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## Variability of Carbon Stock in Florida Flatwoods Ecosystems Undergoing Restoration and Management

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VARIABILITY OF CARBON STOCK IN FLORIDA FLATWOODS ECOSYSTEMS UNDERGOING  
RESTORATION AND MANAGEMENT

by

KATHRYN ELIZABETH BECKER  
B.S. University of Florida, 2008

A thesis submitted in partial fulfillment of the requirements  
for the degree of Master of Science  
in the Department of Biology  
in the College of Sciences  
at the University of Central Florida  
Orlando, Florida

Spring Term  
2011

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## ABSTRACT

The global community is struggling with mitigating the effects of widespread habitat loss and degradation; the effects of which are being further magnified in the face of global climate change. Quality natural habitat is becoming increasingly limited and atmospheric carbon levels continue to rise. Therefore, land managers responsible for multiuse management are often faced with the dilemma of managing ecosystems for biodiversity, as well as optimizing ecosystem services such as carbon storage and sequestration. However, some management techniques used to meet these objectives may yield conflicting results, specifically, the management tool of prescribed fire. Fire is crucial in maintaining species composition and structure in many ecosystems, but also results in high carbon emissions. Thus, it is important for land managers to achieve the most efficient prescribed fire management regime to both preserve plant and animal communities, and optimize carbon storage. A former ranchland at the Disney Wilderness Preserve, Central Florida, USA is being restored to native ecosystems and managed to preserve biodiversity and increase carbon storage. This study quantified the carbon stocks within the aboveground biomass, litter, and top 90 cm of soil in five ecosystems at the Disney Wilderness Preserve, all of which are managed with prescribed fire every two to three years. These carbon stocks were compared in ecosystems in different stages of restoration: bahia grass pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods. The carbon stocks were also compared among three restored flatwoods communities: longleaf pine flatwoods, slash pine flatwoods, and scrubby flatwoods. To determine the effects of the current prescribed fire management, carbon stocks were quantified and compared in recently burned areas (burned 4 months prior) and areas burned

two to three years prior, in all ecosystems. Soil carbon properties were assessed using  $^{13}\text{C}$  isotope analysis. Aboveground biomass and litter carbon stocks were found to increase with higher stage of restoration, and were significantly less in areas with recent fire management. The results of this study did not provide evidence that soil carbon stock was significantly different in different stages of restoration or at different times since fire, but soil carbon stock was found to be significantly different among the flatwoods communities. In un-restored pasture and pasture in restoration sites, the soil was found to be increasingly depleted in  $^{13}\text{C}$  with increasing soil depth. This pattern indicated that carbon in the upper, more labile soil carbon pool had been derived from current C4 pasture or native grasses, while carbon in the deeper, more stable carbon pool is a legacy of the historical C3 forest vegetation that existed prior to conversion to pasture. Additionally, a pattern of less depletion in  $^{13}\text{C}$  with increasing time since deforestation was noted, indicating an increasing loss of historic forest carbon with increasing pasture age. As the pastures in restoration for longleaf pine flatwoods mature, the isotopic composition of the soil profile in the restored longleaf pine flatwoods may serve as a reference value for the soil profiles of these sites. Overall, the mean carbon stock in the aboveground biomass, litter and top 90 cm of soil in the un-restored pasture was  $\sim 13.3 \text{ kg C/m}^2$ , the carbon stock in the pasture in restoration was  $\sim 12.7 \text{ kg C/m}^2$ , the longleaf pine flatwoods had the highest carbon stock at  $\sim 17.7 \text{ kg C/m}^2$ , the scrubby flatwoods had the smallest carbon stock at  $\sim 7.7 \text{ kg C/m}^2$ , and the slash pine flatwoods had a carbon stock of  $\sim 15.8 \text{ kg C/m}^2$ .

I am dedicating this thesis to my brother, Thomas O. Brown, who has been an enduring source of inspiration to me during his life and always. Throughout our young lives, Tommy encouraged me to strive for excellence and he led by example. He shared with me his love for our family and friends, his love of nature, and his desire to protect our natural Florida wonders for future generations. He was and will always be my dearest friend.

## ACKNOWLEDGMENTS

I would like to thank The Nature Conservancy staff at Disney Wilderness Preserve for their support; particularly J. Mikaela Anderson for her contribution to the planning and data collection for this study. I would also like to thank Jesse Abelson and Emily Copeland for help with data collection. I am thankful for the comments and guidance from the department faculty that served on the committee for this thesis: Dr. C. Ross Hinkle, chair, Dr. Pedro Quintana-Ascencio, and Dr. John Weishampel. Thank you to Dr. Graham Worthy and Dr. Pedro Quintana-Ascencio for allowing me access to much of the laboratory equipment used in this study, and to the Light Stable Isotope Mass Spec Lab at the University of Florida for running the elemental and isotopic analyses. Also, I am grateful to my family for all of their support throughout this process, and always. This work was supported in part by the National Garden Club Scholarship to K. Elizabeth Becker and a research grant from The Nature Conservancy to the University of Central Florida.

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## INTRODUCTION

Native ecosystems provide unparalleled opportunities for biodiversity and many environmental and ecological services such as habitat for endemic, threatened, and endangered species. However, the reduction of quality native habitat has drastic negative effects on these ecosystem services worldwide. Currently, habitat degradation, destruction, and fragmentation are also magnifying the effects of climate change on the world's species (Pyke 2005; Van Lear et al. 2005). With limited quality and connected habitat, species attempting to respond to climate change through range shifts are imperiled (Thuiller et al. 2006). As a result, conserving or restoring native ecosystems is crucial to maintain species richness.

The intrinsic value of increased biodiversity that ecosystem restoration and management holds is priceless. Unfortunately, biodiversity is often not sufficient to encourage restoration and management of private lands. As global climate continues to change, offsetting anthropogenic carbon emissions is a foremost concern for many industries, therefore quantifying the carbon sequestered and stored in an ecosystem can be used to assign an economic value to restoration in the form of carbon offsets (Stainback and Alavalapati 2004; Galatowitsch 2009). These carbon offsets may provide private landowners with an economic incentive as well as the financial means to conduct restoration projects and management on their land. It is through private land owner participation that connectivity of these ecosystems can be reestablished, giving many species the opportunity to endure for future generations (Alavalapati et al. 2002).

