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The Effects of Prescribed Fire on Microbial Biomass Abundance in Longleaf Pine Ecosystems

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by

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ABSTRACT

One of the most diverse ecosystems, longleaf pine (*Pinus palustris*) habitats comprise only a small fraction of the habitat they once spanned in the Gulf Coastal Plain Region of the Southeastern United States. To preserve longleaf pine ecosystems, proper prescribed burning techniques and ecosystem management are essential for preventing high intensity wildfires and increasing species diversity. Little is known about the effects of prescribed fires on carbon levels, nitrogen levels, and microbial biomass in longleaf pine forests. These components are crucial for determining how well burned areas are recovering at a microbial level compared to unburned areas. The greater the microbial abundance and diversity that returns to soils after a burn can indicate greater relative recovery rates, making them important to analyze over time. Previous studies have described varied results on the effects of prescribed fires on carbon and nitrogen levels, but their effects on microbial biomass in longleaf pine forests is understudied, presenting a knowledge gap in this area of study. We studied the effects of prescribed fire on levels of soil carbon, nitrogen, and fungal biomass (as ergosterol) prior to a prescribed burn and 2, 3, and 4 months post burn. Samples were collected from 12 burned sites and 12 unburned control sites inside and outside of cattle plots, encompassing 188 total samples. It was hypothesized that carbon and nitrogen levels would increase three weeks after the prescribed burn in burned plots and decrease in the following two- and four-month sample collections. Soil fungal biomass (ergosterol) levels were expected to increase directly after the burn and decrease in the following two- and four-month samples in burned plots. Unburned control plots were expected to have similar levels, dipping three weeks post fire. Effects of the fire were assessed using a two-factor ANOVA. The results demonstrate that carbon levels (which were lower in burned plots prior to the burn) spiked directly after the burn and continually decreased after two months. Nitrogen levels decreased overall, and fungal biomass levels initially spiked, decreased, and then increased again after four months post burn. These results help bridge the knowledge gap on how prescribed burns affect fungal biomass levels and contribute more data to the varied results of research on carbon and nitrogen levels following prescribed burns. Management plans should consider these effects when planning intervals between prescribed fires, as well as what kinds of fires would better benefit longleaf pine ecosystem conservation and restoration.

Keywords: Longleaf pine, *Pinus palustris*, fungal biomass, ergosterol, prescribed fire, conservation, restoration, ecosystem management

DEDICATION

This paper is dedicated to all my friends and family who supported me and aided me throughout my thesis process. Thank you all for believing in me and helping to push me to do anything that I feel might be impossible. This paper is also dedicated to my grandparents, especially my Mère. Even though you all may not be here to see it, thank you for inspiring my love of the world and pushing my love for the sciences.

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DF	Degrees of Freedom
HPLC	High-Performance Liquid Chromatography
LTEC	Lake Thoreau Environmental Center
USM	The University of Southern Mississippi
LS Means	Least Means Square

INTRODUCTION

The longleaf pine tree (*Pinus palustris*) plays a critical role in the longleaf pine ecosystems of the Southern Coastal Plain Region of the United States. Historically, longleaf pine ecosystems were predominantly monotypic stands of *P. palustris* and a few other scattered herbaceous species, such as *Ilex glabra* and *Vaccinium spp*. Longleaf pine forests once spanned a large majority of North America. Now, their range has dwindled to roughly 10% of the estimated 37-38 million hectares they once occupied (Longleaf Pine, n.d.). This decrease is mostly attributed to burning prevention, human settlement, and logging that occurred after the start of the 19th century. Regular burning of these forests is important to maintain plant diversity as well as prevent the accumulation of fuel on the forest floor. Without them, specific species would take over (*Ilex glabra* for example) and decrease overall diversity. Additionally, an accumulation of fuel can lead to more dangerous natural fires. Economic impacts and a decrease in biodiversity have accompanied these anthropogenic impacts, drastically affecting many species that depend on these ecosystems.

One of the most diverse ecosystems outside of the tropics is the longleaf pine ecosystem in the Coastal Plain Region, hosting 309 narrowly endemic taxa across five regions (Weakley & Sorrie, 2006). Likewise, an estimated 900 plant, 100 bird, 36 mammal, and 170 reptile and amphibian species reside in longleaf pine ecosystems (Natural Resource Conservation Service, 2020). This ecosystem includes various habitats ranging from savannas to forests to wet flatlands. Additionally, *P. palustris* is deemed a keystone species due to its continuing relationship with naturally occurring fires. Many endemic species in these ecosystems are also keystone species, such as the gopher tortoise

(*Gopherus polyphemus*), whose burrows upon which many other species rely as their habitat. The continuing destruction of longleaf pine ecosystems (directly and indirectly) has contributed to a trophic cascade of decreased biodiversity across species. Despite the continual decrease in biodiversity over the years, restoration efforts have been identified and implemented to help preserve existing remnant longleaf pine ecosystems.

