegrated under photodegradative conditions (Austin and Ballaré, 2010). Though a ‘bleaching’ effect (litter discoloration) of litter inside several of the FW litterbags was observed, additional replicated litterbag experiments with varying thinning intensities and lignin concentrations would be necessary to explore such mechanism in managed pine plantations.

The first rotation weed control treatment history had a negative influence on pine litter decomposition in the subsequent rotation of this study. Prior studies by Polglase et al. (1992a) and Gurlevik et al. (2003) also reported lower pine litter mass loss for sites receiving weed control treatments. Gurlevik et al. (2003) attributed this reduction in litter mass loss to elevated litterbag temperature and subsequent drying of litter, both of which created unfavorable conditions for decomposition. However, an effect of increased litterbag temperature was not significant for the weed control plots in this experiment (a modest increase of 0.9ºC compared to the CC treatment). Other factors like increased phenolic concentrations in the litter (Polglase et al. 1992b) and higher initial litter lignin content may have contributed to the lower mass loss in the CW treatment ($r^2 = 0.57, F_{(1,4)} = 5.26, p = 0.08$) compared to the CC treatment. In this field experiment, the sustained vegetation control treatment maintained in the previous rotation resulted in shifts in the understory community composition from hardwoods (gallberry-dominated) to graminoids (chalky bluestem-dominated) (Subedi et al., 2017). This shift resulted in differences in the overall litter chemistry of the forest floor. For instance, lignin concentration for chalky bluestem may range from 20-25% (Golley, 1965) compared to about 17% for
gallberry leaves (Halls et al., 1957). Also, gallberry in the Cc treatment had a 2.2- fold higher N concentration than chalky bluestem in the Cw treatment (Subedi 2013). These differences in understory litter type chemistry could have potentially affected pine litter decomposition in the Cw treatment (Hättenschwiler et al., 2005).

**Understory Vegetation and Litter Chemistry Effects on Pine Litter Decomposition**

The positive effect of gallberry leaves on pine litter decomposition observed for all treatments in both experiments highlights the importance that understory vegetation plays in decomposition and nutrient cycling processes (Platek and Allen, 2001; Wardle et al., 2003; Vivanco and Austin, 2008; Qiao et al., 2014). Almost 20-22% higher decomposition rates of pine litter were observed in this study when combined with gallberry leaves (irrespective of gallberry source) (1:1 w/w mix). Gallberry leaves were richer in most nutrients and had 2.8 to 3.6- fold higher N concentrations than pine litter. The addition of gallberry leaves likely increased both labile C sources and other nutrients (N, P, or micro-nutrients) and potentially alleviated decomposer nutrient limitations (Fontaine et al., 2007; Kuzyakov, 2010; Camenzind et al., 2018). In mid-rotation stands of loblolly pine growing on Ultisols in North Carolina, Piatek and Allen (2001) reported a positive effect of mixing broadleaved litter with pine needle (1:5 broadleaves:pine mix) on decomposition rates. Interestingly, there were no significant differences in pine litter decomposition due to the addition of gallberry leaves originating from either the fertilized or control plots in the current study. Nonsignificant difference between the two sources (fertilized or control plots) in nutrients or labile C release from gallberry leaf decomposition may have resulted in similar priming effects. For example, there were no significant differences between either mass loss of gallberry leaves (40% of original from the control vs 35% of original from the fertilizer plots) or N concentration between gallberry leaf types in the untreated carryover experiment.
Numerous studies have described how litter decomposition proceeds as an interaction among abiotic, biotic, and physico-chemical properties of the litter and site characteristics (Aerts, 1997; Hättenschwiler et al., 2005; Zhang et al., 2008; Prescott, 2010). At a global scale, mean annual temperature, latitude, C:N, and N explained significant variation in litter decay rates (Zhang et al., 2008). However, the lack of relationship between mean litterbag temperature and litter decay rates \( (r^2 = 0.004, F_{(1,22)} = 0.08, p = 0.77) \) in this study suggests that temperature may not be a relevant variable to estimate litter decay rates at a local scale. Instead, the litter lignin:P ratio significantly explained the variation in litter decay rates in our study; higher litter decay rates for litter were associated with a lower initial lignin:P ratio. Similar observations have been made in tropical wet forests where the lignin:P ratio was one of the most important factors affecting leaf litter decay (Wieder et al., 2009). The significant negative relationship between the lignin:P ratio and litter decay rates highlights an apparent P control on litter decomposition on nutrient limited Spodosols, where both N and P availability limit aboveground pine productivity (Subedi et al., 2014). These results further support our hypothesis that both previous rotation management and continued management in the second rotation modify pine litter chemistry which drives litter mass loss in southern pine plantations.

**Nutrient Release during Litter Decomposition in Managed Pine Stands**

In nutrient limited southern pine stands, alleviation of growth limiting nutrients, mainly N and P, resulted in greater leaf area development and pine growth (Subedi et al., 2019) in the CF, CFW, F, and FW treatments compared to the untreated controls. Higher litterfall mass and litter N concentrations in the CF, CFW, F, and FW treatments suggest that litterfall represents a major N pool in these stands. For example, almost 16.1 kg ha\(^{-1}\)yr\(^{-1}\) and 13.4 kg ha\(^{-1}\)yr\(^{-1}\) of N was accumulated in the pine litter pool above the mineral soil in the CF and CFW treatments. In addition, subsequent immobilization of N observed in this study suggested that this litterfall pool
was a N sink in these stands. For the F and FW treatments, N was also immobilized in the litter, suggesting similar N release patterns irrespective of legacy or continued management treatments. Other pine litter decomposition studies reported litter N immobilization during decomposition (Piatek and Allen, 2001; Gurlevik et al., 2003; Kiser et al., 2013). However, higher N contents observed in the litterbags by the end of the current study were likely due to contributions from atmospheric N deposition, canopy N leaching, and microbial N fixation (Gholz et al., 1985a; Son, 2001). Gholz et al. (1985a), for example, reported a deposition of 13.5 kg ha\(^{-1}\) of N from precipitation (1455 mm) alone in an 8-year-old slash pine plantation in North Florida. Likewise, McDonnell and Sullivan (2014) reported about 7.3 kg ha\(^{-1}\)yr\(^{-1}\) total atmospheric N deposition between 2000-2012 in north central Florida. In the F and FW treatments, annual fertilization applied around the tree base and away from the litterbags minimized the direct effect of fertilizer N being deposited on the litterbags.

Litter P dynamics observed in this study were different from Piatek and Allen (2001), Sanchez (2001), and (Gholz et al., 1985b), who reported P immobilization during pine litter decomposition in mid-rotation stands. On the contrary, P was mineralized from all treatments in this study, suggesting that litterfall represents an important P source in these second rotation stands. Although increased litter P concentration and litterfall mass translated into about a 2.61-fold increase in the annual litterfall P pool in the C\(_F\) compared to the C\(_C\) treatment, most of this P pool would be more labile (Polglase et al., 1992b). For this same site, Polglase et al. (1992a) reported that the inorganic P fraction in litter comprised about 45 to 64% of the total litter P, and fertilization increased this fraction. In the C\(_F\) and C\(_{FW}\) treatments, more than 50% of the initial litter P was mineralized in the first two months. It is likely that most of the P mineralized in the C\(_F\) and C\(_{FW}\) treatments during the first two months of litter decomposition was the inorganic P.
fraction. Other studies documenting changes in litter P fractions in pine stands also report that fertilization increased the soluble P fraction which was immediately released upon decomposition (Kiser et al. 2013). The magnitude of litter P concentration and P mineralization was increased by active fertilization in the F and FW treatments; almost 61% of the initial litter P in the F and 59% in the FW treatments was mineralized in the first two months.

Potassium and Mg dynamics in decomposing litter suggested that these nutrients were easily mineralized irrespective of continued management or the legacy silvicultural treatments. Because K and Mg are not structural materials and they exist mainly in the solution form in plant cells, rapid mobilization was expected (Osono and Takeda, 2004). Calcium, on the other hand, was generally immobilized in the previously fertilized treatment plots (C_F and C_FW) of the untreated carryover experiment, but was mineralized in the actively re-fertilized treatments (F and FW). Calcium immobilization has also been observed by others in loblolly pine stands, irrespective of silvicultural treatments over the first year (Sanchez, 2001; Gurlevik et al., 2003). In our study, Ca release was smaller in magnitude for other treatments (e.g. 14% in C_C, 10% in C_W). Rapid mineralization of K and Mg suggests that litterfall served as a source for these nutrients. From a forest management perspective, these results highlight that the same litterfall pools may be a sink for one nutrient and a source for another nutrient. Identifying whether the litterfall pool is a source or a sink for nutrients would help guide better nutrient amendment strategies to alleviate growth limitation in managed plantations.

Summary

This study was designed to investigate the carryover effects of legacy fertilization and weed control treatments on loblolly pine litter decomposition in the subsequent rotation, and to contrast these effects to the same treatments imposed in the second rotation. For the first time in intensively managed southern pine stands, these results provide direct evidence that first rotation
fertilization treatments increased litter decomposition in the subsequent rotation. Immobilization of N during litter decomposition suggests that recent litterfall pool served as a N sink and that litter decomposition alone may not support stand N demands on these nutrient limited sites. However, fertilization induced increases in litterfall mass and P release rates during litter decomposition, suggesting that litterfall P pools in the C_F, C_FW, F, and FW treatments were an important P source. On the contrary, pine litter decomposition was lower for treatments that included a history of sustained understory competition control (C_W) compared to the C_F treatment. This trend was also present when the weed control treatments (W) were continued into the second rotation. Considering lower litterfall inputs associated with the C_W and W treatments, N and P cycling in these treatments would be less compared to fertilized sites. Different litter decomposition trends in the C_FW and FW treatments were likely due to the effects of thinning conducted in the FW treatment. Litter chemistry also differed among treatments; fertilization generally increased nutrient concentrations and weed control was generally associated with increased lignin concentrations. Furthermore, facilitation of pine litter decomposition by gallberry leaves reinforced the potential role that understory vegetation may play on nutrient cycling and mineralization processes in these nutrient limited southern pine ecosystems. On a local scale, litter mass loss differences among silvicultural treatments were best explained by the lignin:P ratio. Litter mass loss in this study might be underestimated due to litterbag mesh size, which restricted meso- and macro-faunal access to the litter. An improved understanding of litter decomposition and nutrient cycling in the context of multi-rotational management would require additional long-term decomposition studies that also include all decomposer community components.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>First-rotation treatment</th>
<th>Second-rotation treatment</th>
<th>Treatment abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated carryover</td>
<td>Control</td>
<td>untreated</td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>untreated</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Fertilizer (same as the Fertilizer only treatment) + Weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>untreated</td>
<td>C&lt;sub&gt;FW&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>untreated</td>
<td>C&lt;sub&gt;W&lt;/sub&gt;</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>Control</td>
<td>Control</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>Fertilizer only (Cumulative nutrient additions for the first six years (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B) + Thinning operation at age 6 year</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>Fertilizer (same as the Fertilizer only treatment) + weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>Fertilizer (same as the F treatment) + Thinning operation at age 6 year + weed control (Chemical methods)</td>
<td>FW</td>
</tr>
<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>Weed control only (Chemical methods)</td>
<td>W</td>
</tr>
</tbody>
</table>
Table 4-2. Summary of fixed effects of ANOVA for the pine litter decomposition for 12 months as affected by litter mix types and silviculture for the untreated carryover and actively managed retreated experiment at the IMPAC II study

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Source</th>
<th>Num df</th>
<th>Den df</th>
<th>F Ratio</th>
<th>pValue &gt; F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated carryover</td>
<td>Silviculture</td>
<td>3</td>
<td>22</td>
<td>5.04</td>
<td>0.0083</td>
</tr>
<tr>
<td></td>
<td>Litter type</td>
<td>2</td>
<td>22</td>
<td>6.90</td>
<td>0.0047</td>
</tr>
<tr>
<td></td>
<td>Silviculture*Litter type</td>
<td>6</td>
<td>22</td>
<td>0.35</td>
<td>0.9045</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>Silviculture</td>
<td>3</td>
<td>22</td>
<td>4.55</td>
<td>0.0126</td>
</tr>
<tr>
<td></td>
<td>Litter type</td>
<td>2</td>
<td>22</td>
<td>15.66</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td></td>
<td>Silviculture*Litter type</td>
<td>6</td>
<td>22</td>
<td>1.06</td>
<td>0.4149</td>
</tr>
</tbody>
</table>
Table 4-3. Correlation coefficients for litter nutrients, C/nutrient ratios, lignin, and lignin/nutrient ratios with pine litter decay (k year\(^{-1}\)) (n=24) at the IMPAC II study.

