FINAL REPORT

Title: The consequences of soil heating for prescribed fire use and fire restoration in the South

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List of Abbreviations/ Acronyms

Rs	Soil respiration (μmol CO_2 $m^{-2}·sec^{-1}$)
OSBS	Ordway-Swisher Biological Station
ACF	Austin Cary Forest
LONG	long-unburned units
FREQ	frequently burned units
INTER	intermediate frequency units
FOFEM	First Order Fire Effects Model
TR	Trenched
NT	Not-trenched
B	Basal plot location
D	Dripline plot location

Acknowledgements

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Abstract

Soil heating resulting from prescribed burning in the southern US has potential immediate and long-term impacts. Where fire is being restored to long-unburned sites, the duration and depth of soil heating may be substantial, affecting seed banks, soil carbon cycling, and root and rhizosphere systems with often severe repercussions for overstory tree survival. Where fire has been used frequently, effects on soil quality are assumed benign, but this is not empirically proven. Current understanding of the relationships between fuels, prescribed burning, and soil heating is limited in southern pine ecosystems, even though the region burns a higher percentage of its forests than anywhere else in the US. To fill this knowledge gap, we characterized the relationships among fuels, fire, and soil heating in two widespread forest types of the South: pine flatwoods and pine sandhills. Second, to quantify ecological effects with clear management implications for ecosystem sustainability, we evaluated soil heating impacts on tree stress, vegetation, and soil respiration. Below-ground soil processes are integrated in soil respiration, which reflects the combined biological and physical consequences of soil heating and is a critical component of ecosystem carbon budgets. Overstory tree retention and diverse understory vegetation are principal goals for restoration of these ecosystems and are likely to be influenced by soil heating. We respond to research needs identified in previous studies by quantifying the consequences of soil heating across contrasting soils and fuel conditions. Our approach enables us to provide recommendations to managers and answer the question, ***"Under what conditions should fire managers in the South be concerned about soil heating, and why?"***

Objectives

This project addresses the research needs identified within *JFSP 15-1-05 Funding Opportunity Notice* in that it "Examines the effects of soil heating on second-order fire effects" and evaluates fire behavior and fuels consumption in relation to soil heating and its impacts. Our work characterizes the link between prescribed fire and soil heating under different burning, fuel, and soil conditions in southern pine forests, where little research has been conducted on these important relationships. Second, we examine soil heating effects on multiple second-order effects, including soil respiration; soil C and N; vegetation communities, seed banks; and tree stress/mortality.

The primary question our research addressed was: *"**Under what conditions should fire managers in the South be concerned about soil heating, and why?**"* The following objectives guided our work:

Objective 1) To examine the patterns of prescribed burning-induced soil heating and fuels across two ecosystems, two fire frequencies, and two within-stand locations (basal and open) using multiple *in situ* prescribed fire experiments

Objective 2) To examine ecological responses to soil heating under different fuel conditions including soil respiration, tree stress and mortality, and vegetation response

Objective 3) To evaluate the accuracy of existing soil heating models under different fuel conditions and evaluate current knowledge on how soil temperatures correspond to changes in soil biological activity and survival, to improve capacity for use in fire management and planning using predictive models.

There were multiple changes in three of the PIs' affiliations that delayed the project, along with the accidental burning of two of our original sites and state-wide bans on prescribed burning that limited our ability to fully address each of our original objectives. Our substantial supplemental work made up for these changes. This additional work includes an analysis of tree stress (Slack et al. 2016), contribution to two major state-of-the-science reviews on post-fire tree mortality (O'Brien et al. 2018, Hood et al. 2018), surprising patterns of grass flowering following soil heating in our sites (Shearman et al. 2019), and the first-ever systematic review of scientific literature evaluating soil biological response to soil heating (Pingree and Kobziar 2019).

Background

While many of the South's fire-adapted ecosystems evolved in the context of frequent fire, widespread fire suppression activities to protect human interests has shifted the responsibility of fire's role into the hands of natural resource managers. Prescribed burning attempts to restore or replicate historical fire regimes in ecosystems where its natural occurrence has been disrupted due to fragmentation, land use change, expansion of the wildland-urban-interface, or fire exclusion (Ryan et al. 2013). In the South, prescribed fire can be applied across large areas for relatively low cost (~$30/acre) and can be used where topography or lack of access limit mechanical fuels reduction. In addition to its fuel-reduction effects, in most of the ecosystems of the South, prescribed fire plays a critical role in promoting ecological function (e.g., nutrient cycling, seed scarification, stimulation of food plants for wildlife) and maintaining fire-adapted biological communities (Means 2006, Ryan et al. 2013). These attributes make prescribed burning a popular tool for southern land managers, who in 2011 reported burning over 6 M acres, *ca.* 80% of the national total for non-agricultural prescribed burn area (Melvin 2012).

The majority of pinelands across the South are characterized by short fire return intervals, ranging from 1-5 years for pine uplands, flatwoods, and sandhill communities. Fires that burn less frequently lead to undesirable outcomes due to rapid recovery of encroaching shrubs and hardwood trees, development of ladder fuels, and closed canopies increasing potential carbon loss from wildfire, depletion of functional group diversity in understories, and forest floor accumulation resulting in tree stress or mortality when burned (Varner et al. 2005; Glitzenstein et al. 2012). In roughly half of the existing remnant southern pine ecosystems, fire exclusion has lasted several times the historical fire return interval (Outcalt 2000), raising management concerns about the ecological implications of restoring fire as an ecosystem process (Varner et al. 2005). Alternatively, where fire has been frequently applied over extended periods of time, current understanding reveals inconsistent consequences for ecosystem productivity, carbon flux, soil nutrients, and vegetation (Clark et al. 2004, Godwin 2012, Lavoie et al. 2014, Wiggers et al. 2013). Across the range of fire intervals employed by managers in southern pinelands, evaluation of fire's effectiveness in meeting objectives has primarily focused on above-ground indicators of ecosystem structure and function, which tend to be more easily measured. Yet belowground systems (including soil flora and fauna, roots and rhizosphere symbionts, and nutrient, hydrologic, and seed reservoirs) are fundamental to the restoration of ecosystems (Neary et al. 1999).

The differential consequences of frequent vs. infrequent prescribed burning on belowground southern pinelands has received little research attention, and the degree to which aboveground fuel consumption drives soil heating consequences is unknown. As these relationships can inform management decision- making regarding how and when fire can be successfully applied toward long-term ecosystem maintenance, we present basic research to address soil heating effects on key ecosystem components and processes including soil carbon respiration (Rs), tree stress and mortality, and vegetation species richness. Fire's direct bearing on belowground systems reflects the interaction of aboveground fire reaction intensity with soil characteristics (e.g., texture, moisture content), which drive the degree to which soil is heated along with the duration of heating (Neary et al. 1999). Lower fuel moisture contents, higher ambient air temperatures, and greater available fuels typically result in higher reaction intensity, while fuel packing ratio and moisture content can have mixed effects (Rothermel 1972). To capture variability in and quantify these relationships, we employed a sampling design across sites with contrasting fire return intervals (low, mid, high frequency). As a function of soil qualities (i.e. moisture content, texture, and thermal properties), increased fuel consumption presumably leads to increased soil heating; we quantified this relationship across two dominant contrasting soils- one with a spodic horizon and one without. The positive relationship between soil heating and tree stress is mediated through direct injury to tree root systems (Varner et al. 2009) and potential decreases in water and nutrient availability (O'Brien et al. 2010); while increased root damage decreases soil respiration and may increase respiration sensitivity to temperature (Boone et al. 1998). Finally, the direct relationships among soil heating, soil carbon efflux, and vegetation response are harder to predict given the lack of primary literature from research in the region- we aim to fill these important knowledge gaps for these ecosystems.

Material and Methods

Study Areas and Ecosystems

Both research study sites were in north central Florida where prescribed fire is a dominant tool for forest management. The two ecosystems we evaluated represent some of the most fire-prone and common community types found in the Southeastern Coastal Plain: pine sandhills and pine flatwoods. Flatwoods are located on poorly drained sites typically underlain by Spodosols. Stand structure typically varies from 100-300 trees/ha, with a flammable, continuous vegetative understory component of saw palmetto (*Serenoa repens*) and gallberry (*Ilex glabra*), and an overstory which is generally composed of pines, including both longleaf pine (*Pinus palustris* Mill.) and slash pine (*Pinus elliottii* L.). Sandhills are xeric, well-drained sites with a more open stand structure (30-100 trees/ha). Sites have a diverse herbaceous understory dominated by wiregrass (*Aristida beyrichiana*) which plays an important role in fire spread for this ecosystem. The overstory component of sandhills is dominated by longleaf pine (*Pinus palustris* Mill.). Characteristic site images are shown in Figure 1.

Figure 1. Representative conditions in long-unburned and frequently burned longleaf pine forests in sandhill and flatwoods ecosystems, Gainesville and Melrose, FL.

Both study sites are owned and managed by the University of Florida (Gainesville, FL, USA). The flatwoods site in this study is The Austin Cary Memorial Forest (ACMF), an ~7000-acre teaching and experimental forest located near Waldo, FL, USA, (29° 44' N, -82° 14' W) just north of Gainesville. This site primarily contains flatwoods with variable prescribed fire regimes ranging from annual to long-unburned (>50 years without fire), and is generally flat with no perceptible slope. Soils within this site are Spodosols: poorly drained, sandy, siliceous Hyperthermic Ultic Alaquods of the Pomona series. We utilized an annually burned area (10 ha) which has been burned yearly since 1980, a triennially burned area (30 ha) which has been burned since 1980, and a long-unburned site with no fire for > 50 years. Units ranged from 5-10 ha in size.

The sandhill study site was the Ordway Swisher Biological Station (OSBS) located just east of Melrose, FL, USA (N29° 40' W81° 74'). Soils at the Ordway Swisher Biological Station are excessively drained Quartzipsamments (Readle 1979), and topography generally flat. We conducted our burns in a long-unburned unit (48 years since last fire) utilized in earlier JFSP-funded work (Varner et al. 2009). Adjacent to this unit are two units that represent near-annual (1-2 years between fire) and frequently burned (range 3-5 years between fires) with approximately a decade or more of these frequencies being maintained.

The climate of this region consists of hot, humid summers, short winters, and a mean annual temperature of 20 °C. Annual precipitation is 1200 to 1500 mm, somewhat equitably distributed through the year but concentrated in the summer months. Although these are the average climatic conditions, observed weather during our major burn window was unusual. According to a Fire Weather Outlook produced by the Florida Forest Service, the spring of 2017, "…*will go down as one of the driest on record*," (FFSFWO 2017). The record-breaking lack of rainfall, exacerbated by some of the warmest observed temperatures ever recorded for this time of year, resulted in widespread wildfires, and subsequently caused numerous burn bans across most of Florida. Heavy rainfall then swiftly mitigated widespread drought conditions in the beginning of June, leading to flooding. This time is marked as when the rainy season effectively began for this year, with some areas of Florida receiving more than 254 mm of rain in one day on June 6 (FFSFWO 2017).

Study Design and Treatments

We built on existing datasets from experiments previously conducted in long-unburned sites at the OSBS (Varner et al. 2009, Gill et al. 2012, Kreye et al. 2012), and on sites where Rs has been evaluated at ACF (Godwin 2012). In both the flatwoods and the sandhill sites, our original study design included three fire frequency treatment units: frequent, intermediate, and long-unburned. In each fire frequency treatment unit nine similar (age, diameter at breast height, total height, stature) longleaf pine trees were randomly selected. Circular 2 m diameter plots were established around the base of each pine (using the tree as the center point, "*basal*") and nine beyond the crown drip line (using rebar as a center point when soil collar not present "*dripline*"). Because two of the original units were accidentally burned prior to being equipped for assessing soil heating, we replaced two units with similar units representing two of the three fire frequencies: intermediate and frequent. Due to burn bans followed by extraordinary precipitation during our burn windows, we were limited to burning two of the treatments only in each site: long-unburned and frequently burned (N = 4 units). The remaining units, as well as those accidentally burned, were still utilized for soil respiration and vegetation analyses (N = 8 units). Units were burned within a four-month early growing season period in 2017 when conditions were favorable for fuel consumption (i.e. at least one day after a precipitation event) and maintaining control of the burns. The long-unburned flatwoods unit at the Austin Cary Forest, ACF-L, was burned over two days due to logistical and safety constraints given the extremely hazardous fuel loading and continuity (Figure 1). Fire weather conditions for each burn are detailed in Table 1.

Table 1 – Weather, fire behavior, and surface fuel and soil moistures during 2017 experimental burns in frequently burned and long-unburned longleaf pine sandhill (Ordway-Swisher) and longleaf pine flatwoods (Austin Cary) sites in north Florida, USA.

		Fire Weather				
Site	Date	Temp	RH	Wind	Flame Length	Time[ǂǂ]
	Time[ǂ]					
		°C	*%*	*m sec^{-1}*	*m*	*min*
OSBS-F	23 Jun 14:45	33	41	0-0.5	1.0-6.0	1-3
OSBS-L	20 Jun 14:55	35	49	0-0.5	0.3-1.3	1-11
ACF-F	10 Apr 12:34	26	57	0.9-1.5	0.2-0.6	8-10
ACF-F	10 Apr 14:08	27	52	0.9-1.8	0.3-0.6	2-3
ACF-F	10 Apr 16:36	27	47	0.9-1.8	0.3-0.6	5-10
ACF-L	7 Mar 11:53	25	49	0-1.3	0.5-1.0	5-10

ACF-L	7 Mar 16:13	26	49	0-1.3	0.2-0.3	2-3
ACF-L	10 Mar 11:11	28	72 54[1]	0.8	0.8-1.5	7-14
ACF-L	10 Mar 16:05	29	55	0.6	1.0-1.5	5-20

OSBS: Ordway-Swisher Biological Station; ACF: Austin Cary Forest; F: frequently burned; L: long-unburned; ǂ Ignition time; ǂǂ Duration of flaming at plot locations, [1] RH dropped to 54% within an hour after ignition.