Previous restoration efforts included planting other pine species, such as loblolly pines (*Pinus taeda)*. However, this was found to be as harmful as clear-cutting the area because longleaf pine trees were displaced by the loblolly pines, further limiting their future growth. Current restoration efforts for longleaf pine ecosystems revolve around re-establishing a fire regime, which is usually manmade and monitored. Historically, low-intensity fires would burn through longleaf pine ecosystems every few years, contributing to a mainly clear canopy and herbaceous understory (Natural Resources Conservation Service, 2023). Adapted to recurring fires, longleaf pine trees and seedlings can outcompete other species in the event of a burn (Brethauer et al., 2021). Re-establishing a burn cycle limits the encroachment of numerous hardwood species, allowing longleaf pine seedlings to settle and thrive instead of competing for space. Implementing a consistent burn regime also gives native plants an opportunity to grow instead of nonnative invasive plants, increasing biodiversity.

Prior research has explored the effects of prescribed burning in longleaf pine ecosystems on the different tree species that inhabit the areas, as well as the effects of different intensity fires on various pine ecosystems across the globe. However, little research has analyzed the effects of prescribed fire on microbial diversity, abundance, and microbial biomass in longleaf pine forests, presenting a knowledge gap that is important to understand the collective effects of prescribed fire on longleaf pine ecosystems and its potential long-term effects on other species.

Microbial biomass is the measure of microbial or fungal abundance in soil. For the purposes of restoration and conservation, it is crucial to analyze the living microbial abundance after a fire to get a representative picture of returning fungal populations. As microbial abundance and diversity increase, the health of the soil can too. If microbial abundance decreases, it can show that the applied restorative regime may be causing harm. Measuring living fungal biomass can be done through determining the presence of ergosterol. As a result, measuring ergosterol presence is one of the main focuses of this study.

In the present study, I analyze the effects of prescribed burning on fungal biomass in longleaf pine ecosystems with the goal of understanding the soil microbial effects that this restoration practice has on this habitat. Soil samples were collected from multiple burned and unburned plots over a four-month period at Lake Thoreau Environmental Center. Soil fungal biomass concentrations were analyzed via the extraction and quantification of ergosterol (Gessner, 2020). It was hypothesized that fungal biomass diversity would decrease directly after prescribed fire but drastically increase two months after burning due to the increased nutrients in the soil from the burning of herbaceous material and lower competition with other microbial species. Carbon and nitrogen levels were expected to experience a significant increase post-fire and decrease throughout the 4-month sampling period.

LITERATURE REVIEW

Longleaf Pine Overview

Longleaf pines (*Pinus palustris*) are evergreen conifers, which can grow up to 125 feet tall and produce 6-8 inch long cones (UFIFAS, n.d.). These trees can live to be hundreds of years old and are considered a pioneer species. *P. palustris* typically grows in warm, wet temperate climates but can live in a wide variety of sites (Boyer, n.d.). Historically, their range spanned from Texas through Florida and Virginia (Brockway and Lewis, 2003). Within these regions, *P. palustris* prefers drier, more acidic soils, and needs a large amount of sunlight to grow. These pine trees are resistant to fire, depending on recurring burns for the regulation of their growth cycle.

Once dominating 60 million acres, longleaf pine forests hold great importance both in species richness and economic impact (Croker, 1998). The management of longleaf pine ecosystems is seen as critical to many conservationists, as they have the greatest diversity in temperate North America (Mitchell et al., 2006). Housing over 1,200 species, many of which are endemic, longleaf pine forests are considered biodiversity hotspots (Longleaf Pine Initiative, 2020). The ongoing decrease in longleaf pine forest ecosystems has caused species who rely on this environment to decrease in number, creating a negative chain reaction within these ecosystems. Rebuilding these ecosystems helps ensure that biodiversity increases and is maintained over time.

Historical and Ecological Significance

Longleaf pine forests also have historical significance, having greatly contributed to the naval industry (Outcalt, 2000). When settlement began, longleaf pines were mainly harvested for lumber, causing minimal damage to the existing ecosystems since the harvesting was conducted on a need-based scale. Beginning in the 19th century, longleaf pines were tapped and harvested to produce various pine products, such as pine tar, rosin, pitch, and turpentine (Oswalt et al. 2012). The trade of these products contributed to an economic upturn for early English settlers and increased the need for pine trees and pine products. As more settlements arose and trade increased, railroads were installed to move people and goods around the country. Once the railroad systems were created, harvesting of the pines and their derived materials increased as well. The settlement, trade, and construction of the railroads, fragmented existing longleaf pine ecosystems. However, the harvesting of the trees only contributed to a fraction of the damage done to longleaf pine ecosystems.