Ecosystem uptake and storage of carbon has been identified as crucial in mitigating the effect of the increasing addition of carbon to the atmosphere, as it has been well documented in the literature that terrestrial ecosystems have a great potential to sequester carbon (Pacala and Socolow 2004; Houghton 2007). The vegetation and top meter of soil across the Earth's terrestrial ecosystems holds approximately 2300 Pg C (1 Pg =  $10^{15}$  g = 1 billion metric tons):  $550 \pm 100$  Pg C in the vegetation and  $1750 \pm 250$  Pg C in the top meter of soil. Together, these carbon reservoirs hold almost three times the amount of carbon in the atmosphere (800 Pg C), but they are being reduced by changes in land use, such as deforestation for agriculture (Houghton 2007). Accordingly, the restoration of terrestrial ecosystems has been proposed as a means to both preserve biodiversity and increase terrestrial carbon storage (Galatowitsch 2009).

Land managers responsible for multiuse management of preserving biodiversity and optimizing carbon storage and sequestration often must employ management techniques that yield conflicting results, specifically, the management tool of prescribed fire. Fire is crucial in maintaining species composition and structure in many ecosystems, but also results in high carbon emissions (Abrahamson and Hartnett 1990). As a result, it is important to achieve the most efficient prescribed fire management regime to both preserve plant and animal communities, and optimize carbon storage (Galatowitsch 2009).

Although it is a widely used management tool in the southeastern U.S., the effects of prescribed fire management on ecosystem carbon stock are not fully agreed upon in the literature, and

have been found to vary greatly between land uses such as pine plantations compared to natural pine forests (Johnson 1992; Houghton et al. 2000; Johnson and Curtis 2001; Garten 2006; Alexis et al. 2007; Lavoie et al. 2010). This variation is mostly caused by differences in patchiness, fire return interval, and fire intensity; and these variables are often related (Johnson and Curtis 2001). For example, an ecosystem that is frequently burned, such as every three years, will likely have less intense fires and more patchiness due to lower fuel availability than an ecosystem that experiences fire every ten years (Abrahamson 1984). The ecosystem with a shorter fire return interval will gain less biomass between fires, and will likely lose less biomass during a fire (Garten 2006). Also, frequent low intensity fires will be less likely to result in a loss of mineral soil carbon stock (Johnson 1992).

One ecosystem that is repeatedly the focus of potential carbon storage and restoration efforts is the longleaf pine flatwoods community of the southeastern U.S. (Golkin and Ewel 1984; Houghton and Hackler 2000; Alavalapati et al. 2002; Kush et al. 2004; Stainback and Alavalapati 2004; Woodbury et al. 2006; Han et al. 2007; Diop et al. 2009). Longleaf pine flatwoods are often regarded as one of the most endangered ecosystems in North America with a 97% loss in area as a result of widespread land development, agriculture, fire suppression, and deviation from natural hydrologic regimes (Abrahamson and Hartnett 1990; Ewel 1990; Van Lear et al. 2005). Many studies have encouraged restoring this ecosystem to preserve the biodiversity associated with it, and have suggested carbon storage as an additional incentive to motivate land owners (Alavalapati et al. 2002; Kush et al. 2004; Stainback and Alavalapati 2004; Van Lear et al. 2005). The two most recognized longleaf pine restoration sites are the Joseph W. Jones

Ecological Research Center at Ichauway, Newton, GA, USA and Tall Timbers Research Station, Tallahassee, FL, USA. These sites are managed by for multiple land uses including biodiversity, timber production, and hunting. Currently, research is being conducted at both of these restoration sites to assess the ability of the longleaf pine flatwoods community to reach carbon sequestration and storage objectives, even with the application of prescribed fire management (Joseph W. Jones Ecological Research Center 2011; Tall Timbers Research Station 2011).

Restoration of the longleaf pine ecosystem often occurs in isolated locations, and land use history and current and historical fire dynamics can vary immensely between restoration sites (Van Lear et al. 2005). Therefore, to develop goals of carbon storage for restoration and fire management it is important to quantify and monitor biomass, litter, and soil carbon stocks for the site at different stages of restoration and at different times since fire management. Although biomass and litter carbon stocks are not as large as soil carbon stock, they are often where the most change in carbon storage is realized during restoration and fire management (Woodbury et al. 2006; Alexis et al. 2007; Lavoie et al. 2010). A model presented in Woodbury et al. 2006 estimated that after 40 years, afforestation in areas of the southeastern U.S. will increase litter carbon stock from 0 Mg ha<sup>-1</sup> to 12 Mg ha<sup>-1</sup>, soil carbon content was estimated to increase by approximately 25%, and increased carbon storage in trees was found to be seven times greater than either litter or soil carbon increases. However, this model does not account for fire effects, and these estimations are likely to vary greatly with frequent prescribed fire management. In particular, prescribed fire has been found to increase carbon content in litter



and soil due to the production of degradation resistant pyrogenic carbon, or charcoal (Kuhlbusch 1998; Alexis et al. 2007; Ohlson et al. 2009; Alexis et al. 2010).

Additionally, when evaluating the effects of ecosystem management and restoration on carbon stock it is important not only to assess the overall change in carbon content, but to also assess the change in carbon dynamics. Changes in plant communities associated with deforestation and afforestation often involve a loss or gain of soil carbon and a shift from C3 plants to C4 plants, or vice versa (Rhoades et al. 2000; Del Galdo et al. 2003). The switch between these two photosynthetic pathways is distinguishable in the soil profile using stable carbon isotope analysis (Ehleringer et al. 2000; Rhoades et al. 2000; Wang and Hsieh 2002; Del Galdo et al. 2003). It is the distinct isotopic signatures associated with these two photosynthetic pathways that make this transition detectable. The C3 photosynthetic pathway experiences higher levels of discrimination of heavier carbon ( $^{13}\text{C}$ ) isotopes than the C4 photosynthetic pathway, and results in C3 plants having a  $\delta^{13}\text{C}$  isotopic signature of -21‰ to -30‰ which is more depleted in  $^{13}\text{C}$  when compared to C4 plants which have a  $\delta^{13}\text{C}$  isotopic signature of -10‰ to -15‰ (Farquhar et al. 1989; Ehleringer et al. 2000). Therefore, losses of historic C3 forest carbon pools under C4 pasture vegetation can be detected by changes in the soil's isotopic profile (Peterson and Neill 2003).