<table>
<thead>
<tr>
<th>Variables</th>
<th>( r )</th>
<th>( p ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>0.669</td>
<td>0.000</td>
</tr>
<tr>
<td>P</td>
<td>0.771</td>
<td>0.000</td>
</tr>
<tr>
<td>K</td>
<td>0.765</td>
<td>0.000</td>
</tr>
<tr>
<td>Ca</td>
<td>0.164</td>
<td>0.443</td>
</tr>
<tr>
<td>Mg</td>
<td>-0.663</td>
<td>0.000</td>
</tr>
<tr>
<td>B</td>
<td>0.341</td>
<td>0.102</td>
</tr>
<tr>
<td>Cu</td>
<td>0.331</td>
<td>0.114</td>
</tr>
<tr>
<td>Mn</td>
<td>0.259</td>
<td>0.221</td>
</tr>
<tr>
<td>Zn</td>
<td>0.332</td>
<td>0.113</td>
</tr>
<tr>
<td>C:N</td>
<td>-0.652</td>
<td>0.001</td>
</tr>
<tr>
<td>C:P</td>
<td>-0.694</td>
<td>0.000</td>
</tr>
<tr>
<td>C:K</td>
<td>-0.742</td>
<td>0.000</td>
</tr>
<tr>
<td>C:Ca</td>
<td>-0.070</td>
<td>0.746</td>
</tr>
<tr>
<td>C:Mg</td>
<td>0.678</td>
<td>0.000</td>
</tr>
<tr>
<td>C:B</td>
<td>-0.245</td>
<td>0.248</td>
</tr>
<tr>
<td>C:Cu</td>
<td>-0.163</td>
<td>0.448</td>
</tr>
<tr>
<td>C:Mn</td>
<td>-0.413</td>
<td>0.045</td>
</tr>
<tr>
<td>C:Zn</td>
<td>-0.341</td>
<td>0.103</td>
</tr>
<tr>
<td>Lignin</td>
<td>-0.391</td>
<td>0.059</td>
</tr>
<tr>
<td>Lignin:N</td>
<td>-0.280</td>
<td>0.185</td>
</tr>
<tr>
<td>Lignin:P</td>
<td>-0.728</td>
<td>0.000</td>
</tr>
<tr>
<td>Lignin:K</td>
<td>-0.761</td>
<td>0.000</td>
</tr>
<tr>
<td>Lignin:Ca</td>
<td>-0.252</td>
<td>0.235</td>
</tr>
<tr>
<td>Lignin:Mg</td>
<td>0.604</td>
<td>0.002</td>
</tr>
<tr>
<td>Lignin:B</td>
<td>-0.415</td>
<td>0.044</td>
</tr>
<tr>
<td>Lignin:Cu</td>
<td>-0.188</td>
<td>0.379</td>
</tr>
<tr>
<td>Lignin:Mn</td>
<td>-0.466</td>
<td>0.022</td>
</tr>
<tr>
<td>Lignin:Zn</td>
<td>-0.423</td>
<td>0.040</td>
</tr>
</tbody>
</table>

Note: All data were pooled together. ‘\( r \)’ represents Pearson’s correlation coefficient.
Figure 4-1. Layout of the actively managed retreatment and the untreated carryover experiments on Spodosols near Gainesville, FL.
Figure 4-2. Annual litterfall in the untreated carryover and the actively managed retreated experiment for the year a. 2015-2016, and b. 2016-2017 at the IMPAC II experiment. Within each experiment, treatments with same letter are not significantly different at $\alpha = 0.05$ (Tukey’s HSD).
Figure 4-3. Mean soil surface temperature (°C) on top of the mineral soil surface during the litter decomposition study as affected by silvicultural treatments in the untreated carryover and actively managed retreated experiment at the IMPAC II study. Within an experiment, treatments with similar letter are not significantly different at α = 0.05 (Tukey’s HSD).
Figure 4-4. Litter mass remaining during decomposition as affected by silvicultural treatments in the a. untreated carryover and b. actively managed retreated experiments. Error bars represent standard error (n=3). Asterisk (*) denotes significant treatment effects for a given litterbag retrieval period at p < 0.05.
Figure 4-5. Litter decay rate (k) per year (estimated for each retrievals) as affected by silviculture treatments at the a. untreated carryover, and b. actively managed retreated experiment at the IMPAC II study. * and ** respectively denote significant treatment effects at p < 0.05 and p < 0.1. Error bars represent standard error of the mean.
Figure 4-6. Lignin concentration and lignin:N ratio dynamics during litter decomposition in the untreated carryover experiment (a., b.) and actively managed retreated experiment (c., d.) at the IMPAC II study. Error bars represent standard error of the mean.
Figure 4-7. Initial nutrient concentration in pine litter in the untreated carryover and actively managed retreated experiment. Within each experiment, treatments with same letter are not significantly different at $\alpha = 0.05$. Error bars represent standard error of the mean.
Figure 4-8. C:Nutrient ratio dynamics for different first rotation silvicultural treatments in the untreated carryover experiment. Error bars represent standard error (n=3).
Figure 4-9. C: Nutrient ratio dynamics for different silvicultural treatments in the actively retreated experiment. Error bars represent standard error (n=3).
Figure 4-10. Nutrient dynamics in pine litter during decomposition in pine litter in the untreated carryover experiment. Horizontal dashed line corresponds to 100% of initial nutrient mass. Values below the dashed line suggest nutrient mineralization whereas above the line suggest immobilization. Error bars represent standard error (n=3).
Figure 4-11. Nutrient dynamics in pine litter during decomposition in the actively managed retreated experiment. Horizontal dashed line corresponds to 100% of initial nutrient mass. Values below the dashed line suggest nutrient mineralization whereas above the line suggest immobilization. Error bars represent standard error (n=3).
Figure 4-12. Litter type effects on pine litter mass loss per year when treatments were pooled together. Within each experiment, litter types with same letter are not significantly different at $\alpha=0.05$. Error bars represent standard error.
Figure 4-13. Litter decay rates (k year$^{-1}$) as predicted by initial litter lignin:P ratio in intensively managed pine stands in Florida Spodosols. Solid circles represent actively managed retreated experiment and solid triangles represent untreated carryover experiment.
CHAPTER 5
EX-SITU INCUBATION REVEALS NUTRIENT LIMITATIONS TO MICROBIAL
RESPIRATION AND ORGANIC MATTER DECOMPOSITION IN A FLORIDA SPODOSOL

Background

Decomposition is an important ecological process that drives nutrient cycling and net C storage in soils (Vitousek, 1984; Wardle et al., 2004; Sayer et al., 2007; Chapin et al., 2009). The fate of organic matter being released to the atmosphere as CO₂ flux or retained in the soil as organic carbon depends on the complex interplay of broad regulators of decomposition processes like physico-chemical environment (Aerts, 1997; Zhang et al., 2008), resource quality (Berg et al., 2000; Zhu et al., 2016), and the decomposer community (Wall et al., 2008; Bradford et al., 2017). Many studies show that N and P affect primary productivity, decomposer community composition, and the ability of decomposers to facilitate organic matter decomposition (Elser et al., 2007; Manzoni et al., 2008; Güsewell and Gessner, 2009). The effects of other nutrients on the regulation of decomposition, however, have received less attention than N and P in forest ecosystems (Knorr et al., 2005; Powers and Salute, 2011; Trum et al., 2011; Lovett et al., 2016), and only a handful of these studies have focused on the mechanism behind such regulation (Whalen et al., 2018; Sun et al., 2019). In managed forests, where N, P, and other nutrients are often manipulated to improve primary productivity, a mechanistic understanding of nutrient regulation of decomposition may be of particular interest because of feed back interactions on nutrient availability and ecosystem carbon balance.

Nutrient limited forests in the lower Coastal Plain of the United States support a significant area of southern pine plantations. Nitrogen and P fertilization and competing understory vegetation control via herbicide applications are common management practices that alleviate nutrient limitations to overstory productivity (Fox et al., 2007; Jokela et al., 2010; Albaugh et al., 2015; Subedi et al., 2019). With amelioration of N and P limitations using
silvicultural management practices, novel nutrient limitations to productivity may also be induced [e.g. Cu- (Vogel and Jokela, 2011); Mn- (Jokela et al., 1991); K- (Carlson et al., 2014)]. At the same time, long-term silvicultural practices (e.g. fertilization and weed control) have been shown to affect both litter quality and soil nutrient availability in these managed ecosystems (Gurlevik et al., 2003; Kiser et al., 2013; Subedi et al., 2014; Chapter 2). While management induced differences in nutrient availability may affect productivity and net ecosystem C gain (Maier and Kress, 2000; Bracho et al., 2018), it is unclear whether nutrient availability (or novel nutrient limitations) regulates organic matter decomposition in southern pine plantations.

Previous studies have shown that increases in N, P, and S, which are normally limiting to microbial growth, stimulate microbial degradation of the soluble compounds and holocellulose that are preferentially lost during the early stages of decomposition (Berg and McClaugherty, 2014). During the secondary stage of decomposition (lignin dominated substrate), N additions may negatively affect decomposition; it has a suite of effects, including binding with lignin to form more complex organic compounds, altering the fungal community (Morrison et al., 2016), favoring bacterial pathways over fungal (Freedman et al., 2016), limiting phenol oxidase activity (Carreiro et al., 2000), and affecting genetic regulation of lignolytic enzyme production (Hesse et al., 2015). Manganese, on the other hand, may be important during the late stages of decomposition. Manganese is an essential element that serves as a co-factor for the Mn peroxidase enzyme, a lignin degrading enzyme produced by fungi (esp. basidiomycetes) (Perez and Jeffries, 1992) and actinobacteria (Roberts et al., 2011). Manganese peroxidase facilitates lignin degradation via oxidation of Mn$^{2+}$ present in the substrate to reactive Mn$^{3+}$ capable of breaking down the phenolic structure in lignin (Keiluweit et al., 2015). In the context of southern pine stands, where Mn limitation to stem growth has been observed (Jokela et al., 1991), it is
important to understand whether Mn may also be limiting to organic matter decomposition processes.

Many studies on litter decomposition have demonstrated a positive correlation between Mn concentration in the litter and decomposition rates across various regions (Davey et al., 2007; Berg et al., 2015). Manganese has also been identified as being a strong predictor of soil C storage in coniferous forest humus layers (Stendahl et al., 2017). Some studies have reported stimulated organic matter decay rates with Mn additions (Trum et al., 2011; Whalen et al., 2018; Sun et al., 2019). In the context of southern pines, Mn effects on decomposition are unknown but a strong positive relationship between Mehlich III extractable soil Mn concentration (0-10cm) and mean heterotrophic respiration (a proxy for C mineralization by microbes) \( r = 0.66, \ p=0.019 \) was observed in a nutrient limited in north Florida Spodosol (IMPAC II study, Subedi et al., 2014; Chapter 3). Similarly, Sadowski (2010) reported that foliar Mn concentration was a strong covariate for pine litter decomposition in a Florida Spodosol. These observations in southern pines, taken together with observations made elsewhere, highlight the need to investigate the role that macro- and micronutrient amendments have on organic matter decomposition in intensively managed southern pine ecosystems.

On a nutrient-poor site in north Florida, Mn was observed to be a strong predictor for heterotrophic respiration (Chapter 3). In addition, fertilizer additions tended to increase, and competing vegetation control treatments tended to decrease heterotrophic respiration (Chapter 3). Using the soils from the IMPAC II study site, this study evaluated if silvicultural treatments and additional nutrient inputs (N+P, Mn, and Cu) affected microbial respiration and decomposition of pine litter when environmental variables like soil temperature and soil moisture were controlled. It was hypothesized that Mn limits late stage organic matter decomposition and that
Mn additions would be manifest in increased Mn peroxidase activity. In addition, it was expected that the effects of nutrient additions on microbial processes would vary among the historical silvicultural treatments (fertilization and weed control) imposed at the field study site. Two replicated ex-situ laboratory incubation experiments were used to address the following questions:

- Do fertilization and understory vegetation control treatment histories result in differences in microbial respiration and organic matter decomposition, if other drivers like temperature and moisture were controlled?
- Do nutrient additions, esp. Mn, affect microbial respiration rates and pine litter decomposition in soils having different silvicultural treatment histories?