Longleaf pine ecosystems now cover less than ten percent of their original area (Longleaf Pine, n.d.). Their decrease in range has caused them to become one of the most endangered terrestrial ecosystems in the United States (Zampieri & Pau, 2022). Currently, longleaf pine trees exist in eight states in the southeastern portion of the U.S., 50% of which are in Florida (Zampieri & Pau, 2022). Their habitats are highly fragmented, contributing to multiple species' high-priority conservation status (Oswalt et. al, 2012). Most of the damage done to longleaf pine ecosystems can be attributed to the disruption of their natural burn regime by human settlers. Once the trees are harvested or burned, some longleaf pines are replaced by other species, further decreasing their range. Previous regeneration efforts included planting loblolly and slash pines, which counteracted restoration by limiting the potential growth areas of *P. palustris*.

Relationship With Fire and Restoration

Fire is intricately linked with the lifecycle of longleaf pine trees. Before the 1800s, Native Americans in various regions used prescribed burns to clear the understory of longleaf pine forests and agricultural land (Fowler & Konopik, 2007). When Native Americans were forced to relocate and more people settled near longleaf pine forests throughout the 1800s, the human influence on burning patterns were interrupted. However, Scottish-Irish settlers in south Mississippi continued to burn these forests to improve pasturage for open range cattle farming. In the early 20th century, fire suppression became the official policy of state and federal land management agencies. The Southern Forestry Education Project in the late 1920's and early 1930's involved sending special trucks equipped with movie projectors out to small towns in the Gulf and southern Atlantic Coastal Plains to show films promoting fire suppression. Nicknamed the 'Dixie Crusaders', these members of the American Forestry Association set the tone for future nationwide fire suppression campaigns such as Smokey Bear (Rooney, 1993).

As time went on, prescribed burning was too limited out of fear of further destroying the longleaf pine forests. The decrease in fire played a large role in the decrease of longleaf pine forests (Brethauer et al., 2021). Decreased burning led to the increased growth and spread of various hardwood species. This competition stunted the growth of longleaf pine seedlings as well. Unmonitored hardwood species pose a threat because they lead to more frequent and severe fires, as they catch fire quickly and provide more fuel when burns occur (Mitchell et al., 2006).

Today, prescribed fire is considered by many conservationists to be an effective way to combat many invasive species and ensure the success of longleaf pine seedlings in longleaf pine forests (see, Tomat-Kelly et al., 2021). Longleaf pine seedlings are resistant to fire, having the ability to stay in their grass stage for up to fifteen years (Brethauer et al., 2021). Additionally, longleaf pines are resistant after a few years of being in their bottlebrush stage because by this stage, they have grown tall enough to withstand the fires (Brethauer et al., 2021). After prescribed burns are conducted, the seedlings and younger trees have less competition for resources because they undergo less damage from the fire than other species. Prescribed burning is one of the few events that longleaf pine ecosystems need to remain a keystone habitat. These burns leave behind bare mineral soil, which is also crucial for the growth of longleaf pine seedlings, and without fire they are less likely to germinate (Longleaf pine: When fire fuels a forest, n.d.).

Figure I. Image of a longleaf pine ecosystem taken at LTEC.

Effects of Prescribed Fire on Microbes and Soil

Many studies have been conducted on the effects of prescribed burning on longleaf leaf pine ecosystems, but few studies have examined how these fires affect the ecosystem on a microbial level. Fires have varying effects on soil microbial communities, and these effects are based on the intensity of the fires, duration of the fires, and fire regime (frequency or return interval) (Fox et al., 2022). The environment can also be impacted by the burns and shifting nutrient levels resulting from the fires.

Soil composition is impacted by prescribed fires, indirectly influencing fungal communities. Fire increases soil pH and can cause immediate decreases in nitrogen and carbon levels through combustion reactions (Fox et al., 2022). However, after burning, nitrogen and phosphorus levels have the potential to rise as well. These changes in elemental composition are different in each soil horizon, greatly impacting the litter and topmost horizon (Fox et al., 2022). Both nitrogen and phosphorus are essential nutrients in the overall growth and life cycle of soil fungi. Alongside these chemical changes in the soil, fungal communities can be more directly affected by the effects of prescribed fire on their habitats.

Prescribed burning can also impact fungal habitat and nutrient availability, usually decreasing overall living fungal biomass and increasing necromass (Fox et al., 2022). Increases in the mortality of various bacterial species and protists can also occur, decreasing fungal competition, which can further increase biomass (Fox et al., 2022). Previous researchers have found that to maintain microbial biodiversity, frequent, low-intensity fires should be conducted, decreasing competition without greatly increasing the mortality of numerous fungal species (Fox et al., 2022).