The objective of this study was to establish carbon stock values for a Florida flatwoods restoration site that is being established to both preserve biodiversity and increase ecosystem carbon storage through restoration and management. Ultimately, the results of this study will

be used in combination with the results of other studies conducted at this site, such as carbon flux measurements, to set restoration and management goals for this site in the context of optimized ecosystem carbon storage and sequestration. To meet this objective the research presented in this study quantified the carbon stocks within the aboveground biomass, litter layer, and top 90 cm of soil in five Florida ecosystems at the restoration site, which are all undergoing prescribed fire management. These carbon stocks were compared in ecosystems in different stages of restoration: improved bahia grass pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods, as well as several restored flatwoods communities: longleaf pine, slash pine, and scrubby flatwoods. Soil carbon dynamics were assessed for each of these five ecosystems using stable isotope analysis. The reader should note that although total belowground biomass was not incorporated in this study, it is an important feature for carbon storage in these ecosystems and will be addressed in future research.

### Study Site

The study site was The Nature Conservancy's Disney Wilderness Preserve (DWP) (28° 6' 11" N, 81°25' 31" W). DWP is a 4654 ha preserve located just south of Kissimmee, FL, USA (Figure 1) (Wertschnig and Duever 1995). The climate is subtropical and characterized by hot summers and mild winters, with a wet season from May to October and a dry season from November to April. The lowest monthly mean temperature (15°C) occurs in January and the highest monthly

mean temperature (27°C) occurs in August. Average annual precipitation at DWP is 1142 mm yr<sup>-1</sup> (recorded 1998-2010) (South Florida Water Management District Station WRWX). Elevation of DWP ranges from 50 to 70 m.a.s.l. (TNC 1996).

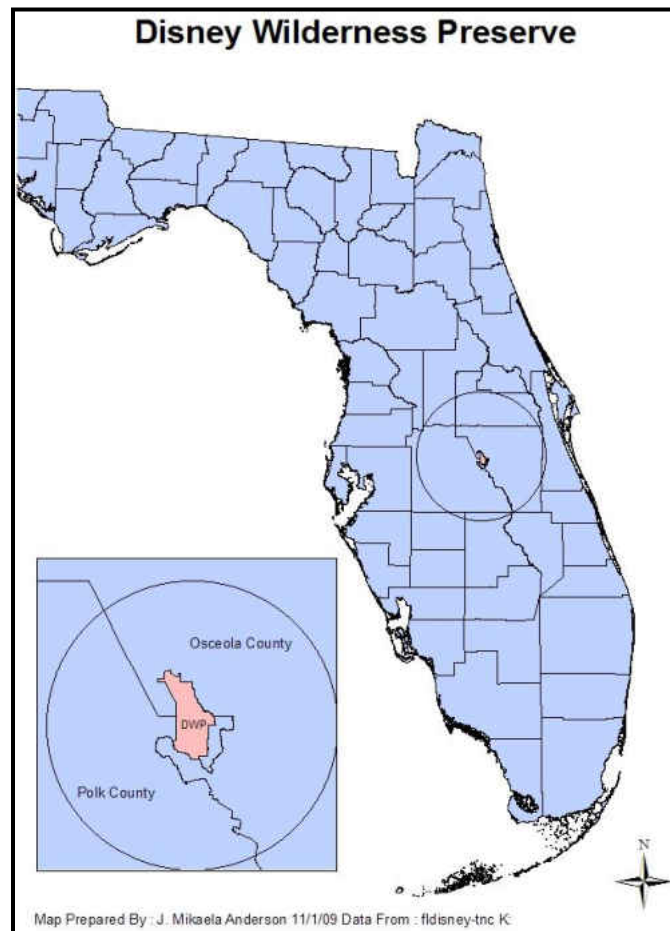


Figure 1. Map of Florida showing the location of the Disney Wilderness Preserve.

DWP is located at the head waters of the Everglades. This locality makes DWP a particularly important site for restoration, and has led The Nature Conservancy (TNC) to add the goal of enhanced water quality for the Everglades system to their objectives for DWP (D. Gordon, personal communication, TNC at DWP). When DWP was purchased in 1992 it was a cattle

ranch dominated by wetlands, various pastures and rangelands including improved bahia grass (*Paspalum notatum* Flugge) pasture, and degraded flatwoods ecosystems that had been grazed by cattle since at least the late 1800s. The ranch was burned in the winter (dormant season) every 3 to 5 years in most areas, and as often as 2 to 3 years in some locations (TNC 1996). Restoration and management of the preserve's flatwoods ecosystems and some pasture land has been underway since 1995 (Wertschnig and Duever 1995).

The restoration and ongoing management activities associated with the flatwoods communities at DWP include the removal of invasive species, such as wild boars (*Sus scrofa*) and cogon grass (*Imperata cylindrical* Beauv.), and growing season prescribed fires every two to three years to reestablish and maintain ecosystem structure, function, and composition. It is assumed that the historical land cover of the pasture areas was longleaf pine flatwoods, and soil classification has indicated the same soil series in the pastures and longleaf pine flatwoods; thus, the restoration efforts in the pastures are focused on returning these areas to this flatwoods community (Wertschnig and Duever 1995; TNC 1996). The restoration efforts in the pastures have also included the removal of non-native plant and animal species and a return of the natural fire regime, as well as plantings of native species, including *Aristida stricta* Michx. (wiregrass), numerous *Andropogon* species, and *Pinus palustris* Mill. (longleaf pine) seedlings (TNC 1996). At the time of this study, the pastures in restoration were dominated by native grasses and herbs, as native woody plant species had yet to reestablish.

Scrubby flatwoods are home to many endemic plants, including 13 federally listed endangered or threatened species, and 22 state listed species (Abrahamson and Hartnett 1990). In addition, numerous endemic, threatened, and endangered vertebrate species use this and the other flatwoods ecosystems for habitat. The most notable threatened endemic to scrubby flatwoods is the Florida scrub jay (*Aphelocoma coerulescens coerulescens*). This bird is geographically isolated to the scrub ecosystem, and has a complex social behavior that makes it extremely vulnerable to habitat degradation and fragmentation (Myers 1990). There are also many threatened and endangered species that use pine flatwoods as habitat. These species include striped newt (*Notophthalmus perstriatus*), red-cockaded woodpecker (*Picoides borealis*), Florida black bear (*Ursus americanus floridanus*), Sherman's fox squirrel (*Sciurus niger shermani*) and Florida panther (*Puma concolor coryi*) (Abrahamson and Hartnett 1990).