**Material and Methods**

**Site Description**

The IMPAC (Intensive Management Practices Center) experiment was established in 1983 to evaluate factors limiting the productive potential of southern pines (Swindel et al., 1988). The study is located approximately 10 km north of Gainesville, Florida (29°30’N latitude and 82°20’W longitude) at an elevation of 45 m from the mean sea level. The climate is warm and humid with a long-term (1984-2017) mean annual temperature of 20.7°C and total annual precipitation of 1207 mm (National Oceanic and Atmospheric Administration, 2018). Pomona fine sands (sandy siliceous hyperthermic Ultic Alaquods) are the predominant soil types at this site. The understory vegetation community was typical of Florida flatwood sites. Woody component of the understory consisted mainly of gallberry (*Ilex glabra* (L.)), saw palmetto (*Serenoa repens* (Bartr.)), fetterbush (*Lyonia ferruginea* (Walt.)), blueberries (*Vaccinium* spp.), and wax myrtle (*Myrica cerifera* (L.)). Chalky bluestem (*Andropogon* spp.), tapered witchgrass (*Dichanthelium* spp.), and nutrushes (*Scleria* spp.) were the graminoid components of the understory (Neary et al., 1990b; Subedi et al., 2017).
Silvicultural Treatment History

First rotation study: The first rotation IMPAC experiment was designed as a $2 \times 2 \times 2$ factorial consisting of species (loblolly and slash pine ($Pinus elliottii$ var. $elliottii$ (Engelm.))), complete and sustained weed control, and annual fertilization arranged in a randomized split-plot design. This resulted in four treatments within each species (species as whole plots): control (C), weed control only (W), fertilizer only (F), and both fertilizer and weed control (FW). The entire experimental area was site prepared using a single-pass bedding treatment. In January 1983, genetically improved (first generation, open pollinated) 1-0 bareroot stock (grown for 1 year in the seedbed) of both loblolly and slash pine were hand planted (Swindel et al., 1988; Colbert et al., 1990). The F and FW treatments received a balanced fertilizer regime including macro- and micronutrients for the first ten years annually. In May 1993, fertilization was stopped and then resumed from 1998 - 2000 (Jokela and Martin, 2000). Competing vegetation in the W and FW treatments was eliminated using a combination of chemical and mechanical methods for the first ten years until canopy closure impeded further understory development (Jokela et al., 2010).

Second rotation study (IMPAC II experiment): In May 2009, the first rotation IMPAC study was harvested and a second rotation experiment was overlaid using the same treatment plots. Prior to the harvest of the first rotation study, the understory vegetation within the C and the F treatments was mulched in place in April 2009 to retain this nutrient pool within the plot boundaries. Mulching was not necessary for the W and FW treatments because of the sustained weed control treatment history from the previous rotation. Following harvest, the entire study area was later bedded in June, with a second bedding pass conducted in August of the same year. Original plots in the first rotation were re-established and those plots were used to examine both the “untreated carryover” and “actively managed retreatment” effects on pine growth and nutrient dynamics in the second-rotation experiment. The IMPAC II experiment now
consists of two randomized complete block designs, with 3 replications each, having four treatments \((C_C, C_F, C_{FW}, C_W)\) for the untreated carryover experiment and four treatments \((C, F, FW, \text{ and } W)\) for the actively managed retreated experiment (Table 5-1). While the untreated carryover experiment, established on the previous slash pine plots, received no treatments in the second rotation, the actively managed retreatment experiment, established on the previous loblolly pine plots, continued to receive similar treatments as in the first rotation (Subedi et al. 2014).

In October 2009, the \(W\) and \(FW\) treatments were treated using a broadcast application of 0.84 kg a.e. ha\(^{-1}\) imazapyr in the form of Chopper (BASF Corp., Research Triangle Park, NC), 1.12 kg a.e. ha\(^{-1}\) triclopyr in the form of Garlon 4 (Dow AgroSciences LLC, Indianapolis, IN), and 0.14 kg ha\(^{-1}\) of metsulfuron methyl in the form of Escort\(^{\circledR}\) (E.I. du Pont de Nemours and Company, Inc., Wilmington, DE).

In December 2009, the entire study was regenerated using containerized seedlings from a second generation single, full-sib loblolly family. Similar to the first rotation, loblolly pines were planted in each plot at a 1.8 m by 3.6 m spacing, with measurement plots consisting of forty trees per plot (arranged as 8 trees each in 5 beds). A treated buffer consisting of three trees and two beds surrounded each measurement plot. Six tree spaces of untreated buffer were provided between two adjacent treatment plots. Across the treatment plots, an untreated buffer of four beds was maintained (Figure 5-1).

In March 2010, all treatments (actively managed retreated and untreated carryover) received a single application of Fipronil (9.1%) in the form of PTM (BASF Corp., Research Triangle Park, NC) to control Nantucket pine tip moth \((Rhyacionia frustrana\) (Comstock)). Later in May 2010, a banded 0.2 kg a.e. ha\(^{-1}\) imazapyr was applied on the beds of in all treatment plots.
(both untreated carryover and actively managed retreated) to control tapered witchgrass and to aid seedling survival. In October 2010, the W and FW treatments received a directed spray application of triclopyr (3%) and imazapyr (1%) to control *Ilex glabra* (L.) and other understory competitors. Later in September 2011, a directed spray of glyphosate (3%) was applied to the actively managed W and FW treatments to maintain a weed free environment.

The actively managed retreated (F and FW) experiment received fertilizer annually. Consistent with the last rotation treatments, the total nutrient additions over the first six growing seasons for the F and FW treatments were (kg ha\(^{-1}\)): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B. As done in the first rotation experiment, the fertilizer was applied in narrow bands (30 cm semicircle) around the base of each tree or planting location. In April 2016, the FW and F treatments were thinned in the actively managed retreated experiment to facilitate other long-term study objectives. The thinning operation removed almost 44.6 Mg ha\(^{-1}\) of aboveground biomass from the FW treatment and 25.7 Mg ha\(^{-1}\) from the F treatment.

**Incubation Study Setup and Sampling:**

An automated incubation system was constructed for this study (Figure 5-2) (Bracho et al., 2016). A total of 96 sequential incubation chambers were placed in a water bath set to the incubation temperature of 26.7°C and connected to an automated incubation system. Air from each sequential chamber was circulated by a pump via a CO\(_2\) analyzer (IRGA, Li-840A Licor, Lincoln, Nebraska) at 0.9 L per minute. A mass flow controller (Mass Flow meter GFM, Aalborg Instruments & Control) was used to maintain a constant flow. The CO\(_2\) concentrations and pressures inside each chamber were measured using a CO\(_2\) analyzer and recorded by a datalogger. To avoid creation of an anaerobic environment and suppression of microbial activity, incubation chambers were flushed with ambient air at any point when the CO\(_2\) concentration in
the chamber headspace reached 10,000 ppm. The temperature inside each incubation chamber was kept constant by submerging the chambers inside the waterbath.

In June 2018, soils were collected from the 0-5 cm depth from 10 randomly located areas inside each field measurement plot. The soil subsamples within each measurement plot were sieved through 2 mm sieve immediately and homogenized before setting up for incubation. The soils were stored at 4°C prior to incubation. Each incubation chamber consisted of four, 30 cm³ vials inside a 1 L mason jar. In each vial, about 30 g of soil were placed into a perforated foil cup over a bed of 3mm glass beads to allow drainage and to maintain the samples at field capacity soil moisture. A small 1mm mesh was placed on top of the soil layer in each vial. About 0.3g of oven dried pine litter collected from the same plot was placed above the mesh (Figure 5-2). The samples were then incubated for six months at a constant temperature of 26.7°C, representing the annual average of daily high temperature for our study site. To control moisture limitations for microbial activity, water was periodically added to keep all samples to maintain them at or near field capacity.

Respiration measurements were automated with a resolution of one measurement every three seconds, with a total of 3.44 million data observations for the study. CO₂ measurements in each incubation chamber were made for eight minutes to ensure that CO₂ in the headspace was well mixed. CO₂ concentration measurements from the last four minutes were used to estimate mean CO₂ concentration in the headspace, thereby ensuring that air in the headspace was well mixed. The rate of CO₂ production for each incubation chamber was estimated as the difference between headspace CO₂ concentrations between two time periods (a cycle). This rate was scaled both to CO₂ production per day per g of soil and CO₂ production per day per g of carbon measured at the start of the incubation.
One sample vial was collected inside each incubation chamber at time 2 months and 4 months. The remaining two vials were retrieved at the end of the incubation period (six months). In addition, pine litter from all treatments were retrieved after 6-months and oven dried to a constant weight at 65°C to estimate litter mass loss during incubation.

**Nutrient Additions Treatment and Experimental Design**

In the laboratory, two (high and low nutrient addition experiments) completely randomized 4×4 factorial experiments for both the actively retreated and the untreated carryover field experiment were used. Silviculture, corresponding to the field treatments (C, F, FW, and W- for the actively retreated; and C\(_{C}\), C\(_{F}\), C\(_{FW}\), and C\(_{W}\) for the untreated carryover) and nutrient additions (Control, Cu, Mn, and N+P) were the main factors in both factorial experiments. The high and low nutrient addition amounts used were based on complete and partial decomposition and recycling rates that would occur over the incubation period. For example, based on the maximum annual litterfall estimates of 5-year-old loblolly pine stands (4.6 Mg ha\(^{-1}\) yr\(^{-1}\); FW treatment; Dalla-Tea, 1990) and average nutrient concentrations measured for the IMPAC II study (all treatments pooled together), complete decomposition of litterfall would recycle about 22.6 kg ha\(^{-1}\) N; 2.4 kg ha\(^{-1}\) P; 984 g ha\(^{-1}\) Mn, and 6 g ha\(^{-1}\) Cu per year. The nutrient addition treatment over the course of the study added equivalent amounts (areal basis) of these nutrients for the high-level factorial experiment. Likewise, the nutrient addition rates for the low-level experiment over a 6-month incubation period was based on a decomposition rate of 40% per year; this would recycle about 4.52 kg ha\(^{-1}\) N, 0.48 kg ha\(^{-1}\) P, 197 g ha\(^{-1}\) Mn, and 1.2 g ha\(^{-1}\) Cu per year. Equivalent amounts (areal basis) of these nutrients were added in the low-level factorial experiment over the course of the study. Nutrients were added as aqueous forms (Cu: Cu chloride; Mn: Mn chloride; and N+P: Ammonium phosphate monobasic + Ammonium nitrate dissolved in ultra-pure water) twice every month during the incubation period. These levels of
nutrient additions were selected to alleviate nutrient limitations without creating toxic conditions (Powers and Salute, 2011). The controls received ultra-pure water equivalent to the volume added in the nutrient treatments.

Chemical Analyses

Litter and soil chemistry

Macro- and micronutrient concentrations of the pine litter were estimated prior to the incubation. Briefly, litter was dried in the oven (65°C) to a constant weight before being ground in a Wiley mill to pass through a 1mm sieve. About 0.5 g of ground samples were dry-ashed in a muffle furnace, and then samples were brought up to volume with aqua-regia (3:1 Nitric/Hydrochloric acid). Inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to analyze the extracts. Total N and C was analyzed using a CNS analyzer (Leco Corp.) and the Dumas method (Campbell, 1992). Macro- and micro-nutrients analyses were conducted at the Micro-Macro International Laboratory in Athens, GA.

Initiat fiber lignin concentration was also estimated for the litter. Briefly, oven dried litter samples were ground in a Wiley mill to pass through a 1mm screen. Acid detergent fiber determinations were made by placing a 0.5 g sample in ANKOM filter bags (F57) and running sample bags through a hot acid detergent solution (Catalog No. FAD20CB; ANKOM Technology, Macedon, NY) for an hour using an ANKOM A200 Fiber analyzer (ANKOM Technology, Macedon, NY). Sample bags were then rinsed, dried, and weighed prior to lignin determination. Sample bags were placed in 72% H₂SO₄ for three hours, agitating periodically in between. The rinsed sample bags were dried, weighed, and ashed in a muffle furnace to estimate initial lignin concentrations.

Soil ammonium and soil nitrate concentrations were estimated for the soil samples. Briefly, 2.5 to 5 g soil was extracted in 25 mL of 1M KCl. The extract was then run through an
automated continuous flow analyser to estimate NH$_4$-N and NO$_3$-N. NH$_4$-N was determined by using a modified semi-automated colorimetric method (EPA Method 350.1), where samples were run without the distillation procedure. For soil nitrate concentrations, the EPA 353.2 method was used. For soil P measurement, 2.5 g of soil samples were extracted using 25 mL of Mehlich III extractant (Mehlich, 1983). The extracts were then run through ICP-AES to estimate P concentrations using the EPA 200.7 method. These analyses were performed at the Analytical and Research Laboratory at the University of Florida. Soil C concentrations in the initial soil samples were estimated using the loss on ignition method. Briefly, 10 g soil samples were dried at 105°C for 2 hours prior to ignition in the muffle furnace at 450°C for six hours. Mass loss after ignition was calculated to estimate % organic matter. Soil C concentration was calculated by multiplying the % organic matter by a factor of 0.58.