Overall, fungal diversity has been found to increase with pyrodiversity, which helps maintain various fungal traits across ecosystems (Fox et al., 2022). Frequent burns also have the potential to drive fungal selective pressure, selecting for fungi that thrive at higher temperatures (Fox et al. 2022). Short-term effects of fire on microbes have been well studied; however, a few studies have examined the effects of prescribed fire in the long-term within various seasons following the burns (Hopkins et al., 2021, Semenoa-Nelson et al., 2019).

Potential long-term effects of prescribed burns on fungi include an overall decrease in nutrients and a change in fungal microhabitat, which favors fire-resistant fungi (Hopkins et al., 2021). Due to the lack of long-term research on the impact of burn regimes on fungal communities in longleaf pine ecosystems, the long-term effects these burns may have on the whole ecosystem are not well understood. Understanding these effects is essential for expanding knowledge on fire ecology and developing the most effective maintenance and restoration processes for longleaf pine ecosystems. By analyzing the effects of prescribed fire on fungal biomass abundance over a span of four months, this research will help bridge this knowledge gap. I hypothesize that fungal biomass would decrease directly after prescribed fire, and then increase after two months due to increased nutrient availability.

METHODS

Site Selection and Soil Sampling

This experiment was conducted between March 2023 and January 2024 at the Lake Thoreau Environmental Center (LTEC) owned by The University of Southern Mississippi (USM). This experiment occurred on the Longleaf Preserve (LLP) in Lamar County near Hattiesburg, Mississippi. The upland portions of the LLP are forested by stands of longleaf pine naturally regenerated after a clearcut in 1916. This uneven-aged forest has trees ranging in age from 1 year old to 120+ years old. In 2009, prescribed fire was reintroduced into three longleaf pine stands in the LLP after a 25+ year absence. Since 2009, prescribed fires have been administered every 1-2 years with the most recent fire occurring in March 2023. Three stands were left unburned so that comparisons could be made with respect to prescribed fire. In 2012, over 300 permanent vegetation data collection points were established throughout the LLP. Twelve of these were selected for long-term vegetation monitoring, six in burned stands and six in unburned stands. At each of the 12 points, four 2m x 2m groundcover plots were established. Soil cores for this study were collected at each groundcover plot (47 plots total; one plot was decommissioned).

Soil cores were collected at each groundcover plot at three different dates: pre-burn, 3 weeks post burn, 2 months post burn and four months post burn. Soil cores were collected using a 1-1/8” x 12” nickel plated, slotted soil probe (AMS, Inc., American Falls ID). Soil cores were collected in butyrate plastic liners (1” x 12”, AMS, Inc., American Falls ID) capped, labeled, and placed into ice chests for transport back to the lab. Soil cores were stored in freezers until further analysis. A total of 188 soil cores were collected.

Soil Analysis

Soil cores were partially thawed, and the soil was extracted from each core using a smaller PVC pipe to push out the top 10 cm of soil. Each 10 cm core was cut in half longitudinally, and each half was placed into an 18 oz. sample bags (Whirl-Pak, Pleasant Prairie, WI). Each bag was labeled with the location, date, and any other defining characteristics of the soil core. After processing, the frozen samples were lyophilized to dryness, then homogenized using a rubber mallet to crush the dried soil within the sample bags.

Ergosterol analysis: Fungal biomass was estimated using the concentrations of ergosterol, a membrane sterol in fungi (see Gessner, 2020). For each sample, 200 mg of lyophilized dried soil was added to a 50 mL plastic centrifuge tube, and ergosterol extracted in 10 ml of alcoholic KOH (0.8% KOH in HPLC grade methanol) for 30 minutes at 80°C. The resultant crude ergosterol extract was then partitioned into n-pentane, evaporated to dryness with nitrogen gas using a N-EVAP, resuspended in methanol, and quantified using a Shimadzu high-performance liquid chromatography (HPLC). Two ergosterol spiked control samples were also extracted and processed to estimate percent recoveries.

Carbon and nitrogen analysis: For each sample, 20 mg of soil was encapsulated (i.e., enclosed) into 8 x 5 mm tin capsules to determine carbon and nitrogen concentrations. The soil encapsulated within the tin capsule was folded and molded into a small ball. Each encapsulated ball was then stored in a 96 well polystyrene assay plate until analyzed. Carbon and Nitrogen was then analyzed using a Costech 4010 elemental combustion analyzer (Costech, Valencia, Cailifornia) following the manufacturer protocols.

Data Analysis

Ergosterol (µg/g dwt), carbon (% C), and nitrogen (%N) data were analyzed separately using a two-way ANOVA with date and burn treatment as fixed factors and location as a block effect. Results were considered significant $\alpha \leq 0.05$. Analyses were conducted using JMP software version 17.2.0.