Soil Heating

To measure soil heating, we inserted thermocouple probes at the base of mature (25-40 cm DBH) longleaf pines ("basal" locations, 15 cm from the bole), and in plots beyond their dripline ("open" locations). Open locations were established within 10 m of one or more of the basal locations, to enable concurrent burning of the group of plots to control for differences in environmental and soil conditions. Each probe consisted of a 36 cm metal shaft encased in fiberglass (to limit heat transfer), and five exposed Type K, 20 AWG fiberglass-sheathed thermocouple wires (Omega Engineering, Stamford, CT, USA) attached to the probe at the following sampling depths: mineral soil surface (0 cm) and at 5, 10, and 20 cm below. We inserted three thermocouple probes at each basal location, at 0, 120, and 240° azimuths (Fig. 2), and one at each open location. Nine basal and nine open locations were selected in each unit for a total of 36 basal and 36 open sampling locations across the study. Thermocouples were attached to CR1000 data loggers via AM 16/32B multiplexers (Campbell Scientific Inc., Logan, UT, USA) for data acquisition. Temperatures were measured at 1 Hz and averaged over 5-min. intervals due to storage capacity of data loggers and expected long-duration monitoring expected.

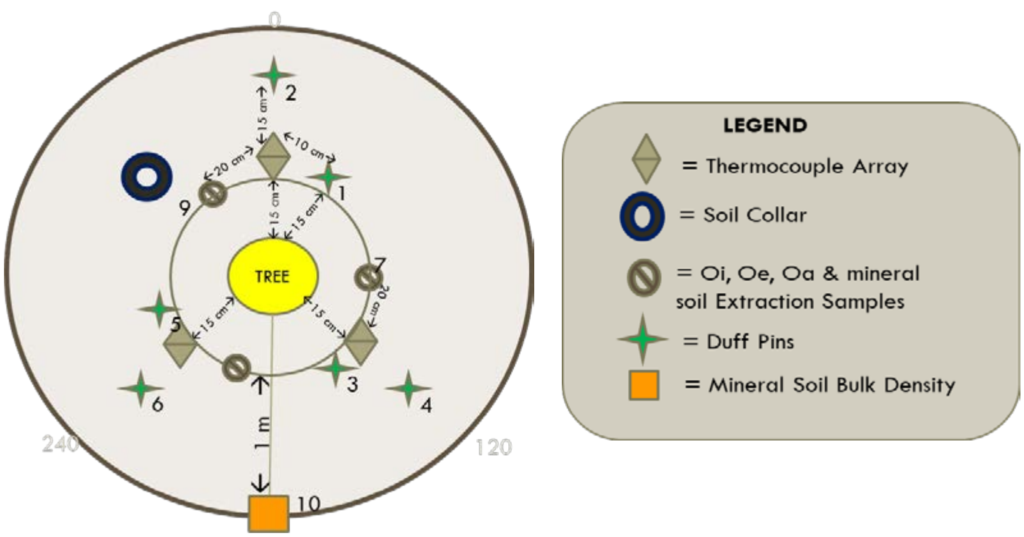

Figure 2. Basal soil heating sampling schematic. Overall plot is 2 m in diameter.

Organic and mineral soil horizons were sampled just prior to burning to quantify day-of-burn moisture contents, with three samples were extracted at each plot location (Fig. 2). Organic horizons were separated into litter (Oi), fermentation (Oe) and humus (Oa) and mineral soil samples were separated into four strata (0-2.5, 2.5-7.5, 7.5-15, and 15-25 cm deep) such that midpoints represented thermocouple locations. At basal locations, samples were extracted 15 cm from tree bases; litter and duff using a 50 mm dia. corer, mineral soil using a 25 mm corer). To limit the effects of extractions on forest floor combustion, extraction locations were offset 20 cm from thermocouple probes (Figure 2) and litter and duff were extracted from nearby trees to replace litter and duff removed from experimental trees. Extractions were taken at open plot locations in the same manner but radiated from plot center rather than tree bases. One mineral soil sample was also extracted using a bulk density sampler at each plot 100 cm away from either tree bases or open plot centers and collected to determine dry soil mineral bulk density. Litter and duff moisture were determined gravimetrically after oven drying at 70 °C until no further weight loss occurred (24+ h). Mineral soil samples were oven-dried at 105 °C for 24 h to determine bulk density and moisture contents of each horizon. Soil moisture was determined gravimetrically using mineral soil, and subsequently converted to volumetric moisture content for modeling.

Forest floor consumption was determined via measurements of consumption pins inserted prior to burning at each plot location. Three consumption pins were inserted 15 cm away from tree bases but were offset 10 cm from thermocouple probes on the opposing side from moisture content extraction locations (Figure 2). Pins were also inserted at open plot locations radiating from plot centers. All consumption pins were inserted flush with the surface of the duff. Litter depths (top of litter to top of duff) were measured at all consumption pin locations. We measured the depth of duff consumed at all pin locations following burns when smoldering had ceased. We subsequently measured the depth of duff remaining at each consumption pin at the flatwoods sites in order to determine the proportion of duff consumed. Consumption pins were removed from the sandhill sites before we could measure remaining duff at those locations.

FOFEM Model Comparisons

The First Order Fire Effects Model (FOFEM) is widely used to predict impacts of burning on soil heating. We compared the soil temperatures observed from our burning experiments with values predicted from the FOFEM fire effects model (Lutes 2019). We examined FOFEM predictions for soil heating at soil heating locations in the flatwoods sites in this study where both duff depth consumed and duff remaining were measured, and where the most substantial soil heating occurred. Model inputs were customized from measured data and default values used where data were otherwise unknown. Measured values used as inputs included duff loading, duff depths, duff moisture, and soil moisture. Batch processing was used in FOFEM to predict maximum soil temperatures across all our soil probe thermocouple locations across study sites, fire regimes, and thermocouple locations. We selected the “Southeast” region for modeling, which relies on a duff consumption model (equation 16 in FOFEM, Lutes 2019) from Hough (1978) that predicts duff consumption from pre-burn litter, woody fuel, duff mass, and duff moisture content. Variables used as inputs to the model from measured values at our sites included litter mass, duff mass, duff moisture, duff depth, and volumetric soil mineral content. Where duff was absent, and the no-duff model is used for soil heating prediction (i.e. litter and herbaceous fuel consumption drives soil heating), herbaceous fuel loading was assumed from the longleaf pine 2-year rough cover type in FOFEM (1.2 tons/ac). The flatwoods and sandhill sites were modeled using “spring” and “summer” seasons in FOFEM, respectively, given the times of experimental burns. We used the “natural” fuel category and the “coarse-loamy” soil family. Output data resulting from batch processing in FOFEM includes maximum temperatures at the soil surface and at 2, 4, and 6 cm depths. Given that we measured soil heating at 0, 5, 10, and 20 cm soil depths we compared observed maximum temperatures at 5 cm with FOFEM predicted maximum temperatures at 4 cm. We did not compare results at the soil surface given that our data were measured at 1 Hz and averaged over 5 minutes for recording; we likely did not observe maximum temperatures at the surface due to residence times, especially where duff was absent.

Soil Respiration (R_s*)*

A complete factorial study design was established for evaluating soil respiration patterns in response to the treatments. Soil respiration (R_s) plots were located within the larger 2 m diameter plots and consisted of 20 cm diameter PVC soil collars. In four units, two flatwoods and two sandhills, soil plots were trenched down to 1 m deep to disarticulate autotrophic respiration from heterotrophic respiration. Control plots (“No Burn” in Table 2) were established to control for the influence of seasonal fluctuations in soil respiration in detecting a treatment effect. Table 2 shows the soil respiration plot replication for each trenched unit (n = 3 per treatment, with two treatments (burn/no-burn and TR/NT) at two locations (basal/dripline), making n=24 soil plots in total for each unit, within 5 trenched units).

Table 2. Factorial design for soil respiration, soil moisture, and soil temperature measurements conducted in all units once a month from January 2016-December 2017.

Treatment	Burn	No Burn
Trench	*Trench + Burn (n=3 basal)* Trench + Burn (n=3 dripline)	*Trench + No Burn (n=3 basal)* *Trench + No Burn (n=3 dripline)*
No Trench	No Trench + Burn (n=3 basal) No Trench + Burn (n=3 dripline)	*No Trench + No Burn (n=3 basal)* *No Trench + No Burn (n=3 dripline)*

In addition, three additional units had the same study design minus the trenching treatment (n=18). *Figure 3* shows an example of plot distributions in a given unit where trenching was established.

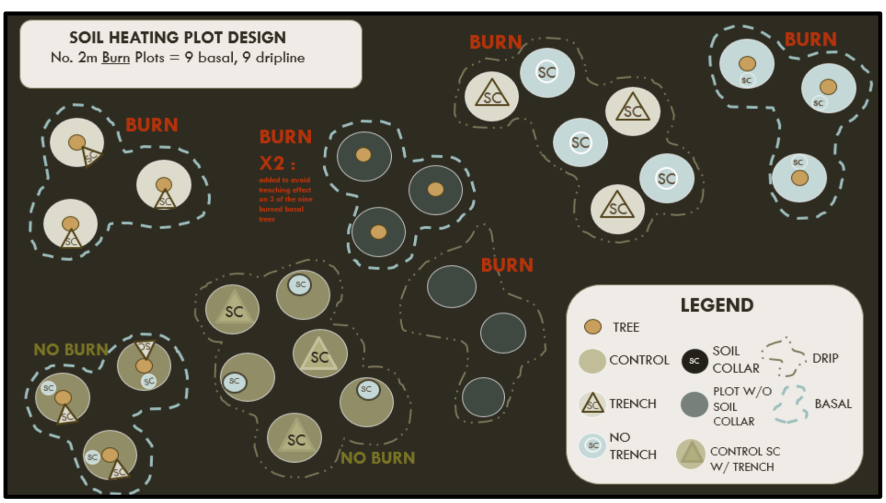

Figure 3. Soil heating and soil respiration study design in each unit. Smaller circles represent trees surrounded by a 2 m diameter plot. There were 18 total R_s plots in non-trenched units, while trenched units (shown here) had a total of 24 R_s plots.

Trench establishment began in the ACCI unit of ACMF in late-October of 2015, and the last trench was finished in mid-December of 2015. Trenches were dug in a triangular shape to a depth of 1m, with dimensions of 1 × 1 × 1 m. Three-five layers of root growth-preventing landscaping fabric were inserted into the fissure. Plants that established in this trenched zone were carefully removed monthly prior to R_s sampling. Three months were allowed prior to the initiation of sampling for severed roots to decompose.

Monthly measurements of soil respiration (R_s) were co-located with soil temperature and soil moisture measurements in both locations: basal (within 30 cm of tree bases), and open locations (i.e. away from any tree canopy dripline). PVC soil collars with dimensions 21.34 cm outside diameter and 11.43 cm tall, were installed at a depth of approximately 8 cm at least 4 weeks prior to R_s measurements for post-installation disturbance to normalize. Using the LI-8100a (LI-COR Inc, Lincoln, NE, USA; Figure 4), R_s measurements (µmol CO_2 m^{-2} sec^{-1}) were recorded monthly pre-burn, immediately prior to prescribed burns, weekly post-burn for five weeks, then returning to a monthly basis. Control plots were measured concurrently throughout. Background levels for R_s at ACMF were obtained from a previous study (Godwin 2017); whereas those for OSBS were obtained by establishing plots in fall of 2015 and taking monthly measurements for at least one-year post-installation following the same normalization period.

Figure 4. Using the LICOR Biosciences LI-8100a (LI-COR Inc, Lincoln, NE, USA), just before the prescribed burn in the OSBS frequently burned unit. Components of the device include: (A) 20-cm soil survey chamber, (B) Omega 8831 type-E soil temperature probe, (C) Decagon Systems EC-5 soil moisture probe (behind temperature probe), (D) LI-8100a device, and (E) 20-cm PVC soil collar.

To account for diurnal variations and capture a representative rate of respiration, measurements were recorded at random plot locations between the hours of 1100 and 1500 EST (Godwin 2017). Concurrently with R_s measurements, ambient soil temperature at 10 cm (°C) [T_s] and soil volumetric water content [M_s] at 5 cm depth is recorded onboard the LI-8100a. T_s is measured using an Omega 8831 type-E T-Handle temperature probe, and M_s is recorded using a Decagon Systems EC-5 probe (Omega Inc., Stamford, CT, USA; Decagon Systems Inc., Pullman, WA, USA). Probes were inserted at random azimuths 5 to 15 cm away from the soil collar and left undisturbed throughout the duration of the 120 sec R_s measurement and 35 sec post-purge period.

Data were subset and tested with generalized linear mixed models (GLMMs) in order to determine the influence from fixed (soil moisture, soil temperature, fire return interval, location-basal or open, and trenching) and random (time, unit) factors. Due to the lack of replication on the site (flatwoods and sandhill pine) and fire return interval (FRI) levels, all analyses were conducted at the unit level to represent the combination of site and FRI. Two units were represented in the flatwoods frequent and intermediate FRI sites and the factor of unit was included in GLMM models. Where necessary, soil respiration rates were transformed and outlier observations deleted in order to meet assumptions of normality. Predictor variables were tested for multicollinearity and regression residuals were tested for assumptions of normality and homoskedasticity before model comparisons with ANOVA. All statistical analyses were conducted in R (R Core Team, 2018) with the *lme4* package (Bates et al., 2015) and assuming a Gamma distribution with a log-link function.