**Mn peroxidase assay**

Soil samples were retrieved after the 4-month and 6-month incubation period for determining Mn peroxidase activity. Soils from the C$_C$ and C$_W$ treatment plots of the untreated carryover experiment and the C and W treatment plots of the actively managed retreated experiments were used. Only samples that received either the Mn (High and low level) or ultrapure distilled and deionized water (control) treatments were analysed. About 5 g of soil was mixed with 30 ml of deionized and distilled water (DDI) and mechanically shaken for 1 hour. After mixing the samples thoroughly with DDI, they were centrifuged for 10 minutes at 10,000 G at room temperature. The extracted supernatant was analysed for Mn peroxidase activity (MnP) using procedures outlined by Fujii et al. (2013). Briefly, phenol red was used as a substrate. The reaction mixture consisted of 2 ml of sodium succinate buffer (50mM, pH 4.5), 2 ml of sodium lactate (50mM, pH 4.5), 0.8 ml of Mn sulfate (0.1mM), 1.4 ml of phenol red (0.1mM), 0.8 ml of H$_2$O$_2$ (50µM), 2 ml of albumin (0.1%), and 1 ml of enzyme extract. The
reaction was initiated by adding $\text{H}_2\text{O}_2$. A 2 ml aliquot of the reaction mixture was removed every two minutes, to which 40$\mu$L of 5M NaOH was added. The reaction was carried out for a total of 10 minutes at room temperature ($25^\circ\text{C}$), and the absorbance was measured at 610 nm wavelength using a UV-1800 Shimadzu Spectrophotometer (Shimadzu Corporation, Kyoto, Japan). An equimolar amount of ethylenediaminetetraacetic acid (EDTA) was used instead of MnSO$_4$ for the control. Activity estimates were made using the difference in absorbance rates between the MnSO$_4$ added reaction mixture and that where MnSO$_4$ was replaced with EDTA. The oxidation rate of phenol red was measured using its extinction coefficient ($\varepsilon = 4460 \text{M}^{-1}\text{cm}^{-1}$). One unit of enzyme activity was defined as the amount of enzyme forming 1 $\mu$mol of reaction product per minute and was expressed as U g$^{-1}$soil ($\mu$mol min$^{-1}$ g$^{-1}$ soil).

**Data Preparation and Statistical Analyses**

The daily microbial respiration rates were fitted with days since incubation for all silvicultural treatments using the following exponential function:

$$y = ae^{-bx}$$

where $y$ is microbial respiration rate, $x$ is number of days since incubation, $a$ is scale parameter, and $b$ is the decay parameter. Repeated measures analysis of variance was used to test the effects of past treatments and nutrient additions on microbial respiration rates (Littell et al., 2006) at the high and low level of nutrient additions for both the actively retreated and the untreated carryover experiments. The following general repeated measures model was used at three time intervals (0-2, 2-4, and 4-6 months after incubation):

$$y = \mu + b + S + N + SN + e$$

where $y$ is the response variable; $\mu$ = overall mean; $b$ = random effect of the block with $b \sim N(0, \sigma^2 b)$; $S$ = fixed effect of the silvicultural treatment; $N$ = fixed effect of nutrient additions; ND = fixed interactive effects between silvicultural treatment and nutrient additions; and $e$ = error term
(random error with \( e \sim N(0, \sigma^2_e) \)). The PROC MIXED procedure in SAS (SAS Institute, 2007) was used for the statistical analyses. Correlations between measurements taken repeatedly for each subject (measurement unit) were modeled using autoregressive (1) error structure.

The effects of silviculture and nutrient additions on pine litter mass loss was investigated using analysis of variance. The following model was used for the study,

\[
y = \mu + b + S + N + SN + e
\]

where \( y \) is the pine litter mass loss response variable; \( \mu \) = overall mean; \( b \) = random effect of the block with \( b \sim N(0, \sigma^2_b) \); \( S \) = fixed effect of the silvicultural treatment; \( N \) = fixed effect of nutrient additions; \( ND \) = fixed interactive effects between silvicultural treatment and nutrient additions; and \( e \) = error term (independent, \( N(0, \sigma^2_e) \)). The PROC MIXED procedure in SAS (SAS Institute, 2007) was used for the statistical analysis.

Analysis of variance was used to investigate if Mn peroxidase activity at two time periods (4 months since incubation and 6 months since incubation) differed with Mn additions (Low level, High level, and Control) for the C and W silvicultural treatments of the actively retreated experiment, and the C_C and C_W treatments of the untreated carryover experiment. The following model was used for this analysis:

\[
y = \mu + b + S + M + T + SM + ST + MT + SMT + e
\]

where, \( \mu \) = overall mean; \( b \) = random effect of the block with \( b \sim N(0, \sigma^2_b) \); \( S \) = fixed effect of the silvicultural treatment; \( M \) = fixed effect of Mn additions; \( T \) = fixed effect of months since incubation; \( SM \) = fixed interactive effects between silvicultural treatment and Mn additions; \( ST \) = fixed interactive effects between silvicultural treatment and months since incubation; \( MT \) = fixed interactive effects between Mn additions and months since incubation; \( SMT \) = fixed interactive effects between silvicultural treatments, Mn additions, and months since incubation;
and e = error term (independent, N (0, σ²e)). Diagnostic reports (residual plots and quantile plots) were used to ensure that the data met assumptions of ANOVA. For all analyses, the effects were declared significant when p values were ≤0.05. Tukey’s HSD test was used to separate significantly different means.

**Results**

**Initial Litter and Soil Chemistry**

Litter and soil chemistry differed among silvicultural treatments for both experiments. In the untreated carryover experiment, the fertilization treatment history resulted in higher N, P, and Mn concentration in pine litter compared to the C_C treatment. For example, the N concentration was almost 1.26-fold higher in the C_F compared to the C_C treatment. Phosphorus concentration was also 1.6- and 1.5- fold higher in the C_F and C_FW treatments, respectively, compared to the C_C treatment. In the 0-5cm soil, there was lower NH₄-N concentration in the C_F and C_FW treatments compared to the C_C treatment. In the actively managed retreated experiment, the F and FW pine litter had higher N, P, K, and Mn concentrations. The pine litter from the W treatment plots had higher lignin concentrations than the C treatment (Table 5-2). Initial soil NH₄-N concentrations were highest for the F followed by the C, W, and FW treatments. Mehlich III extractable P was significantly higher in the F treatment compared to the C treatment. Soil C (%) was also significantly higher in the F treatment compared to all other treatments (Table 5-2).

**Effect of Silviculture on Microbial Respiration**

The previous rotation’s fertilization and weed control treatments affected mean microbial soil respiration rates per g of soil in the untreated carryover experiment. For example, mean soil microbial respiration rate for the C_F treatment (1.49 μmol C/g soil/ day) was significantly higher compared to all other treatments (C_FW= 1.25 μmol C/g soil/ day, C_W=1.02 μmol C/g soil/ day, and C_C= 0.99 μmol C/g soil/ day). Mean soil respiration rate for the C_FW treatment was 1.23- and
1.26-fold higher than the C_C and C_W treatments. The microbial respiration rate declined exponentially as decomposition progressed (Figure 5-3a). Silvicultural treatments, however, did not significantly affect the decay parameter (b) estimate of the exponential function (p = 0.479; ANOVA on parameter). The scale parameter (a) estimate was significantly higher in the C_F (1.87) treatment compared to the C_C (1.38) and C_W (1.43) treatments (p=0.013) (Figure 5-3a). When respiration was normalized with respect to C concentration (%), analysis of the means revealed that the C_W treatment had a significantly higher scale parameter (49.52) and the C_F treatment (0.0021) had a significantly lower decay parameter estimate than the average of all treatments (a=44.13; b=0.0031) (Figure 5-3b).

The continued fertilization treatment in the actively managed retreated experiment had significantly higher mean microbial soil respiration rates per g of soil compared to the other treatments. For instance, the mean soil microbial respiration rate in the F treatment (1.875 µmol C/g soil/ day) was almost 1.8-, 1.8-, and 1.9-fold higher, respectively, compared to the C (1.039 µmol C/g soil/ day), FW (1.056 µmol C/g soil/ day), and W (0.985 µmol C/g soil/ day) treatments. As in the untreated carryover experiment, the microbial respiration rate declined exponentially as decomposition progressed (Figure 5-3c). Silvicultural treatments, however, did not significantly affect the growth/decay parameter (b) estimate of the exponential function (p = 0.482; ANOVA on parameter). The scale parameter (a) estimate was significantly higher in the F (3.00) treatment compared to the W (2.22), FW (2.02), and C (1.98) treatments (p=0.012) (Figure 5-3c). When respiration was normalized with respect to C concentration (%), analysis of the means revealed that the W treatment had a significantly higher scale parameter (69.48) and the F treatment (51.14) had a significantly lower scale parameter estimate than the average of all treatments (56.35). Likewise, the growth/decay parameter estimates were significantly higher for
the W (0.0104) and lower for the F (0.0054) treatments compared to the overall average among treatments (0.0007) (Figure 5-3d).

**Effect of Nutrient Amendments on Microbial Respiration**

The silvicultural treatments affected the mean microbial respiration rate per g of initial C for both experiments (Table 5-3) across all time periods and nutrient addition levels investigated. The low level nutrient addition effects, however, were significant at 0-2 months and 4-6 months in the untreated carryover experiment and 2-4 months in the actively managed retreated experiment. The high level nutrient addition effects were significant at 2-4 months and 4-6 months in the untreated carryover experiment, and 0-2 and 2-4 months in the actively managed retreated experiment. Interactions between silviculture and nutrient additions were present at both the high- and low-level nutrient additions for the untreated and actively retreated experiments over all time periods (Table 5-3).

During the first two months of incubation, the nutrient addition effects on microbial respiration rates per g carbon per day were evident for the Cc treatment in the untreated carryover experiment and the C treatment in the actively retreated experiment (Figure 5-4a, b and 5-5a, b). In the untreated carryover experiment, the high-level N+P addition rate increased microbial respiration by almost 15.5% in the Cc treatment (Figure 5-4b). In the actively retreated experiment, N+P additions at both the low- and high-level stimulated microbial respiration by 48.6% and 63.2%, respectively in the C treatment (Figure 5-5a, b). Nutrient additions in the fertilized plots (F and Cr) did not significantly affect microbial respiration. Copper additions at the low level also resulted in a positive effect on microbial respiration for the Cc and C treatments (Figure 5-4a, 5-5a).

During the 2 to 4-month incubation interval, the Mn addition effect was significant for the Cc, C, and W treatments. Manganese addition at the low level increased microbial respiration
by almost 21.1% in the C<sub>c</sub> treatment (Figure 5-4c). In the C treatment of the actively retreated experiment, both the low- and high-level Mn additions resulted in 33% and 35% higher microbial respiration rates (Figure 5-5c, d). Both the low- and high-level Mn additions also stimulated microbial respiration in the W treatment by 1.3-fold. Positive N+P effects were also present in the C treatment at both levels of addition, and in the W treatment at the low-level. For the C<sub>f</sub> and F plots, N+P addition at the high-level resulted in a suppression of microbial respiration (Figure 5-4d, 5-5d).

From 4 to 6 months, the effect of Mn addition for the C<sub>c</sub> treatment was only significant at the low addition level (Figure 5-4e). A N+P suppression effect was present in the C<sub>f</sub> treatment at the high level of addition, and the C<sub>w</sub> treatment at both levels of additions (Figure 5-4e, f).

Effect of Nutrient Amendments on Pine Litter Decomposition

Nutrient amendments in the laboratory experiment affected pine litter decomposition at 6 months (Figure 5-6). However, these effects were present only in litter from the plots without a fertilization treatment history (e.g. C<sub>c</sub>, C<sub>w</sub>, C, and W treatments). For example, both the N+P and Mn treatments at the low- and high-levels resulted in higher pine litter mass loss for the C<sub>c</sub> treatment (Mass loss: low-level N+P = 27.0% and low-level Mn = 27.2%; high-level N+P = 27.7% and high-level Mn = 27.3%; Control = 24.9%). In the actively managed retreated experiment, N+P significantly increased pine litter mass loss in the C treatment for both the low- and high-level additions. Manganese additions at the high level also improved pine litter mass loss in the W treatment (Mn= 28.8% vs control= 24.6%). No significant effects of N+P, Mn, or Cu on pine litter mass loss were observed for the C<sub>f</sub>, C<sub>fw</sub>, F, and FW treatments.