RESULTS

Soil Carbon

As shown in *Table I*, there was a significant effect of burn treatment on % C (p=0.0011). The block effect (the 12 different plot types) was also significant (p=0.0002). Soil % C did differ among dates. Overall, the mean % C was 21.7% lower in the burned samples than the unburned samples (*Table II* and *Figure I*). Demonstrated in *Figure II*, carbon was lower in burnt samples before the prescribed fire, two months after the prescribed fire, and four months after the prescribed fire. Three weeks after the prescribed fire, the average carbon weight was significantly higher in the burned versus unburned plots.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Date	3	3	0.881110	0.4819	0.6954
Block[Burn Treatment]	11	11	23.554263	3.5136	0.0002*
Date*Burn Treatment	3	3	2.5561	2.5561	0.0577
Burn Treatment	1	1	11.0705	11.0705	0.0011*

Table I. ANOVA Effect Tests results for the average carbon weights with block type, date, and burn treatment as covariates.

Level	Least Sq Mean	Std Error	Mean
Burned	1.3650910	0.09216376	1.39667
Unburned	1.8691559	0.12023733	1.78333

Table II. Least Means Square table comparing average carbon weights of unburned and burned plots.

Figure II. Effect of burn treatment on % C demonstrated in Least Means Square chart. This demonstrates the percentage of carbon in both unburned and burned plots.

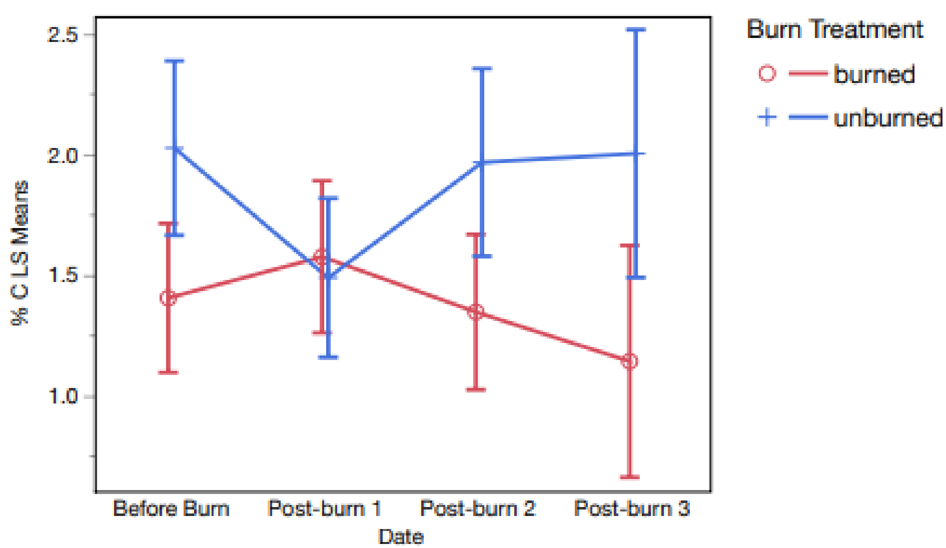

Figure III. Effect of season on % C. This demonstrates the percentage of carbon across pre-burn collections and three weeks, two months, and four months post-burn collections.

Average Nitrogen Weight Analysis

A significance difference was observed between the average nitrogen weight and block type ($p<0.0001$) and burn treatment (0=0.0459, *Table III*). These results aligned with the carbon results, showing no significance in the date and the date combined with the burn treatment. The average nitrogen weight was 11.8% lower in the burned samples compared to the unburnt samples (*Table IV* and *Figure III*). Unlike the average carbon weights, the nitrogen amounts were the same prior to the burn, and steadily decreased in the burned samples (*Figure IV*). There was a decrease in nitrogen three weeks after the fire in the unburned samples, which spiked in the two month and four-month post-burn collections, returning to normal levels.

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Date	3	3	0.00378465	2.1727	0.0939
Block[Burn Treatment]	11	11	0.02735040	4.2822	<.0001*
Date*Burn Treatment	3	3	0.00224165	1.2869	0.2813
Burn Treatment	1	1	0.00235479	4.0555	0.0459

Table III. ANOVA Effect Tests results for the average nitrogen weights with block type, date, and burn treatment as covariates.

Level	Least Sq Mean	Std Error	Mean
Burned	0.04835689	0.00284478	0.049524
Unburned	0.05777393	0.00371132	0.056154

Table IV. Least Means Square table comparing average nitrogen weights of unburned and burned plots.