Vegetation Community Composition

Figure 5. Understory vegetation plots (1 m × 1 m) established at basal locations in the ACF- Long Unburned unit, Gainesville, FL.

Vegetation was assessed at both the basal and dripline locations using two 1 m × 1 m sub-plots per tree or dripline location (Figure 5). Two vegetation plots were established, with one selected at random and the other 180° opposite. The dripline location plots were established at random azimuths from plot centers. In each 1 m × 1 m sampling area, we recorded species, maximum height (cm), Daubenmire cover class, % bare ground, and % of the plot covered in litter. Species richness was compared using ANOVA among fire return intervals, locations (basal or dripline), and ecosystem type (flatwoods or sandhill). All data were tested for assumptions prior to analysis.

Seed Banks

After our initial seed bank germination experiment failed to produce any germinating individuals, we conducted a second seed bank extraction across all units to determine whether soil heating influenced survival and germination of seeds banked in the forest floor and soil. On 27 November 2017 we collected volumetric soil and duff seed bank samples using a 10 × 40 cm bulb planter and trowel from 8 units. Five of these units were located at Austin Cary Forest (ACF-NF, ACF-AFR, ACF-AINT, ACF-NINT, ACF-LONG) and three sites were in Ordway Swisher Biological Station (OSBS-FR, OSBS-INT, OSBS-LONG). The sample locations were randomly associated with two unsampled trees in each site.

Two types of samples were taken at each unit: tree and dripline. Thus, a total of 16 samples were examined in this study. Immediately after collection, the samples were transported to the laboratory in separate sealed plastic bags and cold-stratified at 3.3 °C for 11 months. After stratification, we thoroughly mixed each sample and removed a subsample of 500 ml for germinating in trays (23 cm × 46 cm) using Fafard potting mix in a greenhouse (Figure 6).

Figure 6. Seed bank samples being germinated in germination trays. University of Florida School of Forest Resources and Conservation, Milton, FL.

For germination, we evenly spread the soil seed sample over the mix. Additionally, we placed three trays containing only the Fafard germination mix as the experimental control. The trays were kept in a single tier on iron benches at a height of 1 m and arranged randomly to minimize biases caused by small variations in light and moisture intensities. Trays were watered daily with clear water using a gentle shower head to avoid soil disturbance. We monitored soil moisture in the germination trays using soil moisture sensors. Seedlings were identified to species as they emerged, counted and removed on a biweekly basis for 6 months.

Individual tree responses to soil heating

We capitalized on the initial JFSP-funded soil heating treatments at OSBS LONG (Varner et al. 2009). For each of these 100 pines, we extracted two cores and measured growth as basal area increment and tree defenses via measurements of resin ducts (size and area, Figure 7). We used GLMMs to determine the effects of smoldering duration in the initial fall 2003 burns on the intervening growth (cm^2 yr^{-1}). Core samples were mounted, sanded, and cross-dated for annual resolution of pre- and post-burn ring widths (mm yr^{-1}). Resin duct measures were made using scans of sanded cores followed by ImageJ software analyses. Within each annual ring, we counted the number of resin ducts, their area (mm^2) and then calculated resin duct production (ducts yr^{-1}), mean duct size (mm^2), and total resin duct area (mm^2).

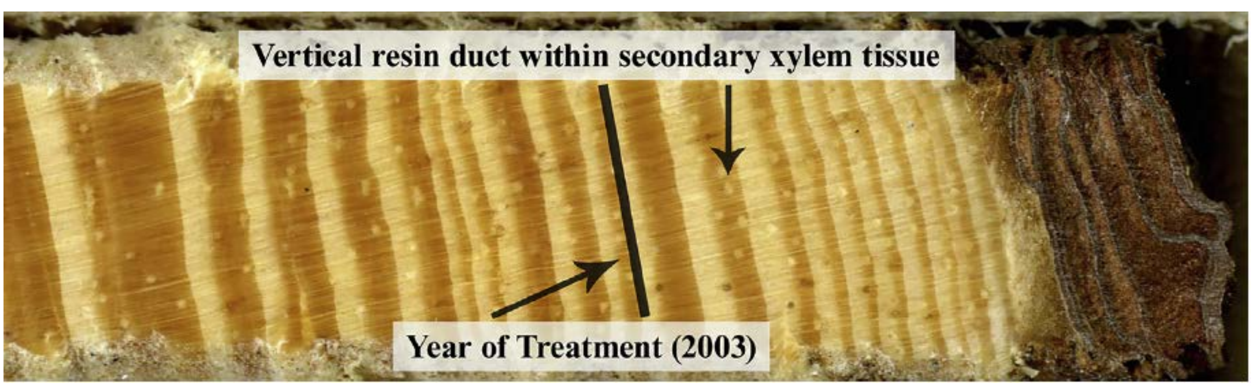

Figure 7. A photograph of a sampled longleaf pine core illustrating resin ducts. The initial reintroduction burn is denoted with the black line. We measured post-fire growth, resin duct production, size, and area. Photograph from Slack et al. 2016 *Forest Ecology and Management*.

Biological Thresholds of Soil Heating

Soil heating caused by prescribed or wildland fire commonly focuses on a single biological thermal threshold of 60 °C for one minute to represent organism death (e.g., displayed as a red line in FOFEM predictions), however this metric severely misrepresents the heterogeneity of the soil environment, the physiological attributes and tolerances of organisms, and the complexity of heat transfer through soils. The disparity between assumed biological responses and the diversity of biological responses after wildland fire events led us to a thorough review of soil biological heating thresholds to help support our interpretation and conclusions drawn from the results of these soil heating experiments. We conducted a systematic review of published literature which included controlled experiments on a variety of soil biota to soil heating and compiled a comprehensive evaluation of whether soil biological thresholds are supported in experimental data. Only 23 peer-reviewed research papers provided reproducible data of biological responses directly paired with soil temperatures, meeting the criteria for inclusion in this review (see full list in Pingree and Kobziar 2019).

Evaluating post-fire tree mortality: syntheses

The unique nature of this project (following two other JFSP funded projects interspersed with other major on-going SERDP and JFSP funded work) allowed us to participate in 1) a summary of the lessons we have learned in our work on southern pines and 2) two state-of-the-science syntheses on post-fire mortality. These efforts focused on how soil heating influences tree physiology and eventual stress and mortality (Varner et al. 2016, O'Brien et al. 2018) and how these injuries in concert with other fire-caused stresses cascade to patterns in tree mortality in fire-prone landscapes more generally (Hood et al. 2018).

Vegetation response to reintroduction fire: wiregrass resilience

We took advantage of a serendipitous opportunity following our experimental fires at OSBS to assess the response of the dominant bunchgrass in longleaf pine ecosystems. In winter 2018, eight months following our summer 2017 burns at OSBS, we noticed abundant post-fire flowering of wiregrass (*A. beyrichiana* syn. *A. stricta*). We assessed flowering response (% of wiregrass clumps flowering), wiregrass density (clumps m^2), and wiregrass biomass (via clipping). We compared these responses to the adjacent (separated by a small woods road) frequently burned unit. We subsequently compared flowering response by wiregrass stature via logistic regression analysis and compared their thresholds of minimum flowering size (Shearman et al. 2019).

Results and Discussion

1) Soil heating

Forest floor fuels differed among ecosystems, fire history, and location (basal vs. dripline/open) and were relevant for soil heating observations (Figure 8). In sandhills, litter (Oi) was deeper at the base of trees versus open locations, however there were no differences between frequently burned and long-unburned sites. Litter was deeper in long-unburned flatwoods sites compared to frequently burned sites and was deeper at basal versus open locations in the long-unburned site, but was shallower at basal locations in the frequently burned site. Duff (Oe + Oa horizons) was deeper in long-unburned sites compared to frequently burned sites and at basal locations versus open locations in both sandhills and flatwoods. Duff was non-existent in open locations in frequently burned sandhills and flatwoods.

During burning (Figure 10), litter (Oi horizon) moisture ranged from 32 to 68 % across sites. Fermentation (Oe) moisture was >60 % at basal locations in sandhill sites, but <60% at basal locations in flatwoods (60 % moisture content is an observed threshold for duff combustion in these fuels, Kreye et al. 2013, 2017); fermentation was driest in the frequently burned flatwoods. Humus (Oe) moisture was ca. 60 % in long-unburned sandhill and flatwoods. Fermentation and humus at open locations ranged from only 24 to 33 % moisture across sites. Mineral soil was wetter in frequently burned sites compared to long-unburned sites in both sandhills and flatwoods at all depths. Surface mineral soil (0-2.5 cm) moisture differences were most prominent in the frequently burned sites: 33% at basal locations, 17% at open locations in the sandhill site; 20% at basal locations, 9% in the open in flatwoods. Deeper mineral soils (>2.5 cm) were drier (<10 %) across all sites and locations.

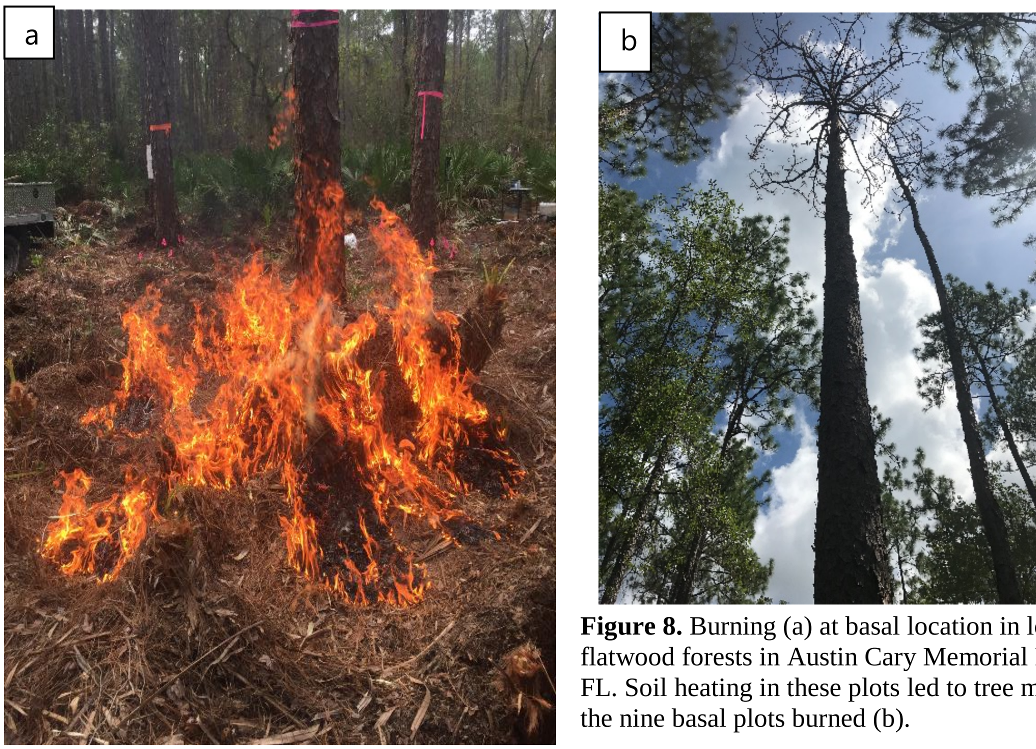

Figure 8. Burning (a) at basal location in long-unburned flatwood forests in Austin Cary Memorial Forest, Gainesville, FL. Soil heating in these plots led to tree mortality of three of the nine basal plots burned (b).

We did not detect soil temperatures ≥60 °C in open locations in the long-unburned sandhill at 5 cm depths or deeper. In basal locations, however, we observed temperatures ≥60 °C that lasted for 181 min at the soil surface and 40 min at 5 cm depths (Figure 9). In the frequently burned flatwoods, temperatures ≥60 °C lasted for 10 min at the soil surface and 5 min at 5 cm depths beneath trees (Fig.1c). As in the sandhills, no lethal soil temperatures were reached in open locations in the frequently burned flatwoods at 5 cm or deeper depths. In the long-unburned flatwoods unit, substantial soil heating was observed for long durations at the base of pines (Fig. 9d). While durations decreased with soil depth, temperatures ≥60 °C lasted for ca. 800 min (>13 h) at soil surfaces, 650 min (>10 h) at 5 cm depths, 340 min (almost 6 h) at 10 cm depths and for 40 min as deep as 20 cm. At the open locations, such temperatures at 5 cm depth were only observed at one probe in one unit (long-unburned flatwoods) and lasted only 5 minutes.