Plant species composition, vegetation structure, and soil composition of the studied ecosystems varies considerably (Abrahamson and Hartnett 1990; Brown et al. 1990). Slash pine flatwoods are found in the southern region of the preserve (Figure 2) on very poorly drained Spodosols and Alfisols, including Ona fine sand and Malabar fine sand that often have standing water (USDA and Soil Conservation Service 1985). The vegetation in this ecosystem is characterized by a low density *Pinus elliottii* Engelm. var *elliottii* (slash pine) dominated canopy, a dense understory comprised mostly of *Serenoa repens* (Bartr.) Small (saw palmetto) with scattered *Ilex glabra* (L.) Gray (gallberry) and *Lyonia* (fetterbush and staggerbush) species, and groundcover of many native grasses and herbs, most notably wiregrass.

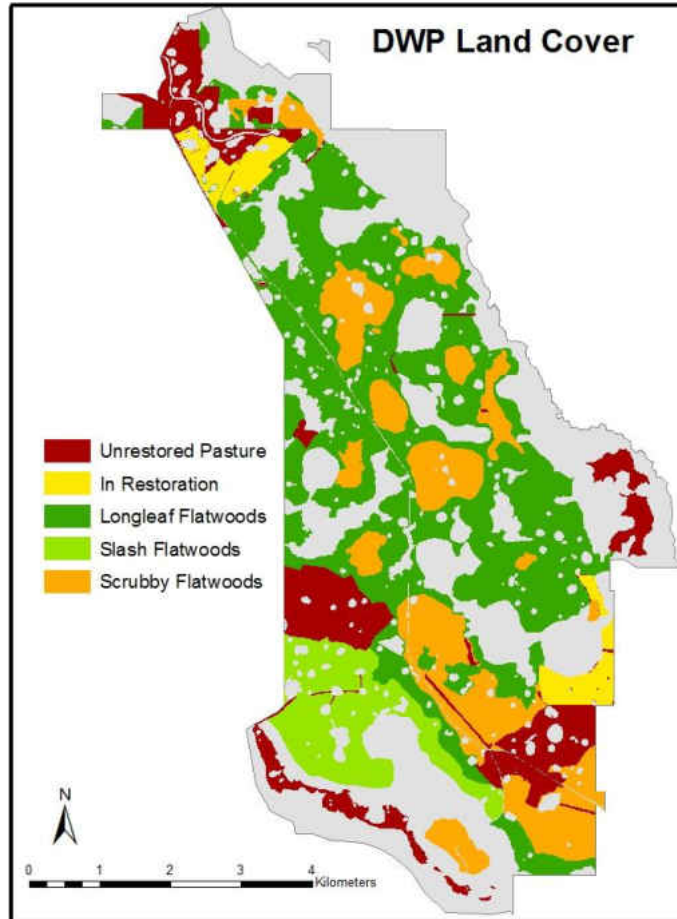


Figure 2. Map of ecosystems of interest at the Disney Wilderness Preserve.

Longleaf pine flatwoods are the most abundant ecosystem type on the preserve (Table 1), and are found on poorly drained Spodosols of the Myakka fine sand, Smyrna fine sand, and Immokala fine sand soil series, but do not experience as much standing water as slash pine areas (USDA and Soil Conservation Service 1976; USDA and Soil Conservation Service 1985). The vegetation composition of this ecosystem is a low density longleaf pine dominated canopy, and like slash pine flatwoods, longleaf pine flatwoods have a dense understory of saw palmetto with scattered gallberry, fetterbush, and staggerbush species, and a groundcover of many native grasses and herbs, in particular wiregrass. Some important differences between longleaf

and slash pines include that longleaf pines are more resistant to frequent low intensity fire than slash pines, they have a longer lifespan than slash pines, and also denser wood than that of slash pines. As a result, longleaf pines have a greater accumulation of biomass throughout their lifespan and also greater carbon content per volume when compared to slash pines (Kush et al. 2004).

Table 1. Areas of ecosystems of interest at the Disney Wilderness Preserve.

Ecosystem	Area (ha)
Un-restored Pasture	470
Pasture in Restoration for Longleaf Pine Flatwoods	123
Restored Longleaf Pine Flatwoods	1262
Restored Slash Pine Flatwoods	288
Restored Scrubby Flatwoods	680
Other	1831
Total	4654

Scrubby flatwoods occur on moderately to well drained, highly permeable Spodosols and Entisols, including Duette fine sand and Satellite sand throughout the preserve (USDA and Soil Conservation Service 1976; USDA and Soil Conservation Service 1985). The species composition of scrubby flatwoods included a very sparse canopy of longleaf pine, and a dense understory of saw palmetto and scrub oak species including *Quercus geminata* Small, *Quercus myrtifolia* Willd., and *Quercus chapmanii* Sargent, and also scattered gallberry, fetterbush, and staggerbush species. In many locations, the understory of the scrubby flatwoods on the preserve is much taller than the understory of the pine flatwoods.

## MATERIALS AND METHODS

### Plot Distribution

The ecosystems evaluated in this study were improved bahia grass pasture, pasture in restoration for longleaf pine flatwoods, restored longleaf pine flatwoods, restored slash pine flatwoods, and restored scrubby flatwoods; all with and without recent fire management (Figure 2). I quantified and compared the aboveground biomass, litter layer, and soil carbon stocks in these ecosystems. I also analyzed the percent carbon and isotopic ( $^{13}\text{C}$ ) profile of the litter layer and soil depths. For the purposes of this study, I will be referring to ecosystems with recent fire management as “burned” and without recent fire management as “unburned.”

Ten random sampling points (five with recent fire and five without) were established in each ecosystem type using Hawth’s tools in ArcGIS (ArcGIS 9.3.1, ESRI Inc.) (Figure 3). Each ecosystem type was buffered by 25 m to prevent points occurring in ecotone regions. Also, parameters were set to ensure that all sample points within an ecosystem type were separated by at least 50 m, to allow for the establishment of independent 400 m<sup>2</sup> circular plots around the sample points. Plots with recent prescribed fire management were established four months after fire, and plots without recent prescribed fire management were established at least two years after fire. These time since fire intervals captured the range of the fire management cycle of DWP, as most areas on the preserve are burned every two to three years. It is important to note that, as shown in Figure 3, the burned and unburned plots were not located in the same



sites on the preserve. As a result, site may serve as a confounding factor with time since fire management in this study.