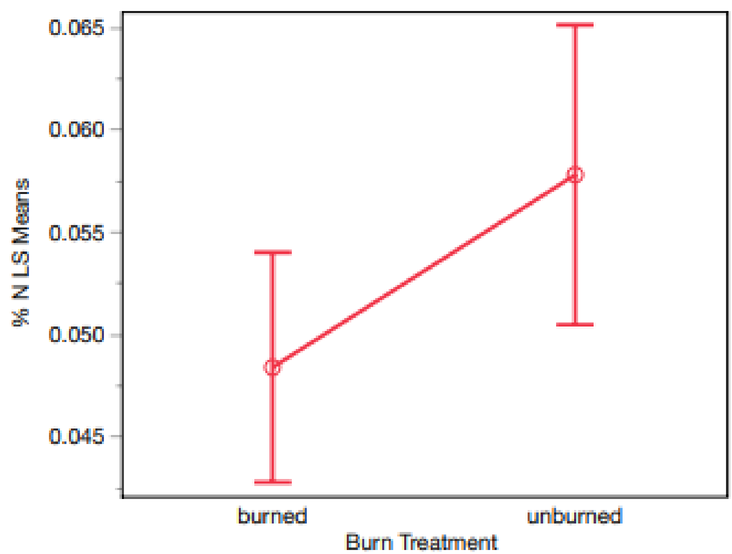

Figure IV. Effect of burn treatment on % N demonstrated in Least Means Square chart. This demonstrates the percentage of nitrogen in both unburned and burned plots.

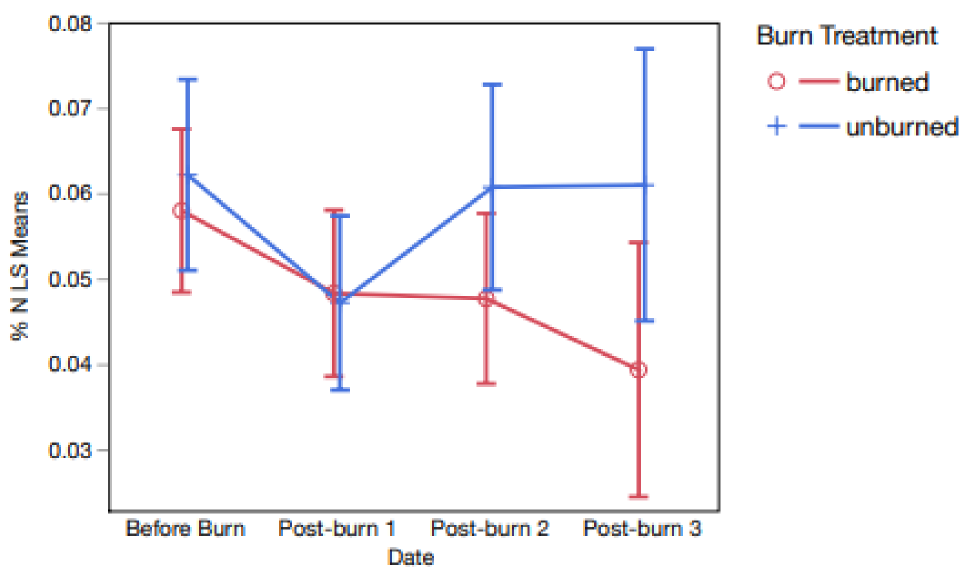

Figure V. Effect of date on % N. This demonstrates the percentage of nitrogen across pre-burn collections and three weeks, two months, and four months post-burn collections.

Ergosterol Analysis

As shown in *Table V*, a significant difference was observed between fungal biomass (ergosterol) present and the date (p=0.0079), block type (p= 0.0162), and the burn treatment (p=0.0046). There was no relationship found between date and burn treatment. The soil fungal biomass (ergosterol) present was 32.8% higher in unburned compared to burned plots (*Table VI* and *Figure V*). Fungal biomass gradually increased in the unburned plots. In the burned plots, fungal biomass spiked three weeks after the prescribed fire, decreased two months after the prescribed fire, and spiked once more after four months (*Figure VI*).

Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Date	3	3	71.04124	4.1068	0.0079*
Block[Burn Treatment]	11	11	140.99650	2.2230	0.0162*
Date*Burn Treatment	3	3	24.63872	1.4243	0.2382
Burn Treatment	1	1	47.90787	8.3086	0.0046*

Table V. ANOVA Effect Tests results for ergosterol present in each sample with block type, date, and burn treatment as covariates.

Level	**Least Sq Mean**	**Std Error**	**Mean**
Burned	2.4304916	0.28348916	2.23271
Unburned	3.7736936	0.36984144	3.23377

Table VI. Least Means Square table comparing ergosterol present in unburned and burned plots.

Figure VI. Effect of fire on soil ergosterol content demonstrated in Least Means Square chart. This demonstrates the amount of ergosterol present in both unburned and burned plots.