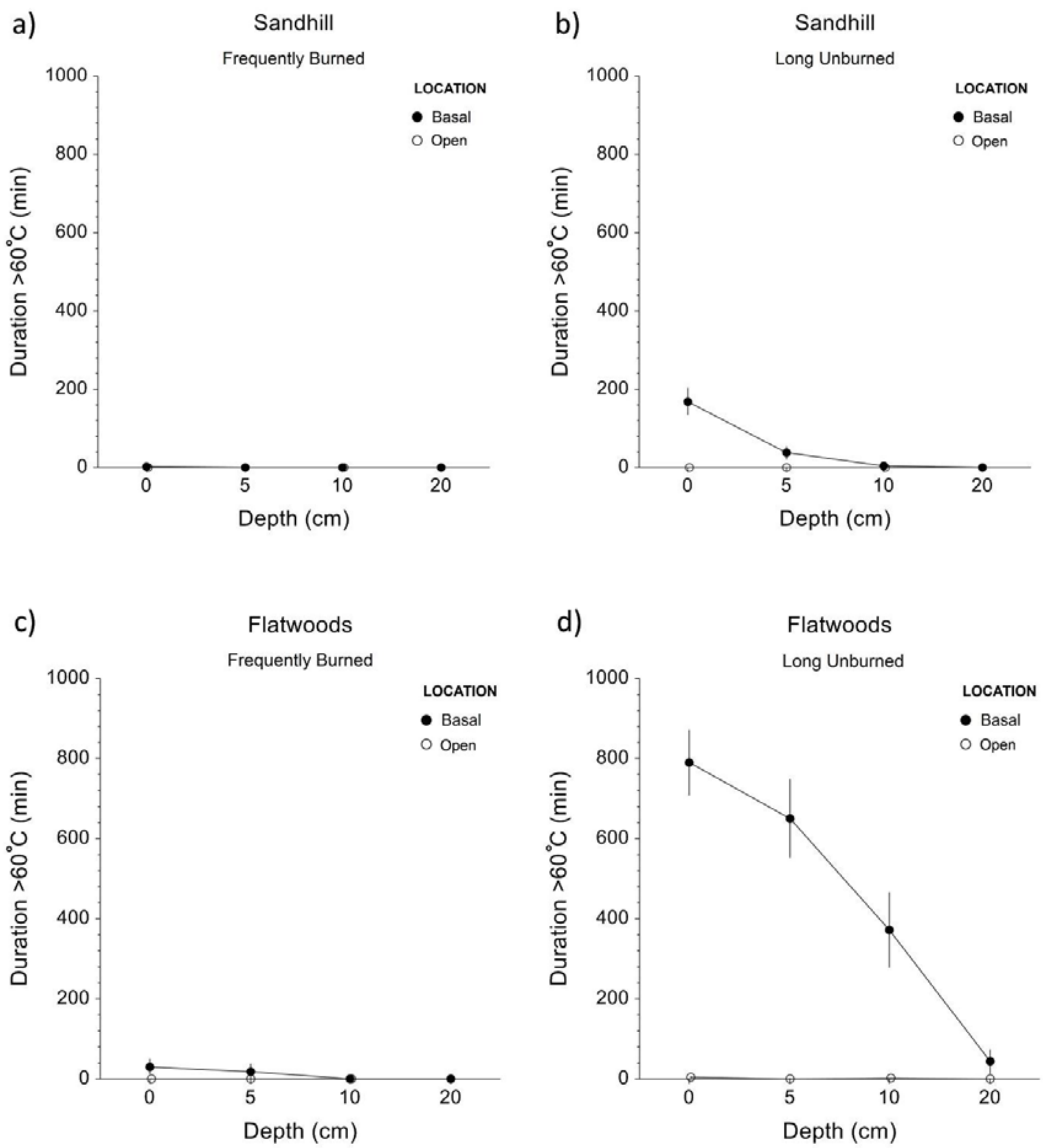

Figure 9. Duration of mineral soil heating >60 °C at the base of longleaf pines (Basal) and beyond their driplines (Open) in frequently burned and long-unburned sandhill and flatwoods ecosystems in north Florida, USA.

At all depths and temperatures, duration of soil heating was greater in the flatwoods site than the sandhills (Figure 10). Soil temperatures approximating potential nitrogen volatilization (≥300 °C) were only observed at the soil surface in the long-unburned sites. Temperatures ≥300 °C occurred for 9 min on average at these basal locations in long-unburned sandhills and for 33 min at basal locations in long-unburned flatwoods.

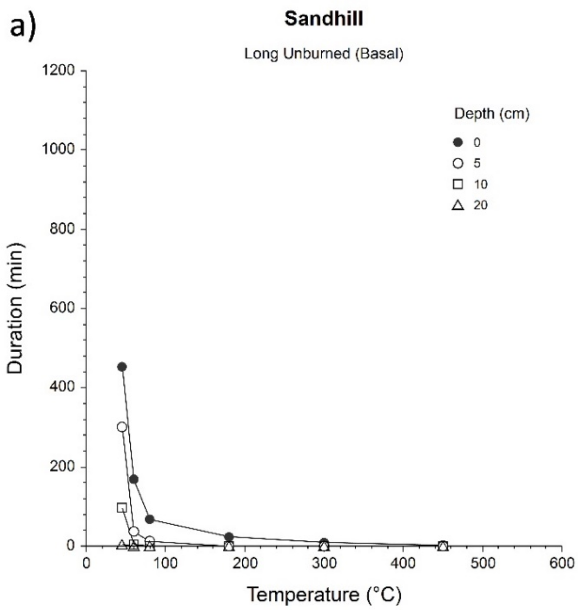

Figure 10. Duration of mineral soil heating across different temperature thresholds at the base of longleaf pine trees in long-unburned sandhill (a) and flatwoods (b) ecosystems in north Florida, USA.

The depth of duff consumed during burns differed across fire history and forest floor locations (basal vs. open) as expected given the differences in pre-burn duff depths. Minor duff was consumed in open locations in either frequently burned (mean = 0.1 cm) or long unburned sandhill units (mean = 0.2 cm). More duff was consumed at the base of pines in the frequently burned sandhill unit, averaging 0.8 cm, and as expected the most duff consumed in the sandhill unit occurred at long-unburned basal locations (3.5 cm). In the frequently burned flatwoods unit, little duff was consumed at basal (0.3 cm) or open (0.4 cm) locations. Duff consumption was greatest at the base of longleaf pines in the long-unburned flatwoods unit, averaging 20.2 cm, but with little duff consumed beyond tree driplines (0.2 cm).

Maximum temperatures reached below the soil surface generally increased with the depth of duff consumed in the pine flatwoods sites where duff consumption pin data were measured, with greater duff consumption, and soil heating, occurring in long-unburned sites (Figure 11). When evaluating lethal heating across all sites and forest floor locations (basal and open), duration of temperatures ≥60 °C increased with depth of duff consumed (Figure 12, $R^2 = 0.732$, $P < 0.001$), highlighting the role of duff consumption in soil heating.

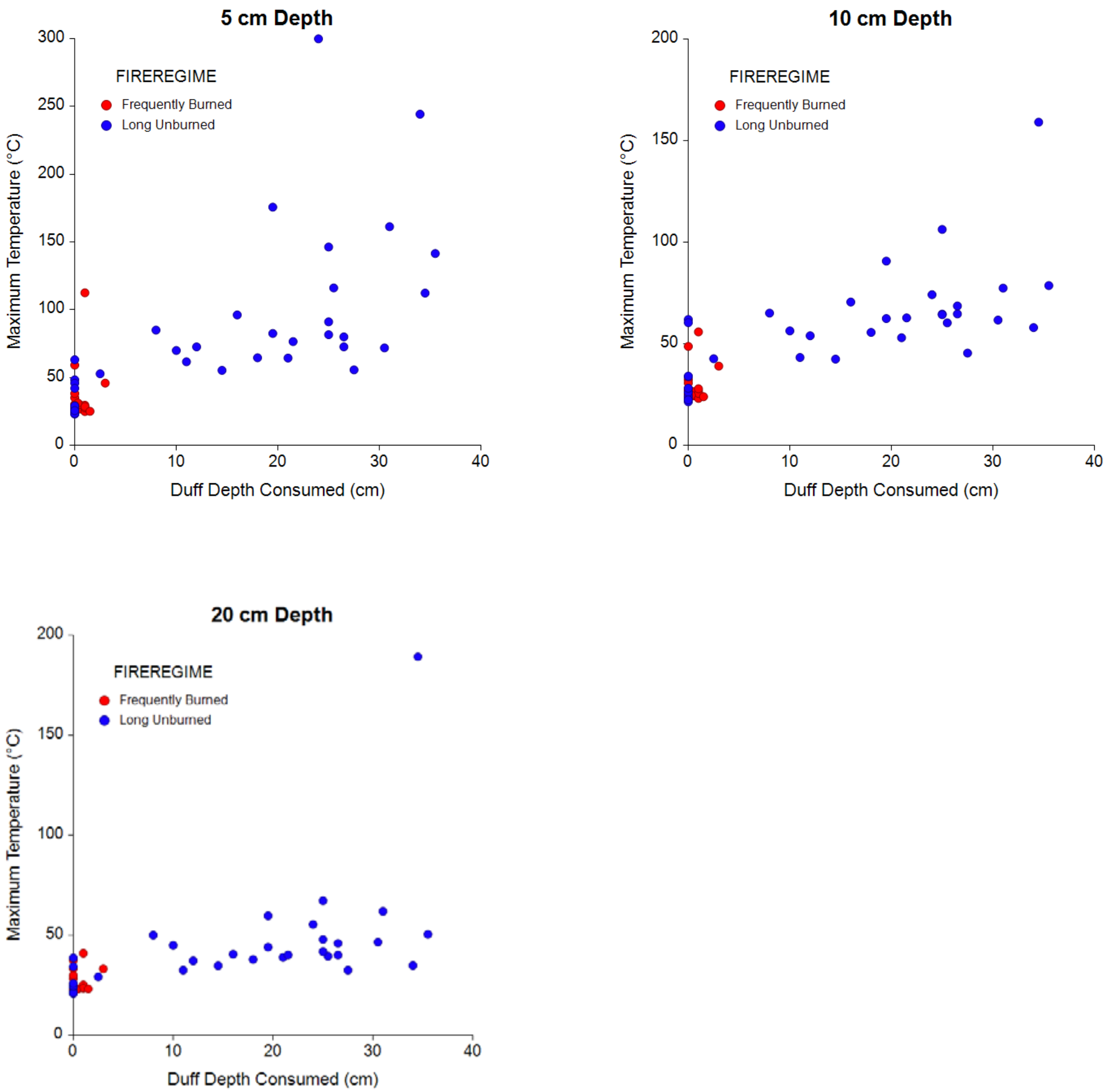

Figure 11. Maximum soil temperatures reached at 5 (top), 10 (middle), and 20 cm (bottom) depths during experimental burns in longleaf pine flatwoods sites in north Florida, USA.

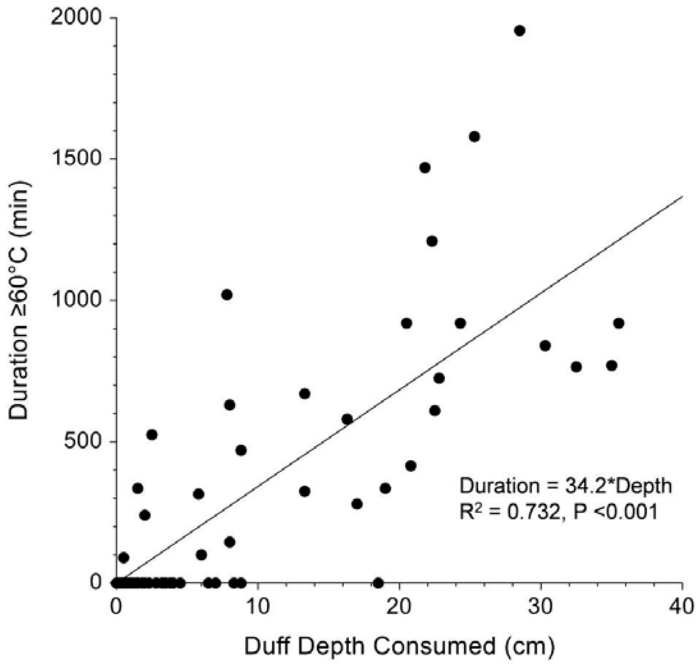

Figure 12. Duration of mineral soil heating >60 °C as a function of duff depth consumed in longleaf pine ecosystems in north Florida, USA.

Duff consumption as a percentage also varied with fire history and forest floor location at the flatwoods site where post-fire duff remaining was examined. At basal locations in the long-unburned unit, duff consumption averaged 88%, while only 5% of duff was consumed in the open. In the frequently burned flatwoods unit, 30% of duff was consumed, on average, in open locations, while only 8% of duff was consumed at basal locations. While depth of duff consumed was largely explained by pre-burn duff depths ($R^2 = 0.96$), shallower duff (< 10 cm) was often unconsumed during burns. The percentage of duff consumed increased with pre-burn duff depth across all flatwoods data pooled with a simple square root model ($R^2 = 0.56$). More detailed results of these soil heating experiments can be found in Kreye et al. (*accepted pending review*).

Mineral soil heating was most prominent in sites where significant duff had accumulated, as a result of fire exclusion, and was consumed during experimental burns. Duff accumulation was greatest at the base of longleaf pine trees and was especially deep in the long-unburned flatwoods. Basal duff accumulations have been observed in fire-excluded longleaf pine sandhill ecosystems (Varner et al. 2007, Kreye et al. 2014) and have been shown to incite duff ignition and consumption at these vulnerable locations (Kreye et al. 2017). Furthermore, the presence of longleaf pine cones, which burn for long durations (Fonda and Varner 2004, Varner et al. 2009), can cause duff to ignite when otherwise too wet (Kreye et al. 2013). Results here highlight that smoldering duff provides sufficient energy for heat transfer into mineral soils where consumption occurs. High soil temperatures were sustained for long durations where deep duff horizons were consumed, whereas minimal soil heating was observed in sites that had been burned frequently, where duff was almost non-existent. Frequent burning likely minimizes belowground impacts given that litter and herbaceous fuels are regularly burned and are consumed quickly with minimal residence times and heat fluxes primarily occurring aboveground. Duff accumulations resulting from fire exclusion present significant challenges for restoring fire to these fire-dependent ecosystems given that manager goals often include duff reduction to promote the establishment of the diverse herbaceous flora indicative of these pyrogenic landscapes (Varner et al. 2005). Based on our results, these impacts may be more problematic in flatwoods ecosystems, although considerably less work has been done to examine duff accumulations across flatwoods sites in the southeastern USA.