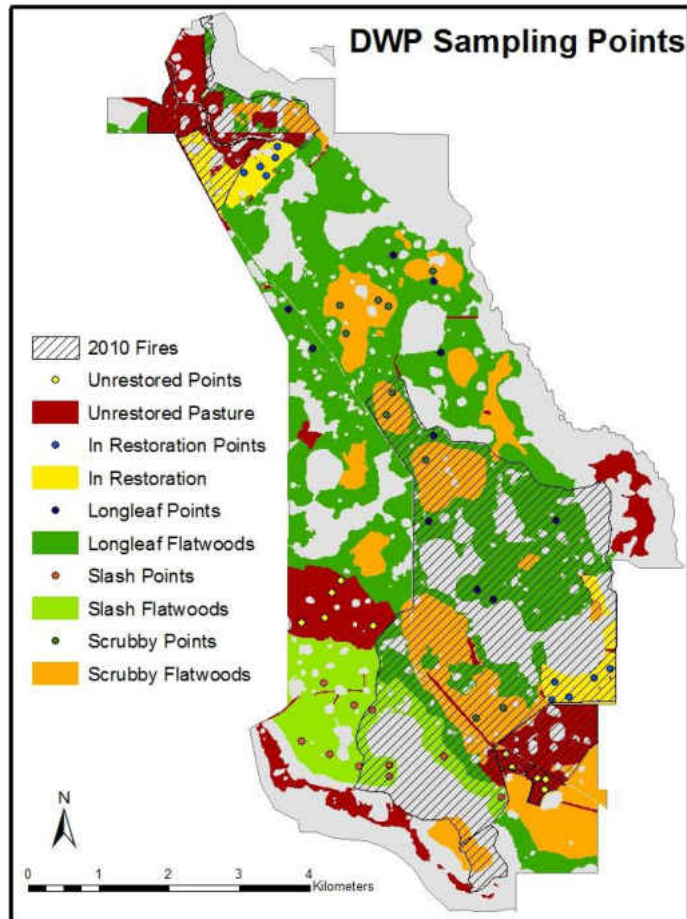


Figure 3. Map of random sampling points established in ecosystems of interest.

### Aboveground Biomass: Measurements and Carbon Stock Calculations

To capture the heterogeneity in each ecosystem type, a stratified sampling design was used. Circular plots with areas of 400 m<sup>2</sup> were established around each of the random points (Figure

4). Within the entire 400 m<sup>2</sup> plot, height and diameter at breast height (DBH) were recorded for live trees and snags with DBH > 7.6 cm. Snags were identified at either the species or genus level, and assigned to one of five decay classes adapted from Thomas (1979). In four subplots, each 1 m in radius, located at the midpoint of the north, east, south, and west axes of the plot, height and diameter at root collar (drc) were recorded for trees with DBH < 7.6 cm, coarse woody debris (diameter > 10 mm), and shrubs (drc > 10 mm). Grasses, herbs, and shrubs (drc < 10 mm) were collected in a 1 m<sup>2</sup> subplot, dried at 60° C for a minimum of 48 hr, and weighed to estimate groundcover biomass (Figure 4).

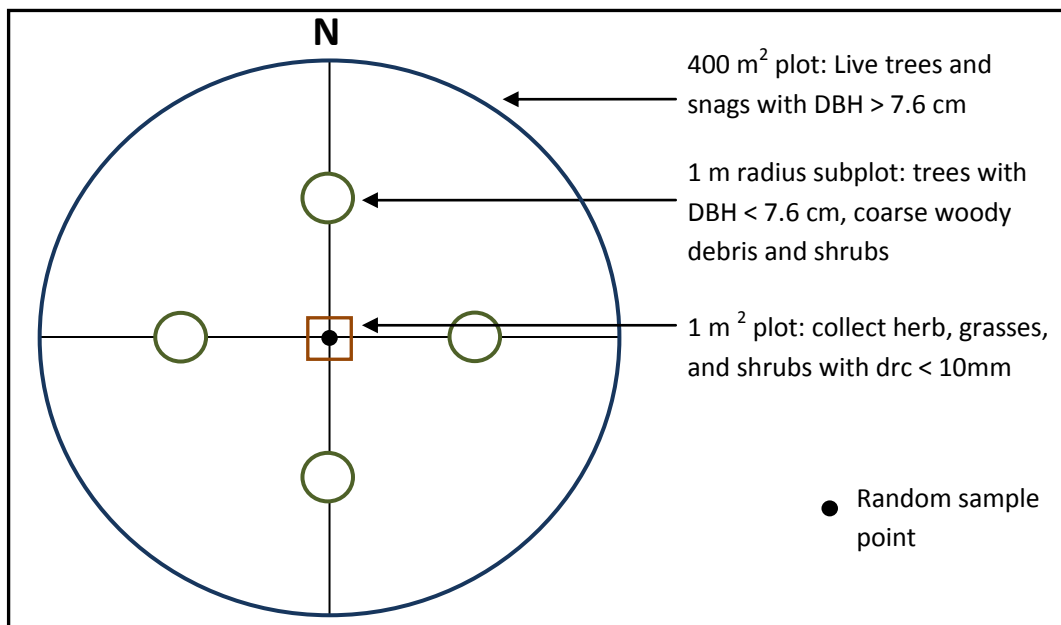


Figure 4. Diagram of stratified sampling design for aboveground biomass.

In each plot, dry mass was estimated for individuals of all tree and shrub species and all snags and coarse woody debris using allometric equations (Table 2). For some species, individuals in the plots at DWP surpassed the diameter reported in the literature for the plants used to

establish these equations. To ensure the allometric equations were applicable for these larger individuals, data were collected from other studies where the diameter of larger individuals had been measured and dry mass had been calculated through destructive harvest (Troy Seiler, University of Central Florida, and Warren G. Abrahamson, Bucknell University, personal communication). Density ( $\text{g/cm}^3$ ) for snags and woody debris was determined for each species and decay class using values from Harmon et al. (2008).

Table 2. Equations used to calculate aboveground dry mass for trees (kg), snags (kg), shrubs (g), palms (g), and woody debris (g).