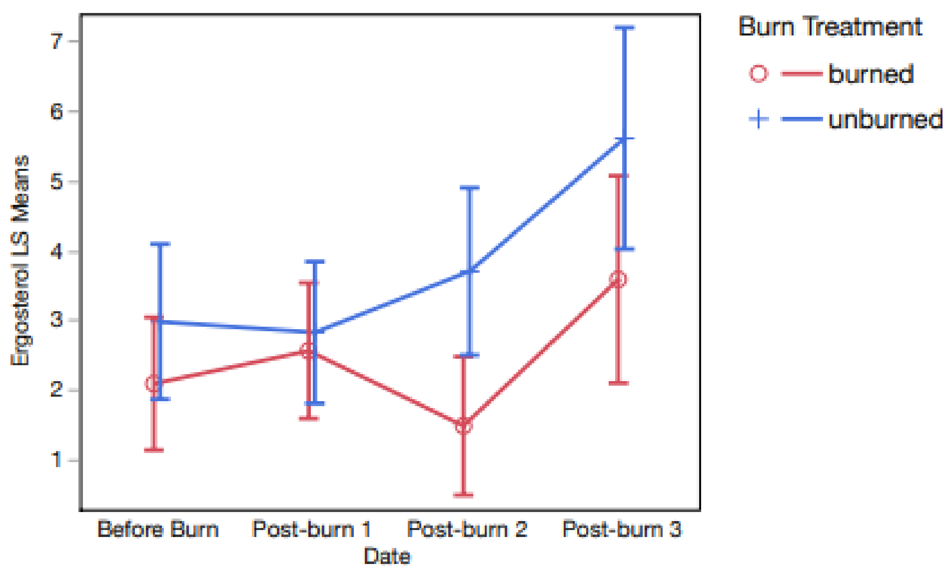

Figure VII. Effect of date on soil ergosterol content. This demonstrates the amount of ergosterol present across pre-burn collections and three weeks, two months, and four months post-burn collections.

DISCUSSION

Determination of Results

I hypothesized that carbon and nitrogen levels would significantly increase immediately after post fire and decrease with the post burn 2 and post burn 3 collections in burned samples. Fungal biomass was expected to increase directly after the prescribed fire due to increased carbon levels and decrease with post burn 2 and post burn 3 collections in burned samples. Unburned samples were expected to have relatively consistent carbon, nitrogen, and ergosterol levels, dipping in the spring.

As seen in *Figure IV*, nitrogen levels were similar in pre-burn and post-burn 1 plots (3 weeks post fire) regardless of burn treatment. This was expected, as three weeks post fire was during spring, so unburned plots should hypothetically decrease in nitrogen levels as plants begin to focus on upward growth. However, in post burn 2 (2 months post fire) and post burn 3 (4 months post fire) plots, average nitrogen weight increased in unburned plots, returning to normal levels. Burned plots decreased steadily in nitrogen since the plants are replacing all parts with little decomposing, focusing on much more than upward growth. These findings are consistent with the hypothesis made for unburned plots. The hypothesis made for burned plots was not supported by these results, as nitrogen levels did not experience an increase directly after the prescribed fire.

Carbon levels mainly come from three different processes: fine root turnover, above ground decomposition, and soluble carbon deposition. Carbon levels for unburned plots were higher in pre-burn samples than in the burned plots. This is likely because a burn was conducted every year for the past three years in accordance with a separate experiment on *Ilex glabra* (gallberry). As a result, the burned plots had less time for carbon to return to normal levels. As expected, carbon levels spiked in post burn 1 and decreased in post burn 2 and 3 in burned samples, supporting the previously made hypothesis (*Figure II*). Additionally, unburned samples also aligned with the hypothesis, dipping in the spring due to upward growth and increasing in post burn 2 and post burn 3 samples as carbon levels returned to normal. This is consistent with research done on low-intensity fires as there are more burned fragments in the soil, increasing total carbon levels (Scharenbroch et al., 2012). However, this research is still debated because the results vary with differing fire severity.

Fungal biomass (ergosterol) levels were lower in the pre-burn samples in burned plots compared to the unburned plots. This is likely due to the small period between prescribed burning as well. However, ergosterol levels in the unburned samples steadily increased over time; whereas ergosterol spiked in post burn 1 samples, decreased in post burn 2 samples, and increased once more in post burn 3 samples (*Figure VI*). This does not support the previously made hypothesis because ergosterol levels were expected to decrease for a longer period after the prescribed fire.