Mn Effect on Mn Peroxidase Activity

For the untreated carryover experiment, Mn addition affected the activity of Mn peroxidase in the C<sub>c</sub> treatment after 4 months of incubation. The high-level addition of Mn...
resulted in about a 65.8% and 90.2% higher Mn peroxidase activity, respectively, compared to the low-level Mn and no Mn addition treatments (Figure 5-7). Although Mn peroxidase activity generally increased with Mn additions, they did not differ significantly from the C_C treatment after six months of incubation (High-level= 0.0334 U g^{-1} soil, Low-level = 0.0272 U g^{-1} soil, Control= 0.0254 U g^{-1} soil). For the C_W treatment, there were no significant differences in Mn peroxidase activity among Mn addition treatments over both incubation periods. In the actively managed retreated experiment, Mn addition at the high level increased Mn peroxidase activity after four months of incubation in the W treatment (0.0365 U g^{-1} soil) by about 85.3% compared to the no nutrient addition treatment (0.0197 U g^{-1} soil). Although the low-level Mn addition rate tended to increase the absolute Mn peroxidase activity in both the C and W treatments, the increases were not significant. Six months after incubation, the Mn addition effects on Mn peroxidase activity for the W treatment was not significant. In addition, a strong positive correlation between microbial respiration rate (per g soil per day) and Mn peroxidase activity at six months (r=0.632; p<0.001) was detected when both the untreated carryover (C_C and C_W) and actively managed retreated (C and W) experiments were pooled together (Figure 5-8).

**Discussion**

Prior studies in managed pine ecosystems suggest that litter decomposition can be altered by common silvicultural practices like fertilization and weed control treatments on inherently nutrient poor soils (Polglase et al., 1992b; Gurlevik et al., 2003; Kiser et al., 2013). Mechanisms for these effects are relatively unknown in Spodosols, a major soil type that often supports southern pine plantations in the US South. Two field experiments in a nutrient-poor Spodosol, one with nutrient amendments (macro- and micronutrients) and understory competition control treatments applied in the prior rotation, and the other with the same treatments continued into the subsequent rotation, provided a unique opportunity to compare and
contrast whether silvicultural treatment histories alleviate nutrient limitations to decomposition processes. In this study, controlled laboratory experiments were designed using soils and pine litter from these field experiments. These laboratory experiments allowed a mechanistic understanding of nutrient limitations to decomposition processes on a Spodosol having a range of nutrient and understory vegetation management histories in Florida.

**Effects of Silvicultural Treatment Histories on Microbial Respiration**

The effects of fertilization on organic matter decomposition and microbial respiration has been widely investigated in the context of atmospheric N enrichment in forest ecosystems across different regions of the world (Berg and Matzner, 1997; Burton et al., 2004; Knorr et al., 2005; Pregitzer et al., 2008; Zak et al., 2011; Frey et al., 2014; Ye et al., 2018). A general observation from these studies suggest that N enrichment reduces microbial respiration and could potentially slow organic matter decomposition. Consistent with that observation, results from the laboratory incubation study of the F and C_F treatment soils collected from managed southern pine plantations in lower Coastal Plains of the US showed lower microbial respiration rates per g of soil C than the overall average respiration rates among common silvicultural tratments.

Existing literature suggests two possible mechanisms that may explain the observation of lower CO_2 release per g of C on fertilized soils. First, higher N and P inputs from fertilizer addition treatments may suppress production of lignin-degrading enzymes by microbial communities (Buswell et al., 1995; Carreiro et al., 2000; Hofmockel et al., 2007; Jian et al., 2016), and thereby reduce fungal decomposition of recalcitrant substrates. A recent meta-analysis of soil extracellular enzyme activity across N fertilized sites, however, suggested that N repression of oxidases was not as strong as limitations by C or P (Jian et al., 2016). Second, nutrient availability likely affected how microbes partition C between respiration and biomass growth (Manzoni et al., 2008; Blagodatskaya et al., 2014). On nutrient limited sites where the
litter C:N ratio is high, microbial efficiency of C use is generally lower, with more allocation to respiration than biomass growth (Manzoni et al., 2012). Therefore, it is likely that lower CO₂ release from the fertilized plots in our study may be due to higher microbial carbon use efficiency compared to the nutrient-limited C and W plots. Likewise, potential differences in microbial community composition between silvicultural treatments applied over a rotation may be responsible for driving such differences in microbial respiration responses (Strickland et al., 2009). One thing to note, however, is that although fertilization reduced respiration rates per g of carbon, the total amount of CO₂ released from these soils was much higher than other treatments because fertilization resulted in higher total carbon in the forest floor and soils. For this study site, in-situ microbial soil respiration rates in the historically fertilized plots contributed to almost 2.3 MgC ha⁻¹ more efflux to the atmosphere per year than the control plots early in the subsequent rotation (Chapter 4).

**Effects of Silvicultural Treatment Histories on Pine Litter Mass Loss**

Pine litter mass loss during the incubation period was rapid compared to the ex-situ incubation for both untreated carryover and actively managed retreated experiments. This observation was consistent with prior observations by Polglase et al. (1992b) who reported relatively lower mass loss rates during an in-situ compared to an ex-situ experiment for this same site. Such differences were expected in this study because controlled environmental conditions (26.7°C and no moisture limitations) likely optimized the microbial activity. There were also consistent mass loss trends in both the ex-situ and in-situ incubation, with lowest pine litter mass loss for the Cw treatment (Chapter 4, Figure 4-4). Although some studies have demonstrated that herbicide treatments (e.g. glyphosate) may affect the microbial community response at very high levels compared to operational application rates (Ratcliff et al., 2006), such an effect was not apparent in the Cw treatment likely because herbicides were applied almost 20 years earlier in
the first rotation. In the actively managed retreated experiment, the ex-situ incubation of pine litter suggested lower mass loss in the FW treatment; unlike that observed during the in-situ incubation experiment. This discrepancy in mass loss trends between the field and laboratory incubation experiments was likely due to differences in the bio-physical and climatic characteristics associated with the thinning activity in the FW treatment compared to consistent environmental conditions used among treatments in ex-situ incubation experiment.

**Microbial Respiration and Pine Litter Mass Loss Response to N+P Additions**

Nutrient availability affects both biotic and abiotic mechanisms that drive complex and dynamic organic matter decomposition processes. Existing studies on litter or organic matter decomposition have shown that N, P, K, and other nutrients affect decomposition processes mainly by changing the decomposer community composition and associated biological processes (e.g. enzyme production) (Compton et al., 2004; Allison and Vitousek, 2005; Hobbie, 2005; Whalen et al., 2018). In our study, microbial respiration responses to nutrient additions were very dynamic and positive in the control plots for both the untreated carryover and actively managed retreated experiments. A positive response to the N+P addition likely suggests an alleviation of nutrient limitations, esp. P, to decomposer communities in Spodosols. Improved pine litter mass loss in the C, W, and Cc treatments with N+P additions further supports the premise that these nutrients may alleviate limitations to microbial decomposition processes in these soils. Our observation was consistent with another study conducted at the same site, which reported higher litter and organic matter decomposition rates on fertilized plots compared to those receiving no fertilizer additions (macro- and micro-nutrient) (Chapter 3 and 4). In addition, the litter lignin:P ratio explained significant variation in these litter decomposition studies (Chapter 4). In many tropical forest studies, P limits organic matter decomposition and alleviating this nutrient limitation has been shown to stimulate the decomposition process (Powers and Salute, 2011).
Interestingly, however, was the suppression of microbial respiration under the high-level N+P addition rate for soils originating from those treatments where nutrients were added either in the previous rotation (C_F) or in both rotations (F). Pine litter mass loss also did not increase with N+P addition in these treatments. Observation of the suppressive effects of N+P only after two months of incubation may be associated with changes in substrate qualities and decomposer communities between these periods. Ramirez et al. (2012) suggested that N additions reduced extracellular enzyme activity in soils and shifted microbial community composition to those that preferentially decompose more labile C substrates. Likewise, a negative effect of P fertilization on litter decay has been observed in P enriched boreal forests (DeForest, 2019). For our soils, it is likely that the relative increase in the recalcitrant portion of the substrate resulted in the suppressive N+P effect on microbial respiration as decomposition progressed over the first two months. Other studies conducted in temperate forests reported reduced organic matter decomposition with N enrichment and suggested that the magnitude of N inputs also affected this response (Janssens et al., 2010; Ramirez et al., 2012; Frey et al., 2014). For instance, Frey et al. (2014) documented that with higher N inputs the magnitude of reduction in microbial activity (proteolytic enzyme activity) in the upper soil horizon and organic layers was increased.

**Microbial Respiration and Pine Litter Mass Loss Response to Mn Additions**

A previous study conducted in a slash pine plantation growing on a Spodosol in north Florida documented a positive growth response to Mn additions (Jokela et al., 1991). Similarly, the current study documented for the first time that microbial respiration and litter decomposition also responded to Mn additions on a nutrient limited Spodosol. Higher microbial respiration rates when Mn was added to soils either from the control or those with a weed control treatment history suggests that the microbes benefitted when Mn limitations were alleviated. More importantly, the positive respiration response to Mn addition early in the decomposition process
suggested that increased Mn availability likely aided in decomposition of recalcitrant substrates in the Cc and C treatments. Evidence from temperate forests showed that increased Mn\textsuperscript{2+} availability accelerated organic matter decomposition in N enriched sites (Trum et al., 2011; Whalen et al., 2018) by facilitating the microbial production of lignin oxidizing extracellular enzymes (e.g. peroxidase, laccase) (Dashtban et al., 2010; Whalen et al., 2018).

Manganese availability has been reported to increase the decomposition of late stage and lignin rich litter in forest ecosystems (Keiluweit et al., 2015; Whalen et al., 2018; Sun et al., 2019). Consistent with these observations, the Mn addition response reported in this study was much pronounced in the Cw and W treatments during the later stages of decomposition (e.g. after two months of incubation). Because the weed control plots had higher initial litter lignin, increased Mn availability likely facilitated the decomposition of more recalcitrant substrate after the labile substrates were consumed in the first few months. The significant positive effects on litter mass loss in the W treatment where the high level of Mn addition rate was applied further supports this interpretation. The non-significant effect of Mn on microbial respiration and litter decomposition for the Cf, F, Cfw, and FW plots, however, suggest that the long-term intensive fertilization treatments imposed on the soils at the IMPAC II experiment likely alleviated Mn limitations to the decomposer community.

Results from this nutrient manipulation experiment, conducted using laboratory incubated soil and litter samples, support our hypothesis that Mn limits microbial activity, enzyme production, and litter decay rates in these soils. Manganese is an essential element used by soil microbes as an enzyme co-factor in Mn peroxidase. Consistent observation of increased Mn peroxidase activity in the Cc, C, Cw, and W treatments following Mn additions also supports a Mn limitation for enzyme production and the decomposition of recalcitrant organic matter. The
strong positive correlation ($r = 0.635$) between Mn peroxidase activity and microbial respiration rates late in the incubation process provides additional and consistent support for this hypothesis (Figure 5-8). Also, the strong positive correlation between soil Mn availability in the surface 0-10 cm and in-situ soil heterotrophic respiration rates ($r = 0.66$, $p = 0.019$) reported in an earlier study conducted at the same study site provides further evidence to support a Mn limitation to decomposition processes in nutrient-poor Spodosols.

Growing evidence of macro and micro-nutrient limitations affecting the growth potential of southern pine plantations have shifted fertilization practices to include both macro- and micronutrient additions on some sites (Albaugh et al., 2018). The interaction of nutrient inputs with silvicultural treatment history for both the untreated carryover and the actively managed retreated experiment highlights how nutrient supply may affect belowground carbon cycling processes in managed plantations. While observations from this study suggested that decomposition processes in the intensively fertilized plots may not be affected as much as those without a fertilization history, alteration of micronutrient cycling in nutrient poor soils will likely affect the stability of soil C pools. The lack of significant difference in microbial respiration responses between the low- and high-levels of nutrient additions at the early or late incubation stages in this study (Early stage: untreated carryover: $p=0.24$, actively managed retreated: $p=0.16$; Late stage: untreated carryover: $p=0.62$, actively managed retreated: $p=0.26$) suggests that micronutrient recycling via litter decomposition at a rate of about 40% would likely alleviate micronutrient limitations to decomposition processes. Nevertheless, management practices such as the control of competing vegetation, which could potentially reduce the abundance of some species that strongly influence Mn cycling (e.g. gallberry, Jokela et al., 1991) could have implications in regulating decomposition processes and C cycling. Manganese
mediated Mn peroxidase production also partly explains why mixing Mn rich gallberry leaves with pine litter improved the decomposition rates in the weed control treatment plots of this same site (Chapter 4). In an in-situ environment, these responses may be confounded by other factors such as movement of microbes and soil fauna, forms of available nutrients, photodegradation, soil moisture and temperature fluctuations, nutrient movement between horizons, and fresh carbon supply. Nevertheless, by controlling environmental variation introduced by soil temperature and moisture, this study provided multi-tiered evidence to help elucidate how soil Mn supply limits microbial respiration and organic matter decomposition in a Florida Spodosol.