Species	Equation	$R^2$	Source
<i>Pinus palustris</i>	$\text{Log}_{10}(\text{mass}) = -0.99717 + 1.00242\text{Log}_{10}(\text{DBH}^2(\text{in}) * \text{Hgt}(\text{ft})) * 0.45359$	0.99	Taras & Clark (1977)
<i>Pinus elliotii</i>	$\ln(\text{mass}) = -2.715 + 1.261\ln(\text{DBH}^2(\text{cm}) * \text{Hgt}(\text{m}))$	0.95	Jokela & Martin (2000)
Snags	$\text{mass} = (\pi(\text{DBH}(\text{cm})/2)^2) * (\text{Hgt}(\text{m}) * 100) * (\text{Density}) / 1000$	NA	Harmon & Sexton (1996)
<i>Quercus myrtifolia</i>	$\ln(\text{mass}) = -1.915 + 2.888\ln(\text{drc})$	0.92	Dijkstra et al. (2002)
<i>Quercus geminata</i>	$\ln(\text{mass}) = -1.423 + 2.599\ln(\text{drc})$	0.91	Dijkstra et al. (2002)
<i>Quercus chapmanii</i>	$\ln(\text{mass}) = -1.439 + 2.574\ln(\text{drc})$	0.97	Seiler et al. (2009)
<i>Lyonia</i> spp.	$\text{mass} = (0.1261\text{drc}^{2.1016}) + (0.0208\text{drc}^{3.1103})$	NA	Alexis et al. (2007)
<i>Serenoa repens</i>	$\text{mass} = \text{Exp}(0.64 \ln(\text{min crown}) + 2.3 \ln(\# \text{ leaves}) + 0.254)$	0.84	Abrahamson (2007)
<i>Sabal etonia</i>	$\text{mass} = 10.71(\text{min crown}) + 332.5(\# \text{ leaves}) - 826.3$	0.86	Abrahamson (2007)
Woody debris	$\text{mass} = (\pi(\text{drc}(\text{mm})/20)^2) * (\text{Hgt}(\text{cm})) * (\text{Density})$	NA	Harmon & Sexton (1996)

Diameter at root collar (drc) measured in mm. "min crown" indicates minimum crown width measured in cm. NA indicates that no  $R^2$  value was reported in the literature. *Serenoa repens* and *Sabal etonia* equations include rhizome mass; all other equations include aboveground mass only.

Total aboveground biomass ( $\text{kg/m}^2$ ) of trees, snags, shrubs and woody debris was calculated at the plot level by summing the dry mass of trees, snags, shrubs and woody debris measured in

the plot and dividing by the area measured. The aboveground carbon stock ( $\text{kg C/m}^2$ ) of trees, snags, shrubs and woody debris was calculated for each plot using an estimate of 50% carbon and the total biomass of the individuals in the plot (Curtis 2008). Ground cover carbon stock ( $\text{kg C/m}^2$ ) was calculated for each plot using the dry mass of the ground cover sample collected, the area sampled, and an estimate of 50% carbon. Total aboveground carbon stock ( $\text{kg C/m}^2$ ) for each plot was calculated by summing the carbon stocks for the trees, snags, shrubs, woody debris and ground cover.

#### Litter and Soil: Sampling and Processing

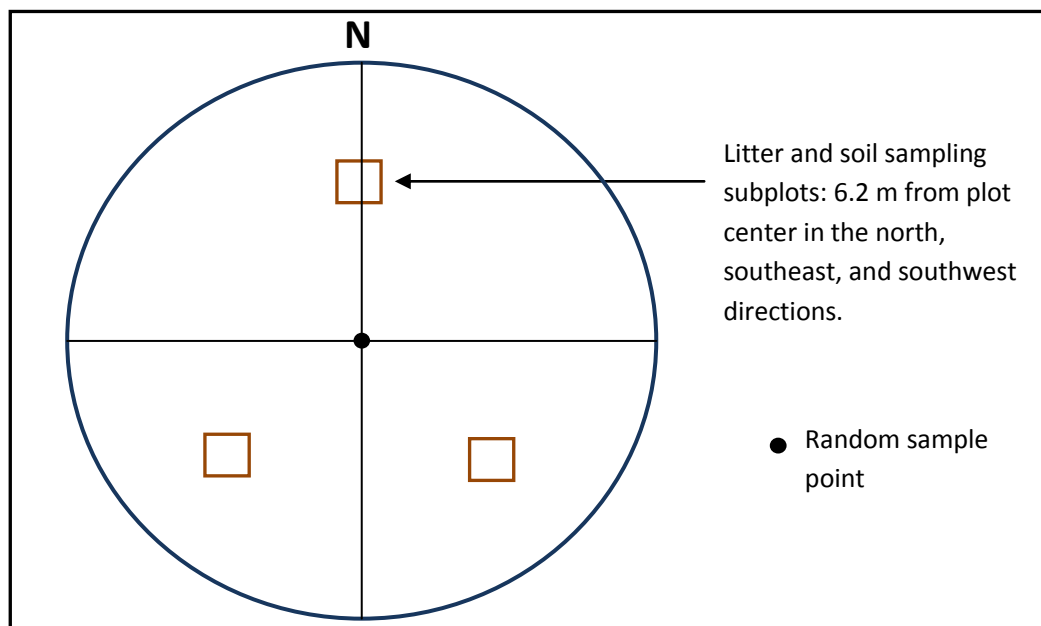


Figure 5. Diagram of sub-sampling design for litter and soil samples.

Within each 400 m<sup>2</sup> sampling plot, three subplots were established 6.2 m from plot center in the north, southeast, and southwest directions (Figure 5). At each of the three subplots, litter was collected within a 400 cm<sup>2</sup> PVC cutting square. Organic horizons were not separated due to the absence of distinguishable O<sub>i</sub> (slightly decomposed) and O<sub>e</sub>+O<sub>a</sub> (highly and intermediately decomposed) horizons (USDA 2006; Burton and Pregitzer 2008). Litter samples were dried at 60° C for a minimum of 48 hr, weighed, and ground using a Spex 8000M Mixer/Mill.

After the litter was collected, a 90 cm deep soil core was taken at each subplot. Each soil core was divided into three depth horizons: 0-30 cm, 30-60 cm, and 60-90 cm. Once sampled soils were immediately placed in a cooler to be transported to the lab. Each soil horizon was homogenized across the three cores, sieved using a 2 mm screen, dried to a constant weight at 60° C, weighed and ground using a Spex 8000M Mixer/Mill. Organic material not passing the 2 mm screen was dried at 60° C for a minimum of 48 hr, separated into charcoal and non-charred organic matter, weighed, ground using a Spex 8000M Mixer/Mill, and analyzed separately (Burton and Pregitzer 2008).