Literature Consistency and Research Importance

It has been found that carbon levels are expected to decrease after prescribed fires if they burn with a high intensity (Alcañiz et al., 2018). With fires of lower intensity, carbon levels may increase over time (Alcañiz et al., 2018). Literature results vary depending on fire intensity specifically, but the results may also vary due to variation in soil organic matter, which depends on topography, fire intensity, fire type, and ecosystem type (Alcañiz et al., 2018). Even so, carbon levels will instantly spike after a prescribed fire due to the burning itself, regardless of the carbon levels measured over longer periods of time. Carbon levels depend on the recurrence of prescribed fires as well as the intensity of these fires. However, one study conducted over thirty years found that yearly prescribed burning decreased overall carbon levels in loblolly and longleaf pine forests (Binkley et al., 1992). This thesis supports the findings of Binkley et. al (1992) because carbon levels decreased overall in post burn 2 and post burn 3, and these plots have been burned yearly for three years. Due to these findings, it could be preferable to increase time between burnings to allow carbon levels to return to normal, which is beneficial to determining fire intervals when constructing forest management plans.

Prior research conducted on nitrogen levels has been relatively inconsistent, more so demonstrating that nitrogen is positively affected by prescribed burning (Alcañiz et al., 2018). For instance, some studies found that nitrogen levels increase in soil after prescribed burning (Alcañiz et al., 2016). However, some researchers have found that nitrogen levels decrease in areas where prescribed burning happens more frequently (Muqaddas et al., 2015). As a result, frequency of prescribed burning seems to play a major role in whether nitrogen levels will continually increase or decrease after a prescribed fire. This thesis supports two research studies demonstrating that reoccurring fires every year led to decreased total soil nitrogen in burned plots (Muqaddas et al., 2015; Blakenship and Arthur, 1999). As the research supports the idea that nitrogen levels mainly increase, this thesis disagrees with this notion, solidifying why there is variance in the results as nitrogen levels steadily decreased after the initial post burn collection. This is important because it demonstrates the need for more long-term studies on soil nitrogen levels in areas with reoccurring prescribed fires as there is no solid consensus about nitrogen levels, which is necessary for proper burn management.

Collectively, these findings are important in future efforts to decide what time variation is best between prescribed fires. As the research shows, nitrogen levels may need less frequent burning to return to normal levels, such as four years in-between (Muqaddas et al., 2015). However, this could be attributed to the burning of organic materials on the forest floor which take significant time to return (Alcañiz et al., 2018). Burning plots when it is wet or replacing some of the organic materials if feasible (such as a small-scale burn), which may mitigate some of the nitrogen loss caused by recurrent fires over short periods of time.

The effects of prescribed burning microbial abundance and biomass are understudied, especially in longleaf pine forests with recurrent burning. Most studies have focused on the effects of high intensity fires in areas where prescribed burns are not recurrent. A study done in 2007 discussed this knowledge gap, and touches on how mycorrhizal abundance and diversity is suggested to reduce with long-term burning (Cairney and Bastias, 2007). However, the authors also state more research should be done before assuming this suggestion is correct. Another study suggests that prescribed burning generally leads to a decrease in microbial biomass (Fox et al., 2022). As a result, this research helps to fill a knowledge gap and is important in showing that fungal biomass (ergosterol) does spike immediately after prescribed fire and begins to increase quite rapidly around the four-month margin, seemingly reaching normal amounts quicker than carbon and nitrogen levels likely due to animals killed during the burn and fresh foliage. This information can help better understand the fire ecology and management of longleaf pine ecosystems.

Data Gaps and Future Research

While this research thesis provided insight into the effects of prescribed fire on fungal biomass, nitrogen, and carbon with short term intervals between burning in longleaf pine ecosystems, certain aspects of the experimentation process should be improved or built upon in future studies. When analyzing the ergosterol amounts in each soil sample after pentane extraction, our HPLC instrument failed. Consequently, 27 samples (mainly from post burn 3) were missing. Additionally, two samples were missing from the carbon and nitrogen analysis due to errors in soil processing. While it is unlikely that this had a large effect on the data, inclusion of these samples may have changed some significant values in the variables tested like date.

Additionally, factors such as burn intensity or soil variation were not measured. Specifically, the soil to sand ratio was not accounted for, and soil variation has the potential to affect carbon and nitrogen levels (Chowdhury et al., 2022). Knowing this information in future experimentation on prescribed burning could provide useful insight into how burn intensity or soil variation contributes to effects on carbon, nitrogen, and ergosterol levels over time. Future experiments should collect post burn samples for longer than four months to achieve a more representative picture of how yearly prescribed burns affect nutrients and ergosterol over time. Additionally, data should be collected on phosphorous in the soil samples and fungal DNA should be analyzed. This data would aid in looking at the effects of prescribed burning on fungal community diversity. Lastly, as these samples were collected inside and outside of cattle plots, the relative locations should be tested in the future to determine if the presence of cattle influences these results.

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