Spatial patterns of soil heating

We conducted *in situ* spatial soil heating measurements during operational broadcast burns at longleaf pine sandhill sites at Ordway-Swisher Biological Station. Our goal was to measure soil temperatures in a spatial sampling scheme (Figure 13) across frequently burned and long-unburned sites. We anticipated that soil heating would be negligible in frequently burned sites, based on our other soil heating experiments, but that we could evaluate spatial variability in soil heating in a long-unburned site and link it to spatial variability in duff patterns using spatial autocorrelation (Kreye et al. 2014). Analysis would provide the ‘patchiness’ of soil heating during prescribed burns. Burning across both fire regimes would also allow us to compare soil heating during operational burns across disparate fire histories, similar to our previous experiments. Due to operational constraints, however, the long-unburned site has not been burned; a lakebed that dried is adjacent to the long-unburned site. Therefore, we were only able to conduct soil heating measurements during burning in a frequently burned site and a site that was fire-excluded for 37 years and then burned in 2003 and subsequently burned 3 times prior to the burn in which we measured soil heating. This last site still included some duff accumulations, although they were shallow and patchy. Preliminary data visualization indicated little soil heating in either site; little to no duff was consumed in the site patchy shallow duff present. We anticipate being able to conduct the spatial sampling in the long-unburned unit, with significant duff accumulations, in 2019 given that significant water levels have increased in the adjacent lake, that had previously been dry, and fire managers are preparing to burn it. We plan to conduct spatial analysis of all soil heating and forest floor consumption data following the burn in the fire-excluded site. In anticipation of potentially minimal soil heating in the more frequently burned sites, we measured temperatures in the mineral soil, but also measured above-ground temperatures and quantified herbaceous fuels at measurement locations. This will allow us to further evaluate spatial variability of above-ground heating with spatial patterns of herbaceous fuel consumption, which is the primary driver of combustion in frequently burned longleaf pine sandhill sites.

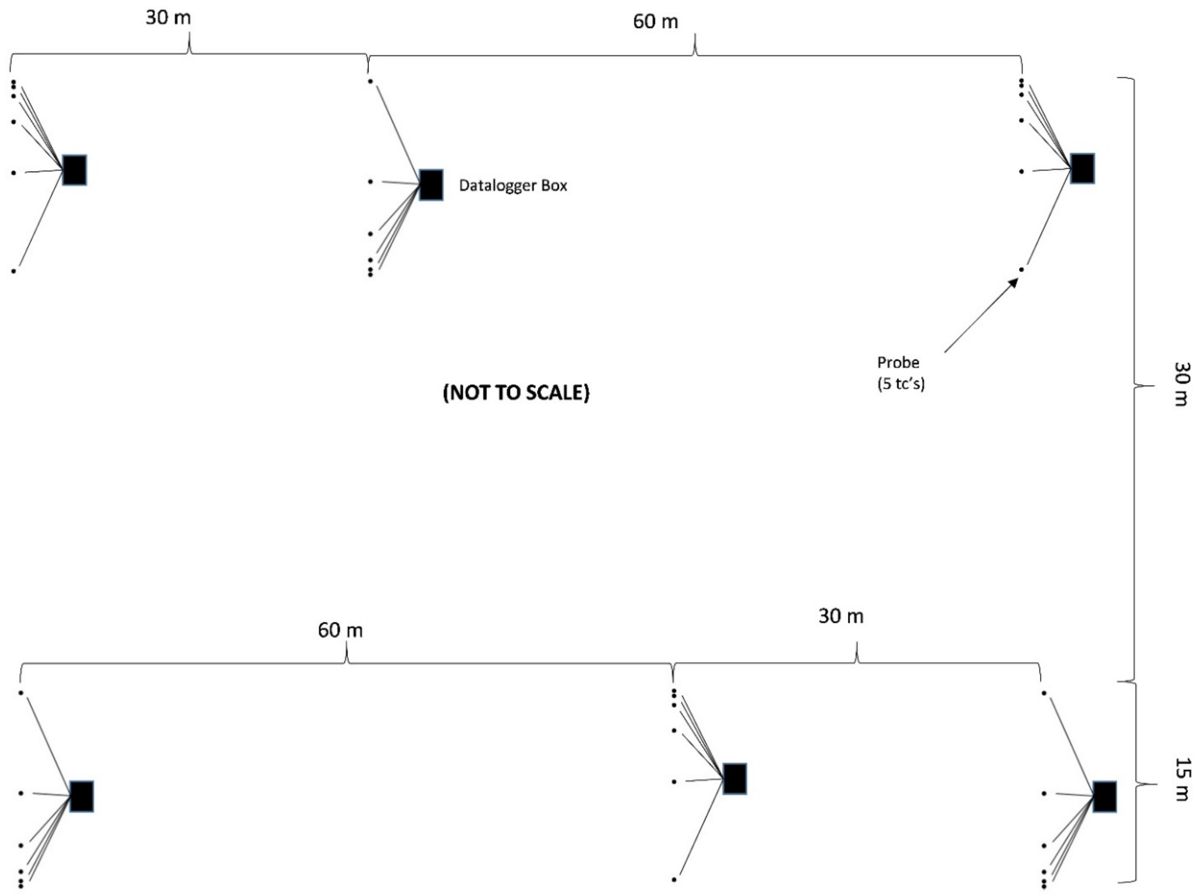

Figure 13. Spatial sampling design to measure above-ground, surface, and soil heating during prescribed burns in longleaf pine sandhill ecosystems.

2) Soil heating models- FOFEM analysis

FOFEM-predicted maximum soil temperatures at 4 cm depths differed from those we measured in our flatwoods units at 5 cm depths, but these differences were primarily a result of a lack of soil heating predicted for plots where duff was present (Figure 14, left). Plots that contained duff resulted in significant soil heating at 5 cm depths, up to 300 °C, as a result of our experimental burns, however, most of these plots were not predicted to result in soil heating according to the FOFEM model outputs. Plots where soil heating was predicted to occur were all, except one, in frequently burned sites where duff depths were shallow (1.0 to 2.9 cm). Temperatures, however, were only predicted to be raised by 4 to 8 °C above ambient, which is 20 °C in FOFEM batch processing. One plot in the long-unburned sandhill site with predicted elevated soil temperatures (2 °C above ambient) also had shallow pre-burn duff (1.0 cm deep). Soil heating was better predicted in sites where duff was not present (Figure 14, right) and herbaceous and litter fuels are assumed to be the energy source for heat flux to the below mineral soils. Although there was variability in differences between predicted versus observed temperatures, the predicted values at 4 cm depths were generally higher than those observed at the deeper 5 cm depths in which soil temperatures were measured during experiments. Results suggest that duff consumption is much greater than predicted in the Hough (1978) model used in FOFEM for predicting consumption and soil heating in the Southeast. Duff moisture contents were generally low enough for duff smoldering to occur in these longleaf pine ecosystems (Kreye et al. 2017) with significant consumption observed. The energy flux provided by smoldering duff resulted in high soil temperatures that were sustained for long durations, yet model results were poor in predicting this phenomenon. It is unclear why the current equation used in FOFEM for the Southeast results in such under-predicted duff consumption, but this seems to result in no heat flux to underlying mineral soils. The use of other duff consumption equations in FOFEM may result in better predictions, however this would need to be further evaluated. We only compared predicted results from the regional inputs used for Southeastern forests to emulate how managers would utilize the program.

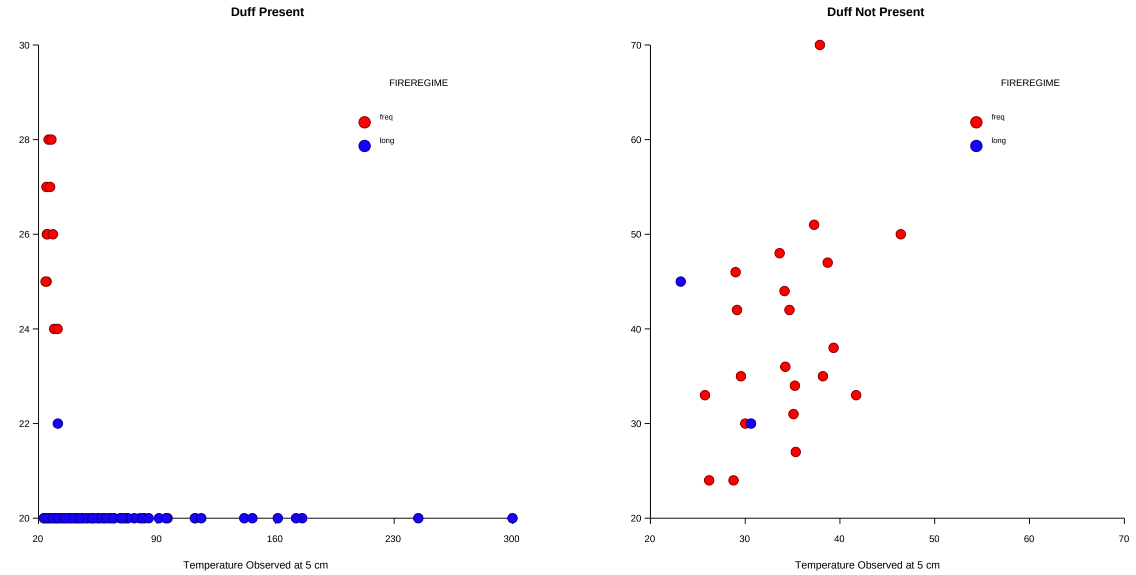

Figure 14. Maximum soil temperatures (°C) predicted at 4 cm depths from FOFEM modeling compared to those observed at 5 cm depths during experimental burns where duff was present (left) and where duff was not present (right).

3) Soil heating effects

a) Soil Respiration

Soil respiration patterns and responses to burning were analyzed both independently and together using an iterative approach:

Q1) Effects of location: How does soil respiration change with respect to moisture, temperature, and location, given monthly fluctuations?
Q2) Effects of trenching: Here, we expected that the location would affect trenching because the proximity to a tree will increase the proportion of heterotrophic respiration.
Q3) Effects of prescribed burning and location (basal vs. dripline/open). We expected the location of plots to influence the effect of the prescribed fire treatment because more fuel was present around trees than in open plots, particularly in the long-unburned units.
Q4) Effects of prescribed burning and trenching: We expected the location of the plots to include the effect of the prescribed fire treatment, same as above. In order to test

trenching, we were only able to use the subset of pre-burn and post-burn plots where trenching was established.

For each question evaluated, units not shown were not significant when full and partial models were compared (*p* value >0.05), or the partial model was singular and could not be tested.

Q1: Effects of Location:

Table 3. Summary of coefficient estimates from Generalized Linear Mixed Model (GLMM) for fixed and random effects to test whether location influenced soil respiration rates.

Unit, Rs transformation	**Moisture**	**Temperature**	**Is location significant? (*p-value* <0.05)**
ACMF FREQ (log x+10)	-0.38	NS	Yes
ACMF INTER (log x+10)	-0.19	0.01	NA
OSBS INTER (log x)	NS	0.03	Yes
OSBS LONG (log x)	NS	0.04	Yes

NA is not-applicable, NS is not significant at p-value <0.05. LONG is long-unburned, INTER is intermediate fire frequency (3-5 years), FREQ is frequent fire (1-3 years return interval), ACMF is Austin Cary Memorial Forest flatwoods, OSBS is Ordway Swisher Biological Station sandhills.

Soil respiration in the frequently and intermediate burned flatwoods units was significantly and negatively related to soil moisture, indicating that increased soil moisture reduced gas exchange in these Spodosol soils. For example, in the ACF Frequent unit, the average change in soil respiration is related to -0.38 units of soil moisture and also decreases from basal to dripline locations. This is likely due to macropore space being occupied by water molecules, thus reducing overall gas exchange, or due to reduced metabolic activity of microbes and, in some cases, extensive root systems located in the basal locations (Figures 15 and 16). Location (basal vs. open) was significant in most of the units analyzed, with the exception of ACF Intermediate. In all other cases, basal respiration rates were higher than those measured in open locations, likely due to the influence of root systems and the autotrophic component of total respiration.

Figure 15. Photos show firing operations and post-fire conditions at basal locations in the Austin Cary Memorial Forest long-unburned unit (ACF-LONG). Note extensive residual saw palmetto rhizome surrounding the tree base. This tree was one of the three that died within three months post-fire.

Q2: Effects of trenching

Table 4. Summary of coefficient estimates from Generalized Linear Mixed Model (GLMM) results for fixed and random effects to test whether trenching influenced soil respiration rates.

	Model: Rs ~ loc + trenching + loc * trenching + Ms + Ts + (1 | date2)				
Unit, Rs transformation	**Location**	**Loc * Trench**	**Moisture**	**Temperature**	**Was trenching significant? (*p-value* <0.05)**
ACMF INT (log x+10)	-0.07	NS	-0.3	0.01	No
ACMF LONG (log x)	-0.44	NS	0.99	0.06	No
OSBS FREQ (log x+1)	-0.4	NS	NS	0.03	Yes
OSBS LONG (log x)	-0.51	0.15	NS	0.03	Yes

NA is not-applicable, NS is not significant at p-value <0.05. Abbreviations as in Table 3.