**Summary**

A controlled laboratory incubation experiment was used to provide multi-tiered evidence of macro- and micronutrient limitations to microbial respiration and organic matter decomposition for soils having a long history of varying silvicultural management practices. Consistent with prior studies, the fertilizer treatments generally resulted in lower microbial respiration rates per g of carbon. This suppression of microbial respiration was accentuated by adding higher amounts of N+P only in the C_F and F treatments. However, the acceleration of CO_2 mineralization rates with N+P fertilization in the C_C and C treatment during the early stages of decomposition highlights the importance of these macronutrients for low fertility soils. Results also present evidence of a Mn limitation to decomposition processes in a Florida Spodosol that supports large areas of southern pine plantations in the southeastern US. The pronounced effect that Mn additions had on microbial respiration rates for soils not having a fertilization history (e.g. the C_C, C, C_W, and W treatments) highlight the importance of Mn limitations for microbial decomposition processes in these soils. Increased Mn peroxidase activity observed for soils from both the control and weed control treatments following Mn additions, and the strong positive relationship between Mn peroxidase activity and microbial
respiration rates suggests that Mn peroxidase production regulated microbial decomposition processes in these soils. From a forest management perspective, multi-element fertilization (e.g., macro- and micronutrients) in plantations growing on Spodosols similar to those found in this study, would likely alleviate nutrient limitations (e.g., Mn) to decomposer soil organisms. Since fertilization treatments in our study site were intensive, Mn limitations to microbial respiration and organic matter decomposition may be more common in operationally fertilized stands growing on Spodosols. Nevertheless, management practices such as intensive and sustained weed control treatments may result in slower organic matter decomposition rates by reducing micronutrient recycling potential of important understory vegetation species such as *Ilex glabra* L.. Results also suggest that the effects of Mn limitations (and other nutrients) on decomposition processes will require further study across a range of soil types to better understand C cycling in managed southern pine ecosystems.
### Table 5-1. Treatments applied to juvenile loblolly pine plantation growing on Spodosols in North Florida at the Intensive Management Practices Assessment Center (IMPAC) II study

<table>
<thead>
<tr>
<th>Experiment</th>
<th>First-rotation treatment</th>
<th>Second-rotation treatment</th>
<th>Treatment abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated carryover</td>
<td>Control</td>
<td>untreated</td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 1088 N, 230 P, 430 K, 108 Ca, 72 Mg, 72 S, 4.1 Mn, 5.4 Fe, 0.9 Cu, 4 Zn, and 0.9 B)</td>
<td>untreated</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Fertilizer (same as the Fertilizer only treatment) + Weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>untreated</td>
<td>C&lt;sub&gt;FW&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>untreated</td>
<td>C&lt;sub&gt;W&lt;/sub&gt;</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>Control</td>
<td>Fertilizer only</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>Fertilizer only (Cumulative nutrient addition for the rotation rotation (kg ha&lt;sup&gt;-1&lt;/sup&gt;): 360 N, 145 P, 300 K, 120 Ca, 61 Mg, 78 S, 3.01 Mn, 0.48 Fe, 0.36 Cu, 3.00 Zn, and 0.36 B) + Thinning operation at age 6 year</td>
<td>Control only Fertilizer only + weed control (Chemical methods)</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>Fertilizer (same as the Fertilizer only treatment) + weed control (combination of chemical and mechanical methods up to age 10 years until canopy closure)</td>
<td>Fertilizer (same as the F treatment) + Thinning operation at age 6 year + weed control (Chemical methods)</td>
<td>FW</td>
</tr>
<tr>
<td></td>
<td>Weed control only (combination of chemical and mechanical methods)</td>
<td>Weed control only (Chemical methods)</td>
<td>W</td>
</tr>
</tbody>
</table>
Table 5-2. Pine litter and soil (0-5 cm) chemistry in different treatments applied in the untreated carryover and actively managed retreated experiments at the IMPAC II study

<table>
<thead>
<tr>
<th>Litter</th>
<th>-------Untreated carryover--------</th>
<th>----Actively managed retreated-----</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (%)</td>
<td>(0.30) b 0.38 a 0.36 ab 0.35 ab</td>
<td>(0.28) c 0.49 ab 0.59 a 0.37 bc</td>
</tr>
<tr>
<td>P (mg kg(^{-1}))</td>
<td>298 b 470 a 459 a 241 b</td>
<td>246 b 785 a 714 a 276 b</td>
</tr>
<tr>
<td>K (mg kg(^{-1}))</td>
<td>412 463 273 260</td>
<td>289 b 1568 a 1298 a 261 b</td>
</tr>
<tr>
<td>Ca (mg kg(^{-1}))</td>
<td>4297 3687 4424 4125</td>
<td>4118 4668 3786 3778</td>
</tr>
<tr>
<td>Mg (mg kg(^{-1}))</td>
<td>1300 a 975 b 1050 b 1281 a</td>
<td>1222 a 917 b 849 b 1277 a</td>
</tr>
<tr>
<td>B (mg kg(^{-1}))</td>
<td>11.4 10.3 12.2 11.2</td>
<td>11.0 13.2 12.9 11.0</td>
</tr>
<tr>
<td>Cu (mg kg(^{-1}))</td>
<td>1.6 1.5 0.8 1.1</td>
<td>1.7 1.7 1.6 0.6</td>
</tr>
<tr>
<td>Mn (mg kg(^{-1}))</td>
<td>117.8 c 211.3 b 295.3 a 100.3 c</td>
<td>103.6 b 195.4 a 175.1 a 103.3 b</td>
</tr>
<tr>
<td>Zn (mg kg(^{-1}))</td>
<td>22.0 b 29.4 ab 37.4 a 22.1 b</td>
<td>25.3 ab 30.9 a 30.4 a 21.3 b</td>
</tr>
<tr>
<td>Lignin (%)</td>
<td>27.4 27.4 28.5 29.7</td>
<td>26.6 b 27.4 ab 27.1 ab 29.0 a</td>
</tr>
<tr>
<td>C:N</td>
<td>175.4 a 137.4 b 137.0 b 151.0 ab</td>
<td>183.0 a 110.6 c 93.1 c 155.2 b</td>
</tr>
<tr>
<td>Lignin:N</td>
<td>90.2 72.1 78.5 84.3</td>
<td>89.1 a 79.6 ab 55.3 bc 49.6 c</td>
</tr>
</tbody>
</table>

Soil (0-5 cm)

| NH4-N (mg kg\(^{-1}\)) | 22.0 a 10.6 bc 9.8 c 18.6 ab | 41.4 ab 50.0 a 25.0 b 37.0 ab |
| NO3-N (mg kg\(^{-1}\)) | 2.4 0.4 0.6 0.5 | 1.5 2.0 0.5 0.5 |
| P (mg kg\(^{-1}\)) | 8.6 6.5 22.1 7.1 | 8.3 b 19.1 a 14.5 ab 8.8 b |
| C (%) | 3.0 4.5 4.2 2.9 | 3.6 b 5.8 a 3.7 b 3.2 b |
Table 5-3. Summary (p Values) of fixed effects ANOVA for microbial respiration (μmol C/g C/day) during incubation as affected silvicultural treatments, nutrient additions, and their interactions in the untreated carryover and actively managed treated experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nutrient Level</th>
<th>Period</th>
<th>Silviculture</th>
<th>Nutrient</th>
<th>Silviculture x Nutrient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated carryover</td>
<td>Low</td>
<td>0-2mths</td>
<td>&lt;0.0001</td>
<td>0.0034</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-4 mths</td>
<td>&lt;0.0001</td>
<td>0.5459</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-6 mths</td>
<td>0.0203</td>
<td>0.0241</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0-2 mths</td>
<td>&lt;0.0001</td>
<td>0.1351</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-4 mths</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-6 mths</td>
<td>0.052</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>Low</td>
<td>0-2 mths</td>
<td>0.0158</td>
<td>0.0514</td>
<td>0.0034</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-4 mths</td>
<td>0.0003</td>
<td>0.0007</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-6 mths</td>
<td>&lt;0.0001</td>
<td>0.0377</td>
<td>0.0112</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>0-2 mths</td>
<td>0.0004</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2-4 mths</td>
<td>&lt;0.0001</td>
<td>0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4-6 mths</td>
<td>&lt;0.0001</td>
<td>0.1493</td>
<td>0.0316</td>
</tr>
</tbody>
</table>
Figure 5-1. Study layout of the IMPAC II field trial on a poorly drained Spodosol in Gainesville, Florida.
Figure 5-2. (a, b) Automated soil incubation system with incubation chambers submerged in a temperature regulated water bath, (c, d) sample set up in vials and inside incubation chambers, (e, f) fungal growth on incubated soil samples and pine litter, and (g) schematics of the incubation system (modified after R. Bracho; Bracho et al., 2015)
Figure 5-3. Effect of silvicultural treatments (Control (C), Fertilizer (F), Fertilizer+Weed Control (FW), and Weed Control(W)) on the mean microbial respiration rates in the incubated soil samples (0-5cm) from the (a, b) untreated carryover, and (c, d) actively managed treated experiment at the IMPAC II study. Exponential functions of the form $y = ae^{bx}$ were fitted for each treatment ($R^2$: $C=0.49$, $F=0.51$, $FW=0.75$, $W=0.83$, $C_C=0.43$; $C_F=0.33$; $C_{FW}=0.47$, and $C_W=0.54$).
Figure 5-4. Microbial respiration rate as affected by silviculture, nutrient additions (Control, Cu, Mn, N+P), nutrient concentration levels (low & high), and time since incubation (0-2 months, 2-4 months, and 4-6 months) in the untreated carryover experiment. Within a silvicultural treatment, nutrient level, and time since incubation, nutrients with same letter are not significantly different at α=0.05 (Tukey’s HSD). Error bars represent ±standard error.
Figure 5-5. Microbial respiration rate as affected by silviculture, nutrient additions (Control, Cu, Mn, N+P), nutrient concentration levels (low & high), and time since incubation (0-2 months, 2-4 months, and 4-6 months) in the actively managed retreated experiment. Within a silvicultural treatment, nutrient level, and time since incubation, nutrients with same letter are not significantly different at $\alpha=0.05$ (Tukey’s HSD). Error bars represent ±standard error.
Figure 5-6. Pine litter mass loss (%) after a six-month incubation period as affected by silviculture and nutrient amendments in (a) untreated carryover experiment, and (b) actively managed retreated experiment. ‘Low’ and ‘High’ denote two levels of nutrient additions. Within a silvicultural treatment, nutrients followed by same letter are not significantly different at $\alpha=0.05$. Error bars represent ±standard error.
Figure 5-7. Mn peroxidase activity (determined using Phenol-red oxidation method) as affected by Mn addition in the soil samples of the control and weed control plots for the untreated carryover and actively managed retreated experiments at 4 months and 6 months of incubation. Floating bars indicate mean activity. Within a silvicultural treatment, floating bars with same letter are not significantly different ($\alpha=0.05$; Tukey’s HSD). One unit of enzyme activity was defined as the amount of enzyme forming 1 μmol of reaction product per minute and expressed as U g$^{-1}$ soil (μmol min$^{-1}$ g$^{-1}$ soil).
Figure 5-8. Relationship between mean microbial respiration rate and Mn peroxidase activity after a six-month incubation period for the control and weed control soils. Solid circles denote the untreated carryover experiment and hollow circles denote the actively managed retreated experiment.
CHAPTER 6
CONCLUSIONS

In nutrient limited Spodosols of north Florida, a productivity gradient created from the long-term application of fertilizer and understory competition control treatments in the previous rotation provided a unique opportunity to examine their effects on the growth dynamics, soil nutrient availability, and organic matter decomposition in a second rotation loblolly pine plantation. In the first rotation, the IMPAC (Intensive Management Practices Assessment Center) experiment was designed as a $2 \times 2 \times 2$ factorial consisting of species (loblolly and slash pine), complete and sustained weed control, and annual fertilization arranged in a randomized split-plot (species as whole plots) design with three replications. This resulted in four treatments within each species: control (C), weed control only (W), fertilizer only (F), and both fertilizer and weed control (FW).

The second rotation experiment (IMPAC II) was overlaid on the same treatment plots of the first rotation treatment plots after the first rotation was harvested in 2009 after 26 years of development. Before harvest, the understory vegetation in the F and C plots were mulched in place to retain this nutrient pool within the treatment plot boundaries. Mulching was not necessary in the W and FW treatments because of the historical weed control treatments used in the previous rotation. The IMPAC II experiment now consists of two randomized complete block designs (RCBD; 3 replications each), having four treatments ($C_C$, $C_F$, $C_{FW}$, $C_W$) for the untreated carryover design and four for the actively managed retreatment design (C, F, FW, and W). The untreated carryover experiment was established on the previous slash pine plots and the actively managed retreatment experiment on the previous loblolly pine plots. In this study, the untreated carryover experiment was allowed to grow without additional fertilizer and weed control treatments. In contrast, the actively managed experiment received the same fertilizer and
weed control treatments in the second rotation as used in the first rotation. A single, full-sib and elite performing loblolly family was used to regenerate the entire study area in December 2009 using containerized seedlings. The initial planting density remained consistent between rotations for both experiments.