#### Litter and Soil: Isotopic Analysis, Elemental Analysis, and Carbon Stock Calculations

Continuous-flow isotope ratio mass spectroscopy (CF-IRMS) was used to determine the percent carbon and isotopic (<sup>13</sup>C) profile for the litter layer and soil horizons of the different ecosystems. The CF-IRMS system used was a Carlo Erba NA1500 CNS elemental analyzer linked to a

Finnigan-MAT DeltaPlus IRMS (Thermo Electron Corporation, Bremen, Germany) at the Light Stable Isotope Mass Spectroscopy Laboratory in the Department of Geological Sciences at the University of Florida. Stable carbon isotope data were reported in delta (‰) notation. Delta notation ( $\delta$ ) is calculated using the ratio ( $R$ ) of  $^{13}\text{C}$  to  $^{12}\text{C}$  in the sample compared to the ratio in Pee Dee Belemnite, a primary standard, as follows (Ehleringer 2000):

$$\delta = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000 \text{ ‰} \quad (1)$$

At all sampled soil depths in the un-restored pasture and pasture in restoration for longleaf pine flatwoods, the percent of soil carbon contributed by the bahia grass pasture and/or native grasses and herbs in the restoration sites (% CC4), most of which are C4 plants, and the percent of soil carbon contributed by the historic longleaf pine flatwoods community (% CC3), dominated by many C3 plants, was determined using the following mixing models (Balesdent and Mariotti 1996, as presented in Hail et al. 2010):

$$\% \text{ CC4} = (\delta^{13}\text{C}_s - \delta^{13}\text{CC3}) / (\delta^{13}\text{CC4} - \delta^{13}\text{CC3}) \times 100 \% \quad (2)$$

$$\% \text{ CC3} = 100 - \% \text{ CC4} \quad (3)$$

where  $\delta^{13}\text{C}_s$  is the  $\delta^{13}\text{C}$  of a given sample,  $\delta^{13}\text{CC3}$  is the mean  $\delta^{13}\text{C}$  of the litter in the restored longleaf pine flatwoods, and  $\delta^{13}\text{CC4}$  is the mean  $\delta^{13}\text{C}$  in the litter of the un-restored pasture and pasture in restoration for longleaf pine flatwoods. Table 3 shows the C3/C4 status and approximate  $\delta^{13}\text{C}$  isotopic signatures of the dominant species that occur in the five studied

ecosystems. The  $\delta^{13}\text{C}$  signatures of these plant species vary with environmental factors such as water availability, but will still retain a characteristic C3 or C4 signature respectively.

Table 3. Dominant plant species that occur in the five studied ecosystems; their C3/C4 status and approximate  $\delta^{13}\text{C}$  isotopic signatures.

	Species	$\approx \delta^{13}\text{C}$	Source
C3 plants	<i>Pinus palustris</i>	- 28.0 ‰	Samuelson et al. 2003
	<i>Pinus elliottii</i>	- 28.5 ‰	Haile et al. 2010
	<i>Serenoa repens</i>	- 27.0 ‰	Foster and Brooks 2005
	<i>Lyonia lucida</i>	- 27.8 ‰	Foster and Brooks 2005
	<i>Quercus myrtifolia</i>	- 28.0 ‰	Foster and Brooks 2005
	<i>Quercus geminata</i>	- 28.5 ‰	Foster and Brooks 2005
	<i>Quercus chapmanii</i>	- 27.5 ‰	Foster and Brooks 2005
C4 plants	<i>Paspalum notatum</i>	- 13.3 ‰	Haile et al. 2010
	<i>Aristida stricta</i>	- 13.8 ‰	Das et al. 2010
	<i>Andropogon virginicus</i>	- 11.7 ‰	Smith and Brown 1973
	<i>Andropogon glomeratus</i>	- 14.1 ‰	Smith and Brown 1973

Percent carbon of the litter, mass of litter sample, and area sampled were used to calculate the carbon stock ( $\text{kg C/m}^2$ ) of the litter layer. Soil carbon stock ( $S_C$ ,  $\text{kg C/m}^2$ ) was calculated using the horizon depth ( $D$ ), percent carbon of materials passing the 2 mm screen ( $C_S$ ), mass of materials passing the 2 mm screen ( $M_S$ ), percent carbon of organic materials 2-10 mm in diameter ( $C_{OM}$ ), mass of organic materials 2-10 mm in diameter ( $M_{OM}$ ), and the total volume of the depth increment ( $V$ ) as follows (Burton and Pregitzer 2008):

$$S_C = D \times (10,000 \text{ cm}^2/\text{m}^2)[(C_S \times M_S) + (C_{OM} \times M_{OM})]/V \quad (4)$$

## Statistical Analysis

Ecosystem types were analyzed as part of two comparisons. The first analysis compared ecosystems in different stages of restoration and at different times since prescribed fire management. This comparison included: un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire management. The second analysis compared different flatwoods communities at different times since prescribed fire management. This analysis included: restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire management.

All analyses were conducted using R version 2.11.1 (The R Foundation for Statistical Computing, Vienna, Austria). A two-way ANOVA was used to compare the carbon stocks of the aboveground biomass, litter layer, and soil of the different ecosystems, as well as the organic matter and charcoal present in the soil, and the percent carbon and  $\delta^{13}\text{C}$  isotopic signature by soil depth. Tukey's HSD test was used for pair wise post-hoc comparisons (Gotelli and Ellison 2004). Paired t-tests were used to compare the percent of total carbon derived from C3 vegetation in the soil horizons of the un-restored pasture and pasture in restoration for longleaf pine flatwoods.



## RESULTS

### Comparing Stages of Restoration and Time Since Fire Management

#### Aboveground Biomass Carbon Stock

Mean carbon stock in aboveground biomass varied significantly with both stage of restoration ( $P = 0.000$ ) and time since prescribed fire management ( $P = 0.014$ ), but no significant interaction was found between stage of restoration and time since prescribed fire management ( $P = 0.728$ ). Tukey's HSD test indicated that the mean carbon stock in aboveground biomass varied significantly between all stages of restoration. Mean carbon stock in aboveground biomass of un-restored pasture was significantly less than that of pasture in restoration for longleaf pine flatwoods ( $P = 0.026$ ), and mean carbon stock in aboveground biomass was found to be significantly higher in restored longleaf pine flatwoods compared to un-restored pasture ( $P = 0.000$ ) and pasture in restoration for longleaf pine flatwoods ( $P = 0.000$ ).

Mean carbon stock in aboveground biomass of unburned un-restored pasture was  $0.27 (\pm 0.14)$  kg C/m<sup>2</sup>, and mean carbon stock in aboveground biomass of burned un-restored pasture was  $0.12 (\pm 0.04)$  kg C/m<sup>2</sup>, showing a loss of 53% of the aboveground carbon stock four months after prescribed fire management (Figure 6). Pasture in restoration for longleaf pine flatwoods also showed a loss of 53% of aboveground carbon stock with recent prescribed fire management.