In all units across fire frequencies, basal locations had higher respiration rates than open locations. Trenching had a significant influence on Rs in the OSBS units, suggesting that autotrophic contribution to total respiration was higher in OSBS than in ACF, and therefore more impacted by trenching. ACF flatwoods have higher organic matter content, deeper O horizons, and more A horizon carbon, which collectively provide better heterotrophic organism habitat than at the OSBS sandhills. Therefore, the heterotrophic contribution to total respiration in the ACF sites is likely higher, as reflected in the lack of significant Rs change post-trenching. Higher temperatures corresponded to higher respiration rates, a common trend in soil respiration studies since increased temperatures enhance metabolic activity of both heterotrophs and autotrophs (Godwin et al. 2017). The effects of soil moisture, however, were ambiguous, increasing soil respiration in the ACF long unburned unit and decreasing respiration in the ACF intermediate unit.

Q3: Effects of prescribed burning and location (basal vs. open).

Table 5. Summary of coefficient estimates from Generalized Linear Mixed Model (GLMM) results for fixed and random effects to test whether prescribed fire influenced soil respiration rates at each location.

	Model ~ log(Rs) ~ unit + loc + trt + Ms + Ts + (1 | month)				
Unit, Rs transformation	**Treatment of Rx Fire**	**Treatment * Location**	**Moisture**	**Temperature**	**Was the location significant?**
ACMF FREQ (log x+1)	0.28	-	-1.9	NS	Yes
ACMF INTER (log x)	0.23	-	-1.67	0.04	Yes
ACMF LONG (log x)	0.23	NS	NS	0.07	Yes
OSBS FREQ (log x)	NS	-	1.56	0.03	NA
OSBS LONG (log x)	NS	NS	2.11	0.06	NA

NA is not-applicable, NS is not significant at p-value <0.05. Abbreviations as in Table 3.

Prescribed burning had a significant impact on all the ACMF flatwoods units, regardless of the fire frequency, and of similar strength across the units. No such effect was found in the OSBS units. Given that ACMF smoldering combustion lasted for multiple days following burns in the long-unburned sites, and that significant consumption of the O horizon occurred, an increase in soil respiration may reflect increased resource (nutrients, water) availability for heterotrophic organisms, and an improvement in habitat related to increased biochar which enhances water retention, cation exchange capacity, and adsorbs nutrients (Pingree 2016). It is unlikely that, especially given the tree mortality that occurred in these sites, autotrophic activity increased immediately post fire- our analysis evaluates the impact of fire over six months post-burn. However, vegetation assessments showed a vigorous flowering response in the dominant shrub, saw palmetto, on the long-unburned sites about one month post-fire, along with prolific regrowth of ferns. Reduction of the O horizon likely freed growing space post-fire, and over time seed banking and sprouting species recovered. Soil heating, also most extreme in the long-unburned ACMF units, did not appear to have a deleterious effect on soil respiration, suggesting that direct mortality of microbes was limited.

Q4: Effects of prescribed burning and trenching

Table 6. Summary of coefficient estimates from Generalized Linear Mixed Model (GLMM) results for fixed and random effects to test whether prescribed fire influenced soil respiration rates in trenched and untrenched plots.

Model: Rs ~loc + tmt + §tmt*loc + trench + Ms + Ts + (1 | date)						
Unit, Rs transformation	**Location**	**Fire treatment**	**Interaction**	**Moisture**	**Temperature**	**Was the effect of trenching significant? (*p-value* <0.05)**
ACMF LONG	-0.38	0.21	NS	1.50	0.06	No
OSBS FREQ	-0.12	NS	-	0.35	0.01	No
OSBS LONG	-0.23	NS	NS	1.07	0.04	Yes

NA is not-applicable, NS is not significant at p-value <0.05. Abbreviations as in Table 3.

As in the location × fire analysis, OSBS units did not show a change in soil respiration as a result of burning (Figure 17). Soil heating in the frequently burned OSBS unit was negligible, so a lack of response is not surprising. ACMF long-unburned again showed an increase in respiration in the burned plots, which was not impacted by trenching. Again, the lack of a trenching effect in these flatwoods soils suggests that heterotrophic organisms drove a significant portion of the overall soil carbon exchange.

Overall, the results indicate that prescribed burning may increase soil carbon cycling rates, regardless of historical fire frequency, in flatwoods but not sandhill pine forests. Increased soil respiration can result from various fire effects, including decomposition of flora, changes in soil environment, stimulation of new growth due to nutrient pulses, and changes in relative contributions of respiration. The trenching we established was aimed at disarticulating the relative influence of heterotrophic vs. autotrophic sources, but only had an impact in the OSBS units. This suggests that autotrophic organisms account for a greater proportion of total respiration in the sandhills sites, likely due to lower overall O horizon development, lower total organic matter and carbon, and generally drier condition of these soils (all elements of habitat for microorganisms). In previous studies, soil respiration has been positively correlated with soil temperature in many ecosystems, and some authors suggest that increases in soil temperature due to climate change may exacerbate landscape-level soil carbon losses (Raich and Schlesinger 1992; Godwin et al. 2017). Previous research, predominantly from forests outside of the South, has shown that fire can influence soil C and N pools, biogeochemical properties, and soil respiration (R_s) rates (Certini 2005; Kobziar and Stephens 2006; Kobziar 2007; Lavoie et al. 2010). As was the case in this study, Kobziar (2007) found that proximity to overstory trees in a Sierra Nevada pine plantation had clear effects on R_s and its sensitivity to soil temperature, suggesting that fire's influence on autotrophic respiration drives changes in soil carbon loss (JFSP project 00-2-30). Assessments of overall impacts of fire on soil carbon cycling must therefore take into consideration the high degree of spatial variability which can be captured by systematically locating sampling plots in large-root influenced and less-influenced locations in proportion to stand densities, or by quantifying distance to large trees as covariates in all analyses of overall ecosystem soil carbon flux.

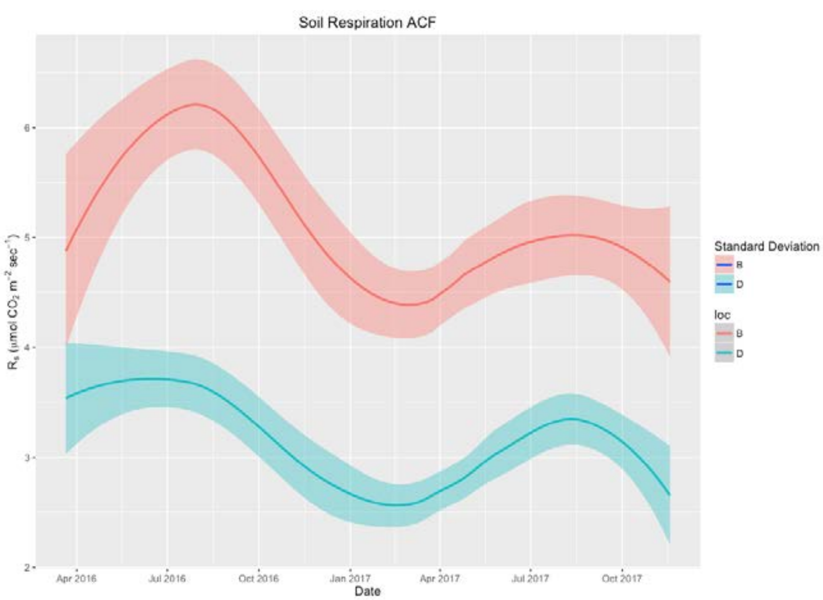

Figure 16. Visualization of soil respiration rates in Austin Cary Memorial Forest Long-unburned plots indicating differences between basal (B) and dripline (D) locations within the forest stand. Mean values are shown with a smoothed curvilinear fit and shading reflects standard deviations.

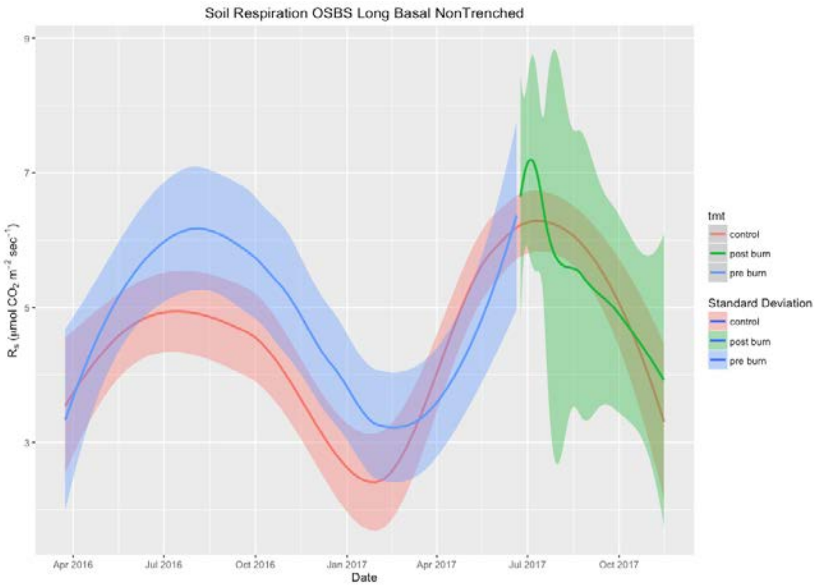

Figure 17. Visualization of soil respiration rates pre- and post-burn in OSBS sandhill Long-unburned un-trenched plots showing increases in variability of respiration rates but little overall change. Mean values are shown with a smoothed curvilinear fit and shading reflects standard deviations.

b) Vegetation

1. Community composition

Sandhills and flatwoods sites differed in their species richness and community composition. Flatwoods had a slightly higher species richness and was dominated in the understory by saw palmetto (*Serenoa repens*), fetterbush (*Lyonia feruginea*), shiny blueberry (*Vaccinium myrsinites*), and many flowering perennials. Sandhills had less dense vegetation, often with patches of bare sand (or forest floor) and dominated by shrubby oaks (mainly *Quercus laevis*). Wiregrass (*Aristida beyrichiana*) was the only species that was abundant across both pine savanna types. Because sandhills and flatwoods share few species, the two habitats were analyzed separately.

We found significant effects of fire frequency and proximity to trees (i.e. directly under trees vs. in open locations) on species richness (Table 7). In all comparisons (Figure 18 A-D), species richness was greatest in the frequently burned units and decreased as the fire frequency decreased from frequent to intermediate to long-unburned. Plots in the open locations were more diverse in the frequently burned plots, but this difference decreased with fire frequency.

The long-unburned sites had similar and low species richness regardless of proximity to overstory pine trees. In fact, some of the long-unburned plots were devoid of understory vegetation altogether. Increased species richness with higher fire frequency is not a surprising result in these ecosystems and has been reported elsewhere for both flatwoods and sandhill ecosystems, especially with regards to graminoids and shrubs (e.g., Freeman et al. 2019; Kirkman et al. 2004).

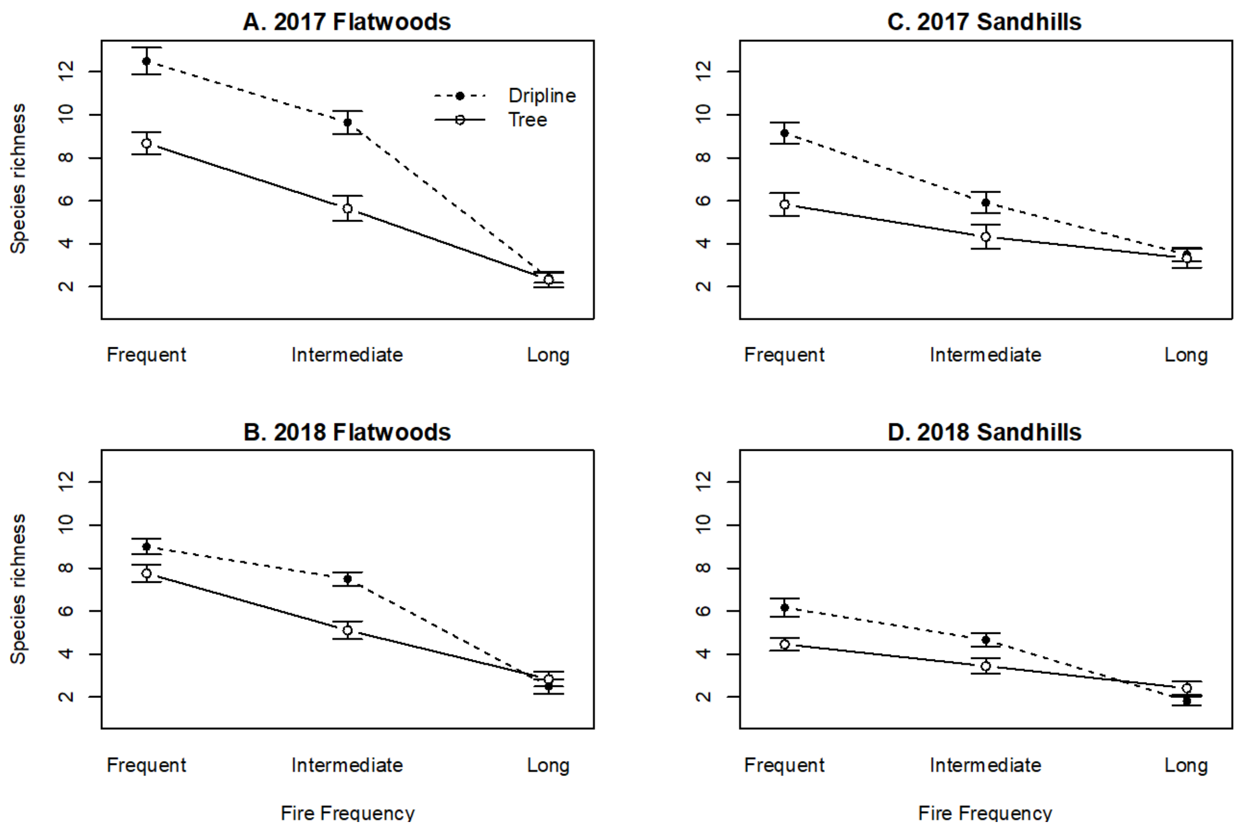

Figure 18. Relationship between fire frequency and plot-level species richness in A-B) flatwoods and C-D) sandhills pine savannas during fall of 2017 and 2018. Bars are standard error.