Comparisons of total height and current annual increment across rotations indicated that the second-rotation stands were more productive than the first rotation. This study also brings to light the importance that improved cultural practices (e.g., advanced genetics, seedling stock, site preparation), improved nutrient use efficiency, enhanced soil nutrient availability, and environmental factors (e.g., elevated atmospheric CO\textsubscript{2}) can have on inter-rotational differences in site productivity. In the current rotation of the untreated carryover experiment, treatments that received fertilizer in the first rotation (C\textsubscript{F} and C\textsubscript{FW}) accumulated significantly more aboveground biomass compared to the C\textsubscript{C} treatment [i.e. $C\textsubscript{F} (63 \text{ Mg ha}^{-1}) = C\textsubscript{FW} (60 \text{ Mg ha}^{-1}) > C\textsubscript{C} (40 \text{ Mg ha}^{-1})$]. From the third to fourth year, biomass accumulation in C\textsubscript{F} exceeded C\textsubscript{FW}, but by the fifth to seventh years the two treatments were similar; a change that likely occurred because of root development into the lower solum for the C\textsubscript{FW} or increased understory competition in the C\textsubscript{F} treatment. Gradual reductions in early growth differences observed over time between the C\textsubscript{F} and C\textsubscript{FW} treatments in the untreated carryover experiment (Subedi et al. 2014) also suggest a diminishing “assart” effect associated with the C\textsubscript{F} pre-harvest understory mulching treatment. In the actively managed retreated experiment, cumulative total aboveground biomass accumulation followed the trend: FW (90.6 Mg ha\textsuperscript{-1}) > F (71.8 Mg ha\textsuperscript{-1}) > W (55.1 Mg ha\textsuperscript{-1}) > C (31.8 Mg ha\textsuperscript{-1}). Comparison of upper quartile height gains due to fertilization between the first- and second-rotation experiments suggested that fertilizer added in the second rotation only provided growth gains after the fourth year. Thus, managers may consider delaying fertilizer additions until after
establishment in the second rotation. Formulation of future silvicultural practices in southern pine plantations should consider site management history, timing of nutrient amendments, and the role that understory vegetation plays in site nutrient retention and/or as competitors affecting long-term productivity and site sustainability.

The unique design of the study also allowed us to investigate the effect of prior rotation management practices on current rotation soil and heterotrophic respiration. Examination of soil respiration and organic matter decomposition dynamics (important proxies for C dynamics), climate, and nutrient cycling in a second rotation, juvenile loblolly pine plantation in north Florida suggested significant silvicultural treatment effects. Repeated measurements of Rs made over 13 months suggested that both the historical fertilization (C_F, 4.56 μmol CO_2 m^{-2} s^{-1}) and the combined fertilizer+weed control treatments (C_{FW}, 4.49 μmol CO_2 m^{-2} s^{-1}) increased mean Rs by 29% and 27%, respectively, compared to the control (C_C, 3.53 μmol CO_2 m^{-2} s^{-1}). The Rh in the historical weed control treatment (C_W, 2.97 μmol CO_2 m^{-2} s^{-1}) was significantly lower compared to the C_F (4.02 μmol CO_2 m^{-2} s^{-1}) and C_{FW} (3.66 μmol CO_2 m^{-2} s^{-1}) treatments, despite having warmer soil temperatures than the C_F treatment, and no differences in soil moisture contents. Instead, a strong correlation between soil Mn availability and heterotrophic soil respiration (r = 0.66) suggests that organic matter pools accumulated in the previous rotation due to fertilizer treatments may be decomposed more easily in the subsequent rotation because of micronutrient cycling. Decomposition rates for a common substrate were also lower in the C_W compared to the C_F treatment (p = 0.027), potentially because removal of understory plants in the C_W treatment affected Mn cycling compared to the C_F treatment where micronutrients were added as fertilizers. Results also suggested that fertilized treatment increased CO_2 release from the soil in
the successive rotation because of how past silvicultural treatments altered micronutrient cycles and organic matter decomposition dynamics.

A litterbag decomposition study conducted at the IMPAC II study showed that the first rotation fertilization treatments increased litter decomposition in the subsequent rotation. However, litter decay trends among treatments were different in the untreated carryover and retreated experiments. Observed differences in decay rate trends in the FW vs CFW treatments compared to their respective controls occurred because of differences in soil temperature ($r^2 = 0.69$, $p = 0.04$). Fertilization increased P mineralization, but N was mostly immobilized. Immobilization of N during litter decomposition suggested that the recent litterfall pool was a N sink and that litter decomposition alone may not support stand N demands in a nutrient-limited soils. However, fertilization induced increases in litterfall mass and P release rates during litter decomposition, suggesting that litterfall P pools in the CF, CFW, F, and FW treatments were important P sources.

In contrast, pine litter decomposition was lower for plots having a history of sustained understory competition control (CW) compared to the CF treatment. This trend was also present when the weed control treatment (W) was continued into the subsequent rotation. Considering the lower litterfall inputs associated with the CW and W treatments, nutrient cycling rates in these treatments would be expected to be less than those receiving fertilizer treatments. When all data were pooled together, only the lignin:P ratio was a significant modifier of pine litter decay rates ($r^2 = 0.53$, $p < 0.0001$). Pine litter decomposition increased by 20-22% when mixed with gallberry (*Ilex glabra* L.) leaves. Facilitation of pine litter decomposition by gallberry leaves reinforced the potential role that understory vegetation may have on nutrient cycling and mineralization processes in nutrient limited southern pine ecosystems.
An ex-situ incubation experiment using litter and soil suggested that fertilized plots generally resulted in more microbial respiration per g of soil, and likely reflected an inherently higher soil carbon content. When microbial respiration was normalized by carbon concentration, the fertilization plots tended to have lower microbial respiration rates. A nutrient addition experiment further suggested that N+P additions suppressed microbial respiration in the C_F and F treatments, but accelerated it in the C_C and C treatments. This observation suggests that during the early stages of decomposition, N and P were limited to those soils without a silvicultural treatment history. A positive Mn fertilization response for the C_C, C, C_w, W treatments suggested that Mn limitations affected microbial decomposition processes in these soils. Furthermore, in the W plots, Mn additions resulted in higher decomposition of pine litter. Evidence was also presented where activity of the Mn peroxidase enzyme was increased with Mn addition. A strong correlation between Mn peroxidase activity and microbial respiration rates for this Florida Spodosol, therefore, suggests a Mn limitation to enzyme production and subsequent decomposition of soil organic matter.

Many questions regarding the mechanisms controlling the long-term productivity of intensively managed southern pine ecosystems still remain unanswered. Continuous monitoring of growth and yield, soil nutrient supply and stand demand, and the competitive role of understory plants for macro- and micronutrients should help frame improved nutrient management regimes for loblolly pine plantations growing on Spodosols in the lower Coastal Plain. In addition, understanding the main and interactive effects of commonly used silvicultural practices (i.e., fertilization, weed control) on belowground processes, root development, soil nutrient supply, and the understory vegetation community will help refine and improve silvicultural practices accordingly. Similarly, replicated field studies investigating genotype ×
site, genotype × silviculture, and their interaction would prove useful in germplasm deployment considerations necessary to realize the full biological potential of southern pines.

Results from this study indicated a negative effect of repeated herbicide application on organic matter decomposition. On nutrient poor sandy soils, where nutrient mineralization following organic matter decomposition plays a pivotal role in maintaining soil nutrient availability, the role of understory species on nutrient recycling will require further investigation. Differences in nutrient cycling (e.g. N, P, and Mn) potential by understory communities (e.g. graminoids vs shrubs) requires further investigation to understand their effects on organic matter decomposition and mineralization processes for nutrient limited Spodosols. While this investigation characterized differences in background nutrients levels, litter chemistry, and understory vegetation, it remains unclear how microbial community composition responds to these common nutrient manipulation practices in managed forest ecosystems. Future work should also focus on direct assessments of microbial community dynamics and microbial carbon use efficiency in these soils to more fully understand nutrient limitations to C cycling. Because Spodosols cover almost 5.6 million ha in the US South, additional nutrient manipulation studies should also be conducted across multiple sites to investigate if nutrients other than N and P limit C cycling. Finally, additional process driven studies should focus on nutrient effects on microbial community dynamics to disentangle the complex relationship between cultural treatments, organic matter decomposition, soil nutrient availability, and sustained productivity of southern pine ecosystems over multiple rotations.
APPENDIX A
HEIGHT AND DIAMETER EQUATIONS FOR LOBOLLY PINES GROWING AT THE IMPAC II STUDY

Table A-1. Parameters for linear equations to determine tree height for loblolly pine stands growing on the IMPAC II study. Annual inventory data at the IMPAC II study were used for the parameter estimates. All treatments within an experiment were pooled for parameter estimation.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age (yr)</th>
<th>BH Value</th>
<th>P Value</th>
<th>BH Value</th>
<th>P Value</th>
<th>R²</th>
<th>SE</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actively managed retreated</td>
<td>5</td>
<td>0.746</td>
<td>&lt;0.01</td>
<td>0.594</td>
<td>&lt;0.01</td>
<td>0.865</td>
<td>0.076</td>
<td>163</td>
</tr>
<tr>
<td>Untreated carryover</td>
<td>5</td>
<td>0.793</td>
<td>&lt;0.01</td>
<td>0.583</td>
<td>&lt;0.01</td>
<td>0.857</td>
<td>0.072</td>
<td>168</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>6</td>
<td>0.822</td>
<td>&lt;0.01</td>
<td>0.595</td>
<td>&lt;0.01</td>
<td>0.869</td>
<td>0.068</td>
<td>162</td>
</tr>
<tr>
<td>Untreated carryover</td>
<td>6</td>
<td>0.956</td>
<td>&lt;0.01</td>
<td>0.549</td>
<td>&lt;0.01</td>
<td>0.823</td>
<td>0.072</td>
<td>168</td>
</tr>
<tr>
<td>Actively managed retreated</td>
<td>7</td>
<td>0.923</td>
<td>&lt;0.01</td>
<td>0.579</td>
<td>&lt;0.01</td>
<td>0.867</td>
<td>0.068</td>
<td>168</td>
</tr>
<tr>
<td>Untreated carryover</td>
<td>7</td>
<td>1.191</td>
<td>&lt;0.01</td>
<td>0.485</td>
<td>&lt;0.01</td>
<td>0.788</td>
<td>0.074</td>
<td>168</td>
</tr>
</tbody>
</table>

Note: The equation is of the form: ln(y) = β₀ + β₁ ln(x), where y is the tree height (m) and x is the diameter at breast height (cm).
APPENDIX B
ALLOMETRIC EQUATIONS FOR THE ESTIMATION OF ABOVEGROUND BIOMASS COMPONENTS OF LOBLOLLY PINE

Table B-1. Parameters for an allometric equation for estimating foliage, stem wood, branches, and bark for 4-year old loblolly pine stands growing on Spodosols of the southeastern United States.

\[ \ln(y) = \beta_0 + \beta_1 \ln(x) \]

<table>
<thead>
<tr>
<th>Biomass</th>
<th>Age (yr)</th>
<th>Value ((\beta_0))</th>
<th>P-Value</th>
<th>Value ((\beta_1))</th>
<th>P-Value</th>
<th>(R^2)</th>
<th>SE</th>
<th>Observation (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Foliage</td>
<td>4</td>
<td>-1.691</td>
<td>&lt;0.01</td>
<td>1.458</td>
<td>&lt;0.01</td>
<td>0.94</td>
<td>0.27</td>
<td>33</td>
</tr>
<tr>
<td>Stem wood</td>
<td>4</td>
<td>-2.340</td>
<td>&lt;0.01</td>
<td>1.808</td>
<td>&lt;0.01</td>
<td>0.97</td>
<td>0.22</td>
<td>33</td>
</tr>
<tr>
<td>Branch</td>
<td>4</td>
<td>-3.138</td>
<td>&lt;0.01</td>
<td>1.975</td>
<td>&lt;0.01</td>
<td>0.92</td>
<td>0.41</td>
<td>33</td>
</tr>
<tr>
<td>Bark</td>
<td>4</td>
<td>-3.045</td>
<td>&lt;0.01</td>
<td>1.564</td>
<td>&lt;0.01</td>
<td>0.97</td>
<td>0.18</td>
<td>33</td>
</tr>
</tbody>
</table>

Note: y is the biomass component (kg dry wt.) and x is the diameter at breast height (cm). Biomass component data from Colbert (1988) were used.
APPENDIX C
NUTRIENT USE EFFICIENCY INDICES FOR 4-YEAR OLD LOBLOLLY PINES