We are unaware of prior analyses in these ecosystems that employed a spatially explicit study design to evaluate the effects of canopy cover, root systems, and environmental factors influencing vegetation and soils by separately measuring basal and open locations. In Freeman et al. (2019), species richness across sites considered "restored" by managers was affected by both soil type and tree density, indicating a potential role of canopy cover as a driver of species richness. Our results show a clear negative canopy cover effect in the frequent and intermediate frequencies across both ecosystems, whereby shading, growing space, soil conditions (e.g., depth of the O horizon), or other factors characterizing the growth environment at the bases of trees may be limiting species richness at the plot level.

Table 7. Results of ANOVAs examining the effects of Proximity to pine trees (i.e., directly under trees and outside tree driplines) nested within three fire frequencies (frequent, infrequent, and long unburned) on plant species richness.

2017	Flatwoods- ACF				Sandhills- OSBS			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Frequency	1	169.7	21.9	<0.001	1	41.3	10.3	0.002
Proximity/ Frequency	4	1228	307.1	<0.001	4	67.9	17	<0.001
Residuals	120	928.9			87	4		
2018	**Flatwoods- ACF**				**Sandhills- OSBS**			
	df	MS	*F*	*P*	df	MS	*F*	*P*
Frequency	1	35.8	13.1	<0.001	1	7.49	3.77	0.05
Proximity/ Frequency	4	138	50.7	<0.001	4	202.9	25.5	<0.001
Residuals	97	2.72			95	189		

2. Seed Banks

A total of 13 germinants representing 5 plant species were observed across the sites and treatments over the 6-month period (Table 8).

Table 8. Species and number of germinants observed in the soil seed bank of longleaf pine forests at eight sites with two (Basal or Dripline) treatments

Site location	Type/Treatment	Species	Number of germinants
ACF-NF	Basal		
ACF-AFR	Basal		
ACF-AINT	Basal	*Oxalis* sp.	1
ACF-NINT	Basal	*Eupatorium capillifolium*, *Oxalis* sp.	1, 1
ACF-LONG	Basal	*Linaria* sp	1
OSBS-FR	Basal	*Linaria sp.*	1
OSBS-INT	Basal		
OSBS-LONG	Basal		
ACF-NF	Dripline		
ACF-AFR	Dripline		
ACF-AINT	Dripline		
ACF-NINT	Dripline	*Digitaria* sp.	1
ACF-LONG	Dripline		
OSBS-FR	Dripline	*Eupatorium capillifolium*	1
OSBS-INT	Dripline	Unknown species	1
OSBS-LONG	Dripline	*Eupatorium capillifolium*	5

Only 8 out of 12 samples had one or more germinants. *Eupatorium capillifolium* (dogfennel), a native ruderal, was the dominant species representing 7 of the total 12 germinants. The other species observed were *Oxalis* sp., *Linaria* sp., *Digitaria* sp., and one unknown species. Of the limited seed bank germination responses observed from the sample, the Dripline samples showed higher number of germinants (8) than the Basal treatments (5). Only one sample (ACF-NINT-Basal) had more than one species germinated.

Although this represented our second attempt at germinating seeds from the seedbank, the results prove inconclusive. At a minimum, they suggest that seed banking species may not be plentiful or easily germinated *ex situ* from these soils.

c) Wiregrass flowering

Wiregrass flowered in both longleaf pine sandhill stands following the experimental burns at OSBS, but the frequently burned stand was had greater wiregrass density, basal area, and percent of clumps flowering. Wiregrass density averaged 3.2 plants m^{-2} v. only 1.8 plants m^{-2} in the frequently v. long-unburned sites, respectively (t = 5.0653, *p*-value = 0.001). Wiregrass basal area averaged 212.9 m^2 ha^{-1} in the frequently burned sandhill but only 32.2 m^2 ha^{-1} in the long-unburned site (t = 5.0841, *p*-value = 0.006). Wiregrass flowering in the frequently burned site was almost 6 times the long-unburned stand (57% v. 10.3%; *p*-value = 0.006).

Results reveal important differences between wiregrass clump size and flowering size between the long-unburned and frequently burned reference (Figure 19). Small wiregrass flowered readily in the frequently burned site, but flowering was rare below all but large wiregrass clump sizes in the long-unburned site.

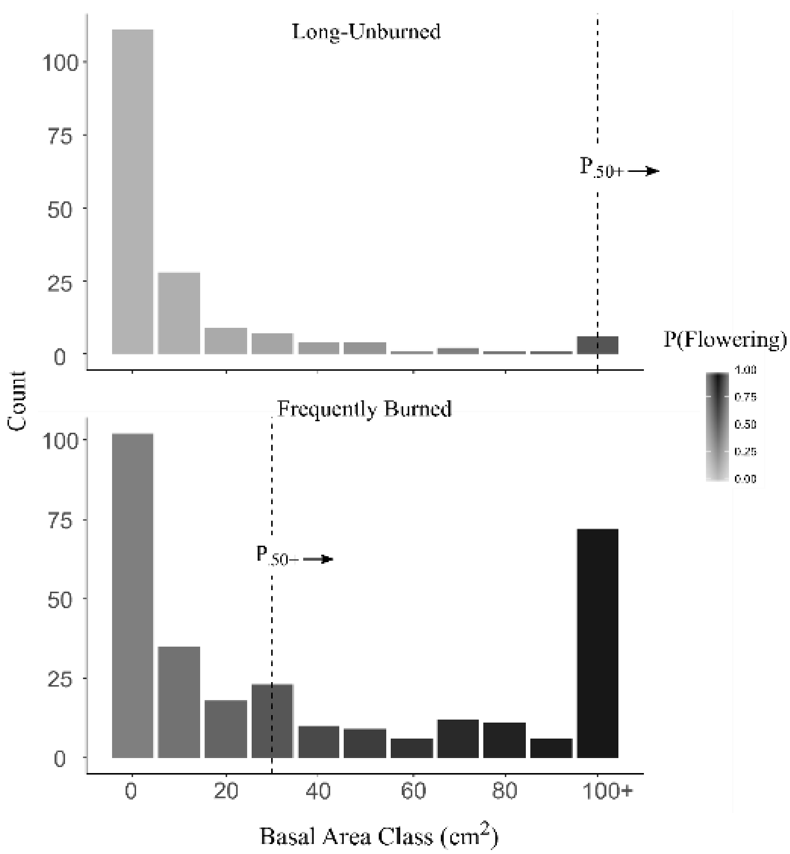

Figure 19. Comparison of threshold clump sizes for wiregrass growing in long-unburned (top) and frequently burned (bottom) sandhills. The dashed vertical lines indicate the minimum size class where the probability of flowering was 50 %.

d) Tree stress, mortality and review

As hypothesized, pines that suffered the greatest smoldering and below-ground heating (as published in Varner et al. 2009), continued to show the effects of this stress post-fire. Growth and defense during the recorded drought years post-fire were diminished (Figure 20). The overall growth and all measures of resin duct defenses, however, were unaffected by initial soil heating. These results suggest that pines injured by soil heating can tolerate these injuries and short-term stress, except during dry periods when their growth and defenses are reduced.

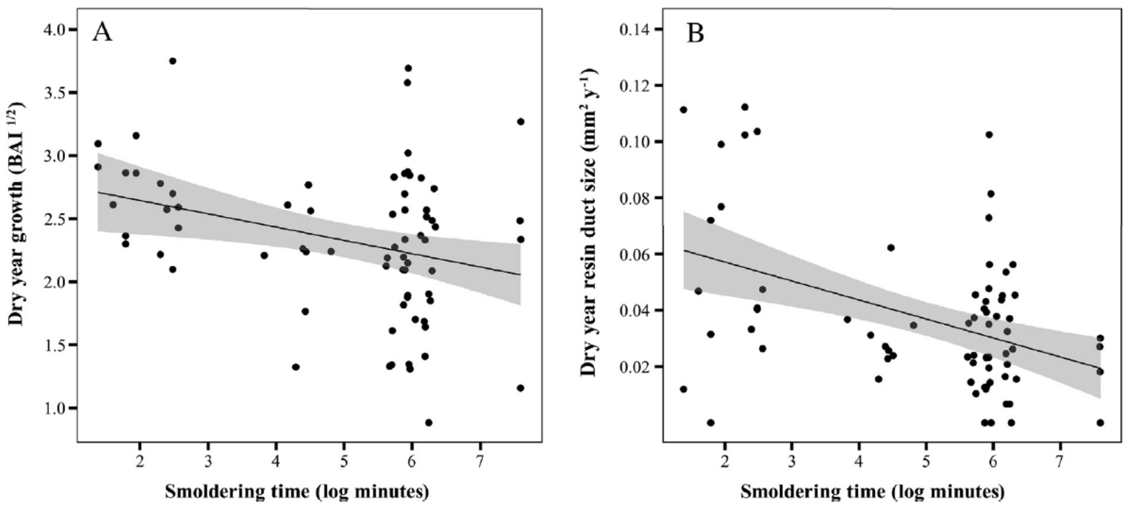

Figure 20. Relationship between soil heating duration (minutes > 60 °C) and A) drought year growth (as square root of basal area increment) and B) drought year resin duct size.

We summarized our work to date, specifically on longleaf pine ecosystems. In this initial review, we found shortcomings in data on vegetation response (with our current efforts answering some of those lingering questions) and on long-term overstory tree responses (again, with our current work filling in some unknowns for growth and defense). The two global syntheses illustrated where this work fits in with other post-fire tree mortality research. First, our work on this topic fills a major knowledge gap: little work on below-ground tree injury from fire has occurred in spite of the well-acknowledged role of roots to tree function and the effects of soil heating on below-ground plant nutrient dynamics.

e) Biological thresholds to soil heating

Our review examined literature that directly related soil heating temperatures and durations to biological responses, compared reported thresholds for soil organisms, and provided recommendations for soil heating data and its application to wildland fire and ecosystem management. For no single study or group of organisms was a threshold of 60°C for one-minute duration evidenced (Figure 20). All soil organisms reviewed, which included roots, mesofauna, bacteria, fungi, microbial biomass, and soil respiration, displayed both positive and negative responses to soil heating across temperature and duration gradients. Importantly, in a number of studies, increases in abundance were observed following heating in comparison to controls, indicating that soil temperature alone is not an effective predictor of soil biological responses to fire. We, therefore, discourage the use of the traditionally accepted metric of 60°C for the duration of one minute. Instead, we invite interdisciplinary efforts from researchers and managers to directly measure biological responses on a case-by-case basis.

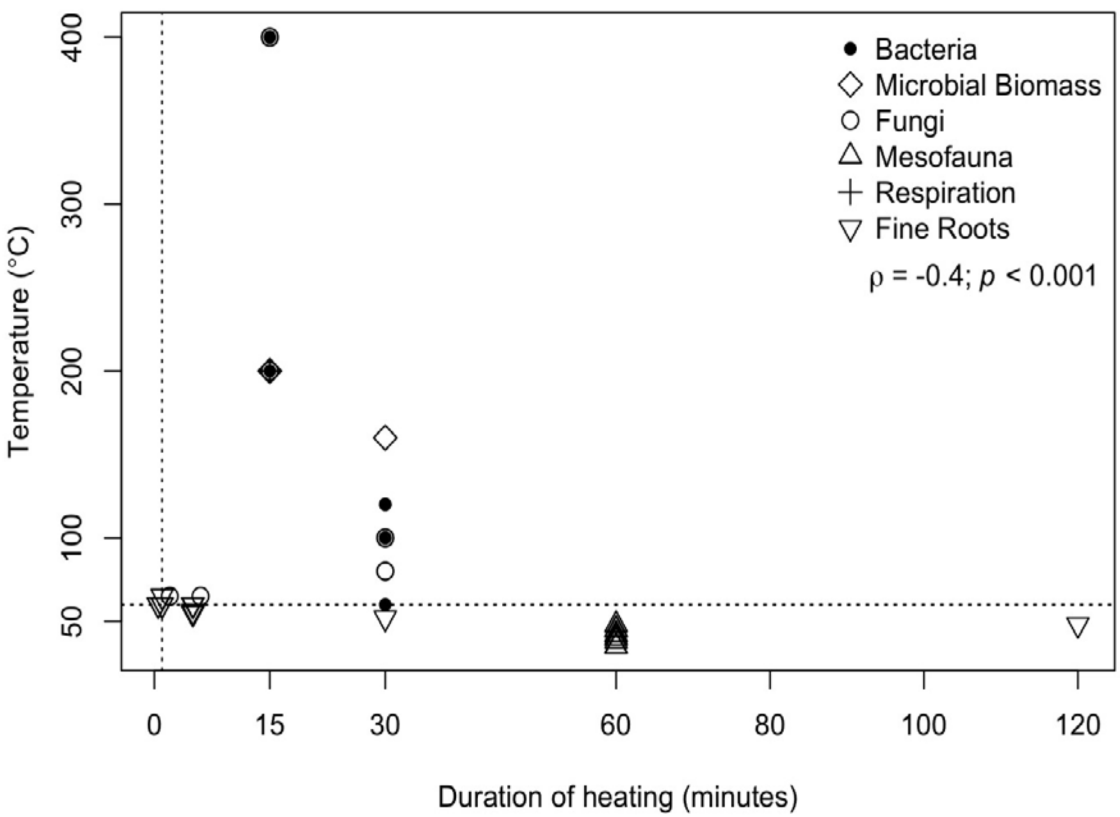

Figure 21. Relationship between mortality threshold temperature (where 100% mortality was observed when compared to controls) and duration established for various soil organisms. Overlapping points occur at 0.5 minutes (2 at 60°C), 1 minute (2 at 60°C), 5 minutes (8 at 55°C, 13 at 60°C), 15 minutes (5 at 200°C, 4 at 400°C), 30 minutes (2 at 80°C, 6 at 100°C, 3 at 120°C), and 60 minutes (4 at 35°C, 6 at 40°C, 2 at 42°C, 4 at 45°C). Dotted lines denote the presumed threshold of 60°C and one-minute duration. N= 71 (from: Pingree and Kobziar 2019).

Conclusions and Implications

Overview

Three themes emerged from our analysis of soil heating patterns and effects on biological components and processes in these flatwoods and sandhill forests of the southern coastal plain:

1) Spatial patterning: results are not consistent across stands and reflect that soil heating and its consequences are spatially patchy, with areas around trees differing from sites in the open;

2) The degree of soil heating and many of its ecological impacts diminish as fire frequencies increase;

3) These fire-adapted communities are generally resilient to repeated fire use, but care must be taken when conducting fire restoration in long-unburned forest stands, especially near tree bases.

Reintroduction fires are a major focus for land managers across the southern pine forests and beyond where past fire exclusion has been dominant. These fires have unique responses for tree stress and mortality as well as long-term resilience. Our data and syntheses support these findings in these ecosystems and beyond.

Implications of Results to Management and Policy

Vegetation response

Our work shows long-term tree stress and diminished resilience where fire is reintroduced after long periods of suppression (Slack et al. 2016) and use these and accumulated findings in the syntheses (Varner et al. 2016, Hood et al. 2018, O’Brien et al. 2018). The implications for management are that fire exclusion’s effects continue after reintroduction of fire- reduced growth in drought years and reduced defense. These effects emphasize that managers should continue to apply fire in a conservative (i.e. minimize stress) approach near tree bases in stands where fire was excluded.

Our plant community results also point to several novel outcomes of interest to land managers. Our serendipitous discovery of wiregrass flowering following fire exclusion (Shearman et al. 2019) was surprising. Wiregrass has been planted and sowed in many restoration treatments across its range. Our results suggest that wiregrass is more resilient to protracted fire exclusion followed by reintroduction fires than many have thought. Our analyses of species richness showed similar resilience, even in the severely burned basal sites in long-unburned units. Across fire frequencies, there were no significant differences between control plots and burned units a year following burning, although further analyses may reveal some nuances regarding diversity and functional groups. Following fire, recovery by mostly sprouting species in the newly exposed growing space likely outcompeted any potential seed-banking or colonizing species. Seed bank results were largely inconclusive but suggested that few viable seeds existed in the seedbank across all fire frequencies. The eventual increase in species richness expected to correspond with increased fire frequency when restoring fire to longleaf pine-dominated coastal plain forests (Freeman et al. 2019) may therefore require conservative firing techniques or timing of burn to correspond to plant phenology, especially where high understory diversity of fire-sensitive species is evidenced.

Soil Respiration

At landscape scales, given that soils contribute such large fluxes to atmospheric CO_2, even small changes in soil respiration rates could have substantial impacts on overall atmospheric CO_2. Because of the importance of soil respiration in local, regional, and global carbon cycles, it is critical to understand how prescribed burning influences these rates. We found that fire use in *frequently* burned flatwoods and sandhills had no measurable effect on soil carbon flux in the six-eight months following burning. Soil temperature measures during the burns in these units suggested minimal soil heating, and with lower fuel loads to begin with, consumption was also not high. In contrast, where soil heating was highest and of the longest duration, soil respiration increased post-fire. Likely due to increased food and nutrient availability and improved habitat for microbes and other soil biota, these increases- most strongly found at tree bases- plus direct carbon losses due to combustion of soil organic horizons suggest that carbon is lost both immediately and longer-term. In other words, soil carbon continues to be cycled at higher rates for a period of time following fire. Total ecosystem carbon recovery in intermediate frequency (3-year interval) flatwoods has been estimated to take approximately 3 years post fire (LaVoie et al. 2010). Our results did not indicate a change in soil respiration rates in similar stands in the same site, suggesting that soil respiration carbon losses are more resilient than above-ground losses over the same time period. In summary, our results suggest that managers should be aware that restoring fire to long-unburned stands has the potential to increase carbon losses and reduce carbon sequestration, while maintaining fire frequencies in intermediate or frequently burned stands is unlikely to have such an impact.

Biological Thresholds

Positive responses across all soil microorganisms illustrate the capacity of these organisms to resist and recover from temperatures above the traditionally accepted threshold for living soil organisms (i.e. 60°C for 1 minute; Pingree and Kobziar 2019). Microbes provide essential ecosystem services such as decomposition and C cycling, nutrient cycling, controls on greenhouse gas fluxes, soil structure and maintenance, and biological population control. These diverse, abundant, and adaptable organisms are likely to be integral drivers of resilience and recovery in frequent-fire ecosystems such as flatwoods and sandhills, especially as natural and human-induced changes to disturbance regimes continue. The responses of bacteria, fungi, and microbial processes in relation to heating temperatures do little to provide a consistent and, therefore, useful threshold temperature and duration for wildland fire management planning and predictions. Instead, fire restoration and frequent fire use effects on soil biota should consider both short and longer-term impacts using direct measures rather than unsubstantiated thresholds as guidance. Even in the most severely burned plots in our sites, where tree mortality indicated loss of fine root activity, soil carbon respiration increased following fire, suggesting that no degree of soil "sterilization" occurred.

Soil Heating Predictive Models

Our analysis of the commonly-used FOFEM fire effects model, which uses the Campbell soil heating model, suggests that the equations used for predicting duff consumption and subsequent soil heating are in need of comprehensive evaluation and amendment, especially for southeastern fuel types. Little duff consumption was predicted by the model, resulting in limited soil heating, and where duff consumption was predicted it, too, underestimated resulting soil heating. Our data show clearly that soil heating is indeed related to duff consumption, but existing models do not capture this relationship well. Application of the Campbell soil heating model to aid in management decision-making could give managers false security that burning under a wide range of moisture conditions will not result in duff consumption or soil heating, potentially leading to tree mortality in stands where duff has accumulated during long durations of fire suppression (Varner et al. 2007).

Opportunities for direct implementation by end users

Concern over tree mortality, stress, and soil carbon losses at the bases of long-unburned longleaf pine in both flatwoods and sandhill stands can be mitigated by burning under 1) moist duff conditions or 2) reducing duff loading or consumption at tree bases prior to burning. Past efforts at tree-level treatments (raking or leaf blower removal) have had mixed results and operational application of fire tends to ignore these approaches (Varner et al. 2007). Existing fire effects prediction models, such as FOFEM, should be carefully evaluated prior to use in southeastern pine-dominated forests, especially where fire is being reintroduced to long-unburned stands and potential consequences for tree mortality are high. Our vegetation response results reveal wiregrass' resilience and offer promise for fires reintroduced to those sites where wiregrass flowering is a goal.

Implications for future research

An overarching theme of the project results was that spatial distribution of mature trees has a strong effect on fire consumption, soil heating, soil processes, and ecological effects. Spatial patterning, here demonstrated by proximity to trees, is not often taken into consideration when fire effects are being evaluated, and only a few studies have taken these spatial considerations into account when drawing conclusions for fire effects on soil processes explicitly (e.g., Varner et al. 2009, Wiggers et al. 2013, Kobziar 2007). Assessments of overall impacts of fire on soil carbon cycling should be careful to consider the patterns of spatial distribution of carbon sources, which can be captured by systematically locating sampling plots in large-root influenced and less-influenced locations in proportion to stand densities, or by quantifying distance to large trees as covariates in all analyses of overall ecosystem soil carbon flux. Measurements that do not include both near-tree and open locations are likely to either underestimate or overestimate total soil carbon flux, and extrapolating soil heating effects in one location is likely inaccurate for the other.

An understanding of microorganism responses to soil heating is essential for appropriate prediction of fire effects on soils and the ecosystems for which they are the foundation. In many of the plots where mineral soil temperatures greatly exceeded the commonly cited threshold of 60°C for 1 minute duration, we saw no significant impact on post-fire soil carbon efflux or vegetation communities, indicating that soil fauna and flora were not negatively impacted. The only significant results occurred in long unburned flatwoods at basal locations, where temperature-durations exceeded the traditional threshold many times over. That soil carbon respiration increased, however, indicates that even at these high doses of heat energy input, soil communities are resilient.

Rather than assuming that a significant response will occur when soil biota are exposed to 60°C for 1 minute, we recommend that future researchers make simple measures of soil biota that combine soil processes and the attributes of the biotic community (abundance and structure) to provide more useful and appropriate guidance that is specific to each ecosystem. These findings should encourage interdisciplinary research efforts that explicitly pair soil biotic changes and soil heating temperatures in order to better understand the integrated above- and below-ground responses to the use and restoration of wildland fire in these and other fire-frequent forests.

In order to improve capacity for prediction of fire effects on important ecosystems processes and components, improved soil heating models are needed. Our simple FOFEM-modeled vs. observed comparison revealed shortcomings found elsewhere. Incorporating recent soil heating research (funded by JFSP) along with greater attention to linking soil heating with vegetation responses in trees and other vegetation will be critical advances in predicting fire effects.

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Appendix B: List of Deliverables

Publications in Peer-reviewed Journals		
Completed	The Myth of the Biological Threshold: A Review of Biological Responses to Soil Heating Associated with Wildland Fire	Forest Ecology and Management
Completed	Pyrogenic flowering of Aristida beyrichiana following 50 years of fire exclusion: resilience of a foundational fire-facilitating bunchgrass	Ecosphere
Completed	Fire and tree death: Understanding and improving modeling of fire-induced tree mortality.	Environmental Research Letters
Completed	Contingent resistance in longleaf pine (Pinus palustris) growth and defense 10 years following smoldering fires	Forest Ecology and Management
Completed	Basal duff smoldering beneath old pines: a distinctive pattern of ground combustion	Forest Science
Completed	Advances in mechanistic approaches to quantifying biophysical fire effects.	Current Forestry Reports
Accepted, In revision	Long-duration soil heating resulting from forest floor duff smoldering in longleaf pine ecosystems: fire history and tree proximity matter	Forest Science
In preparation	Soil heating impacts on soil carbon cycling in frequently burned versus long-unburned pine forests	Soil Science Society of America Journal

Master's Thesis	Status	Title	Academic Institution
	Completed	Effects of Partitioning and Prescribed Fire on Soil CO2 Flux Rates in Flatwoods and Sandhills	University of Idaho

Other Publication	Status	Title	Pub Series
	Completed	Recent advances in understanding duff consumption and post-fire longleaf pine mortality.	eGTR-SRS-212

Presentations/ Workshops/ Field Tours		Title
Invited Paper/Presentation	Completed	Basal duff smoldering beneath old pines: a distinctive pattern of ground combustion .
Invited Paper/Presentation	Completed	Forest floor fire behavior and moisture dynamics: management concerns and ecological functions.
Invited Paper/Presentation	Completed	Restoring Fire to Fire Adapted Ecosystems
Invited Paper/Presentation	Completed	Another look at analyzing post-fire tree mortality
Invited Paper/Presentation	Completed	Bridging the gap between managers and scientists: the Southern pine duff story.
Invited Paper/Presentation	Completed	Consequences of long-duration soil heating for tree stress and mortality.
Invited Paper/Presentation	Completed	Ecological consequences of restoring fire following prolonged fire exclusion
Invited Paper/Presentation	Completed	Advances in understanding duff fires in longleaf pine forests

Training Session	Completed	Tree mortality module in National Advanced Silviculture Program
Field Demonstration/Tour	Completed	Duff Fire Science Symposium
Invited Paper/Presentation	Completed	Forest Floor Fire Behavior: Ecological Implications and Ecological Consequences
Invited Paper/Presentation	Completed	Smoldering in Forest Floor Duff: A Restoration Challenge
Training Session	Completed	RX 310: Combustion of organic soils and consequences for tree mortality
Invited Paper/Presentation	Completed	Soil heating effects on autotrophic vs. heterotrophic soil respiration rates across fire regimes
Invited Paper/Presentation	Completed	Pyroaerobiology: The transport and characterization of viable microorganisms by wildland fire smoke
Invited Paper/Presentation	Completed	Patterns of soil heating during prescribed burns across contrasting fire regimes in widespread south
Field Demonstration/Tour	Completed	The life of smoke: catching aerosolized microbes during prescribed burns in frequently burned forests
Field Demonstration/Tour	Completed	Consequences of fire management for duff and why you should care
Training Session	Completed	RX 310: Fire Effects on Soil Processes
Field Demonstration/Tour	Completed	Restoring sandhill ecosystems: lessons from fire regime restoration and management

Appendix C: Metadata

For all aspects of the project, metadata were recorded digitally for each of three subject areas: soil carbon respiration, soil heating, and vegetation surveys using a Microsoft Excel database. Both data and corresponding metadata were collected by hand in the field, digitized in Microsoft Excel format, and examined using standard methods to ensure quality control. All data and corresponding metadata have been submitted to the USDA Forest Service Research Data Archive, and the recommended FGDC CSDGM format Microsoft Word Form for metadata was used to compile information about data collection and preservation.