Table C-1. Mean nutrient use efficiency indices for 4-year-old loblolly pine stands growing on Florida Spodosols at the IMPAC II experiment

<table>
<thead>
<tr>
<th>Experiment Treatments</th>
<th>Nutrient use efficiency index</th>
<th>--- Kg (oven dry wt). kg⁻¹(nutrient)---</th>
<th>Kg (oven dry wt.). g⁻¹ nutrient-</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>P</td>
<td>K</td>
</tr>
<tr>
<td>Actively retreated C</td>
<td>179</td>
<td>2617</td>
<td>787</td>
</tr>
<tr>
<td></td>
<td>(27)</td>
<td>(144)</td>
<td>(179)</td>
</tr>
<tr>
<td>F</td>
<td>197</td>
<td>1930</td>
<td>506</td>
</tr>
<tr>
<td></td>
<td>(18)</td>
<td>(67)</td>
<td>(36)</td>
</tr>
<tr>
<td>FW</td>
<td>185</td>
<td>2072</td>
<td>555</td>
</tr>
<tr>
<td></td>
<td>(20)</td>
<td>(152)</td>
<td>(150)</td>
</tr>
<tr>
<td>W</td>
<td>199</td>
<td>2441</td>
<td>635</td>
</tr>
<tr>
<td></td>
<td>(27)</td>
<td>(212)</td>
<td>(120)</td>
</tr>
<tr>
<td>Untreated carryover Cc</td>
<td>179</td>
<td>2342</td>
<td>642</td>
</tr>
<tr>
<td></td>
<td>(22)</td>
<td>(216)</td>
<td>(32)</td>
</tr>
<tr>
<td>Cf</td>
<td>167</td>
<td>2359</td>
<td>650</td>
</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(302)</td>
<td>(70)</td>
</tr>
<tr>
<td>CfW</td>
<td>175</td>
<td>2067</td>
<td>609</td>
</tr>
<tr>
<td></td>
<td>(4)</td>
<td>(59)</td>
<td>(77)</td>
</tr>
<tr>
<td>Cw</td>
<td>177</td>
<td>2463</td>
<td>602</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(184)</td>
<td>(97)</td>
</tr>
</tbody>
</table>

Note: Standard deviations are provided inside parentheses.
Table C-2. Relative nutrient use efficiency index of 4-year-old loblolly pines in the untreated carryover and actively managed retreated experiments compared to the first rotation IMPAC study

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Untreated carryover</th>
<th>Actively managed retreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td>N</td>
<td>-7.6</td>
<td>-14.6</td>
</tr>
<tr>
<td>P</td>
<td>41.5</td>
<td>17.7</td>
</tr>
<tr>
<td>K</td>
<td>12.6</td>
<td>23.9</td>
</tr>
<tr>
<td>Ca</td>
<td>134.7</td>
<td>70.4</td>
</tr>
<tr>
<td>Mg</td>
<td>127.1</td>
<td>134.2</td>
</tr>
<tr>
<td>S</td>
<td>-14.5</td>
<td>-25.3</td>
</tr>
<tr>
<td>B</td>
<td>35.8</td>
<td>36.9</td>
</tr>
<tr>
<td>Cu</td>
<td>25.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Mn</td>
<td>44.5</td>
<td>-9.2</td>
</tr>
<tr>
<td>Zn</td>
<td>98.6</td>
<td>14.3</td>
</tr>
</tbody>
</table>

Note: First rotation NUE index estimates were reported by Colbert (1988). Relative NUE index (%) for each treatment were estimated as:

Relative NUE index (%) = \( \frac{\text{NUE index}_{\text{second rotation}} - \text{NUE index}_{\text{first rotation}}}{\text{NUE index}_{\text{first rotation}}} \times 100 \)
Figure C-1. P uptake rates for the 2 and 4-year-old loblolly pine stands at the untreated carryover experiment. Error bars represent standard errors. Second year P uptake was estimated by using component tissue concentration and component biomass estimates reported by Subedi (2013).
Figure C-2. Relationships between NUE index and Mehlich III extractable soil nutrient concentration (ppm) in 0-50 cm observed for four-year-old loblolly pine stands growing in the untreated carryover experiment at the IMPAC II study.
APPENDIX D
NUTRIENT CONCENTRATION OF GALLBERRY (ILEX GLABRA L.) LEAVES AT THE IMPAC II STUDY

Table D-1. Nutrient concentration of gallberry leaves in the untreated carryover and actively managed retreated experiment at the IMPAC II experiment.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Untreated carryover experiment</th>
<th>Actively managed retreated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td>N (%)</td>
<td>Mean</td>
<td>1.08</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.01</td>
</tr>
<tr>
<td>P (%)</td>
<td>Mean</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.00</td>
</tr>
<tr>
<td>K (%)</td>
<td>Mean</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.01</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>Mean</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.04</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>Mean</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.02</td>
</tr>
<tr>
<td>S (%)</td>
<td>Mean</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.03</td>
</tr>
<tr>
<td>B (ppm)</td>
<td>Mean</td>
<td>25.32</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>1.59</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>Mean</td>
<td>3.77</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>0.08</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>Mean</td>
<td>65.81</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>17.66</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>Mean</td>
<td>61.70</td>
</tr>
<tr>
<td></td>
<td>SE</td>
<td>18.34</td>
</tr>
</tbody>
</table>

Note: Within each experiment, asterisk (*) after mean nutrient concentration represents significant difference between treatments α=0.05. SE denotes standard error (n=3).
## APPENDIX E

LITTER NUTRIENT CONCENTRATIONS FOR 4-YEAR OLD LOBLOLLY PINE STANDS AT THE IMPAC II STUDY

Table E-1. Macro- and micro-nutrient concentration in the litterfall of 4-year-old loblolly pine plantation in the untreated carryover experiment at the IMPAC II study.

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Untreated carryover experiment</th>
<th>Actively managed retreated experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C&lt;sub&gt;C&lt;/sub&gt;</td>
<td>C&lt;sub&gt;F&lt;/sub&gt;</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>N (%)</td>
<td>0.43a</td>
<td>0.02</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.03b</td>
<td>0</td>
</tr>
<tr>
<td>K (%)</td>
<td>0.02a</td>
<td>0</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.94a</td>
<td>0.07</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.15a</td>
<td>0.01</td>
</tr>
<tr>
<td>S (%)</td>
<td>0.05a</td>
<td>0.06</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>56.3a</td>
<td>10.2</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>157.4b</td>
<td>29.9</td>
</tr>
<tr>
<td>B (ppm)</td>
<td>9.4a</td>
<td>0.3</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>1.1a</td>
<td>0.1</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>24.1b</td>
<td>2.9</td>
</tr>
<tr>
<td>Mo (ppm)</td>
<td>&lt;0.1a</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>N:Mn ratio</td>
<td>28.07a</td>
<td>4.32</td>
</tr>
<tr>
<td>N:P ratio</td>
<td>12.63a</td>
<td>0.34</td>
</tr>
<tr>
<td>Ca:N ratio</td>
<td>1.12a</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Note: For nutrients within each experiment, means followed by same letter are statistically not different at α=0.05. SD represents standard deviation.
### APPENDIX F
MICRONUTRIENT CONCENTRATIONS IN FLORIDA SOILS AND LOBLOLLY PINE FOLIAGES

Table F-1. Average micronutrient concentration (ppm) in soils (except for Florida soils, which is concentration range in ppm) and loblolly pine foliage in Florida. Standard deviations are provided inside parentheses. BD denotes below detection. ‘NA’ indicates not available.

<table>
<thead>
<tr>
<th>Sites</th>
<th>Horizon</th>
<th>B</th>
<th>Cu</th>
<th>Mn</th>
<th>Mo</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMPAC II soil*</td>
<td>A</td>
<td>3.7 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.4 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.6 (0.3)</td>
</tr>
<tr>
<td>(Gainesville, FL)</td>
<td>E</td>
<td>3.7 (0.4)</td>
<td>0.2 (0.3)</td>
<td>0.3 (0.2)</td>
<td>0.2 (0.5)</td>
<td>0.7 (0.6)</td>
</tr>
<tr>
<td></td>
<td>Bh</td>
<td>3.6 (0.3)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.1)</td>
<td>0.1 (0.1)</td>
<td>0.3 (0.2)</td>
</tr>
<tr>
<td></td>
<td>E2/Bt</td>
<td>3.6 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.1)</td>
</tr>
<tr>
<td>Bunnell soil (Vogel and Jokela, 2011)*</td>
<td>A</td>
<td>1.1 (0.10)</td>
<td>0.3 (0.0)</td>
<td>0.1 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.8 (0.0)</td>
</tr>
<tr>
<td>(Bunnell, FL)</td>
<td>E</td>
<td>1.2 (0.20)</td>
<td>0.2 (0.0)</td>
<td>0.1 (0.0)</td>
<td>0.0 (0.0)</td>
<td>0.7 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Bh</td>
<td>0.6 (0.0)</td>
<td>0.2 (0.0)</td>
<td>BD</td>
<td>BD</td>
<td>0.6 (0.0)</td>
</tr>
<tr>
<td></td>
<td>E2/Bt</td>
<td>0.6 (0.0)</td>
<td>0.2 (0.0)</td>
<td>BD</td>
<td>BD</td>
<td>0.6 (0.1)</td>
</tr>
<tr>
<td>SSIGNS soil*</td>
<td>A</td>
<td>BD</td>
<td>0.1 (0.0)</td>
<td>0.7 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.5 (0.1)</td>
</tr>
<tr>
<td>(Gainesville, FL)</td>
<td>E</td>
<td>BD</td>
<td>0.1 (0.0)</td>
<td>0.3 (0.1)</td>
<td>0.0 (0.0)</td>
<td>0.2 (0.0)</td>
</tr>
<tr>
<td></td>
<td>Bh</td>
<td>BD</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.0)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.1)</td>
</tr>
<tr>
<td></td>
<td>E2/Bt</td>
<td>BD</td>
<td>0.1 (0.1)</td>
<td>0.2 (0.2)</td>
<td>0.1 (0.1)</td>
<td>0.1 (0.0)</td>
</tr>
<tr>
<td>Florida soils (Chen et al. 1999)*</td>
<td>A</td>
<td>NA</td>
<td>0.22-21.9</td>
<td>1.74-236</td>
<td>0.13-6.76</td>
<td>0.89-29.6</td>
</tr>
<tr>
<td>IMPAC II foliage*</td>
<td>8.24 (1.10)</td>
<td>1.9 (0.9)</td>
<td>114.2 (19.3)</td>
<td>0.1 (0.1)</td>
<td>27.2 (2.6)</td>
<td></td>
</tr>
<tr>
<td>Bunnell foliage (Vogel and Jokela, 2011)*</td>
<td>15.00 (NA)</td>
<td>2.6 (NA)</td>
<td>159.0 (NA)</td>
<td>1.5 (NA)</td>
<td>39.9 (NA)</td>
<td></td>
</tr>
<tr>
<td>Reported foliar critical levels (Jokela, 2004; Others)</td>
<td>4-8</td>
<td>2-3</td>
<td>20-40</td>
<td>0.10</td>
<td>10-20</td>
<td></td>
</tr>
</tbody>
</table>

*a* Mehlich 3 extractable soil nutrient concentration in the controls of second rotation 2 year old loblolly pine plantation, *b* Mehlich 3 extractable soil nutrient concentration (Vogel and Jokela, 2011), *c* Mehlich 3 extractable soil nutrient concentration in 2 year old loblolly pine plantation, *d* HNO₃-HCl-HF digested soil nutrient concentration range across florida soils representative of all soil types (Spodosol- 28%, Entisols- 22%, Ultisols-19%, Alfisols- 14%, Histosols- 10%, Mollisols- 4%, and Inceptisols- 3%) (Chen et al., 1999), *e* Foliar nutrient concentration in 4-year-old untreated loblolly pine, *f* Foliar nutrient concentration (Vogel and Jokela, 2011)
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BIOGRAPHICAL SKETCH

Praveen Subedi was born in Pokhara, Nepal in 1986. After graduating from high school, he joined the Institute of Forestry for his undergraduate degree in 2004. In 2009, he received a Bachelor of Science in forestry degree from the Institute of Forestry, Tribhuvan University, Nepal. In 2010, he joined the School of Forest Resources and Conservation at the University of Florida to pursue his graduate study. In 2013, he received Master of Science degree in Forest Resources and Conservation from the SFRC at the University of Florida. In the fall of 2013, Praveen joined the Ph.D. program at the University of Florida pursuing his interests in mechanistic understanding of ecological functions in managed plantations of the US South. He received his Ph.D. from the University of Florida in the summer of 2019.