Mean carbon stock in aboveground biomass of unburned pasture in restoration was 0.43 ( $\pm 0.06$ ) kg C/m<sup>2</sup>, while mean carbon stock in aboveground biomass of burned pasture in restoration was 0.20 ( $\pm 0.03$ ) kg C/m<sup>2</sup>. The largest aboveground biomass carbon stock was measured in unburned restored longleaf pine flatwoods, which had a mean carbon stock of 2.27 ( $\pm 0.63$ ) kg C/m<sup>2</sup>. Restored longleaf pine flatwoods that had experienced recent prescribed fire management had a mean aboveground biomass carbon stock of 0.95 ( $\pm 0.21$ ) kg C/m<sup>2</sup>. Thus, prescribed fire had the largest effect on aboveground biomass in restored longleaf pine flatwoods, resulting in a loss of 58% of aboveground biomass carbon stock at four months after fire.

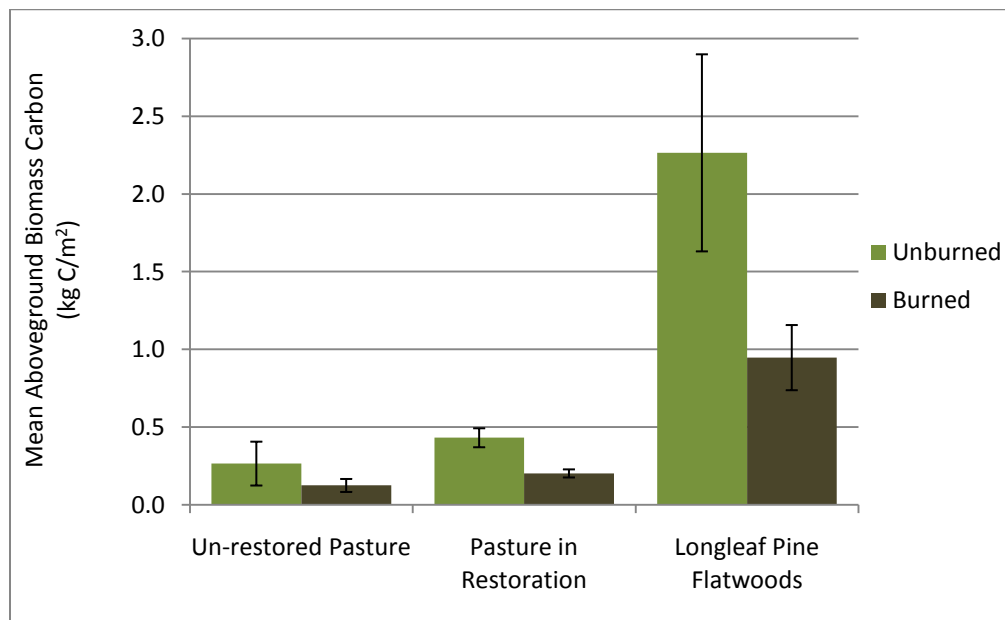


Figure 6. Mean carbon stock (kg C/m<sup>2</sup>) in aboveground biomass of un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the mean (n=5).

## Litter Carbon Stock

Mean litter carbon stock also varied significantly with both stage of restoration ( $P = 0.043$ ) and time since prescribed fire management ( $P = 0.001$ ), but no significant interaction was found between stage of restoration and time since prescribed fire management ( $P = 0.507$ ). Tukey's HSD test indicated that the mean litter carbon stock of un-restored pasture did not differ significantly from that of restored longleaf pine flatwoods ( $P = 0.117$ ) or pasture in restoration for longleaf pine flatwoods ( $P = 0.900$ ), but the mean litter carbon stock of restored longleaf pine flatwoods was significantly higher than that of pasture in restoration for longleaf pine flatwoods ( $P = 0.048$ ).

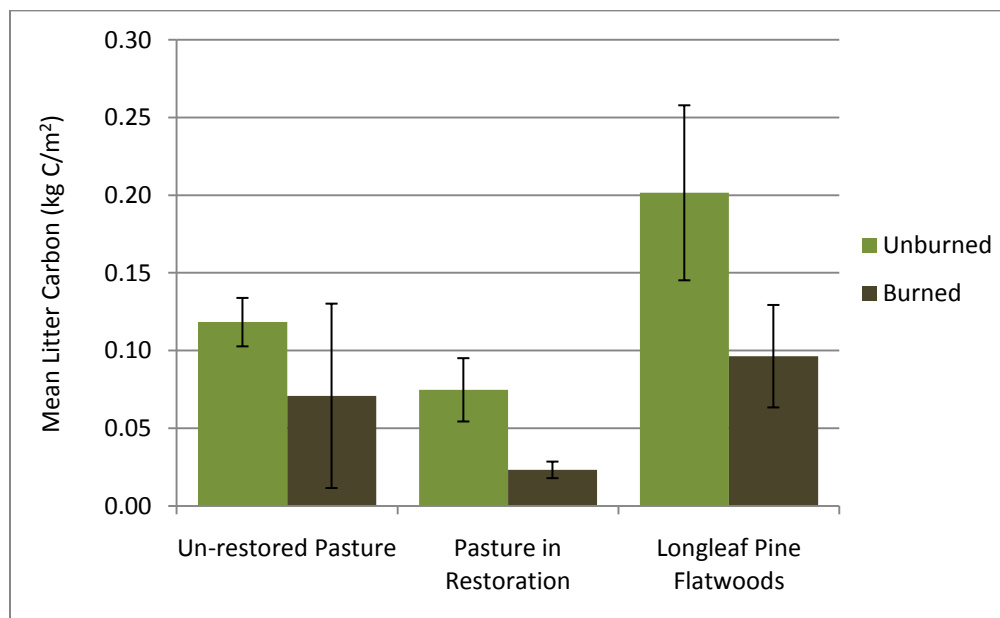


Figure 7. Mean carbon stock (kg C/m<sup>2</sup>) in litter of un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means (n=5).

Un-restored pasture had a mean litter carbon stock of 0.12 ( $\pm 0.02$ ) kg C/m<sup>2</sup> in plots that were unburned, and a mean litter carbon stock of 0.07 ( $\pm 0.06$ ) kg C/m<sup>2</sup> in plots that were recently burned (Figure 7). This resulted in a 40% loss of litter carbon stock four months after prescribed fire management. The greatest loss of litter carbon stock was 69%, and occurred in pasture in restoration for longleaf pine flatwoods. Unburned pasture in restoration for longleaf pine flatwoods had a carbon stock of 0.07 ( $\pm 0.02$ ) kg C/m<sup>2</sup>, while burned pasture in restoration for longleaf pine flatwoods had a carbon stock of 0.02 ( $\pm 0.01$ ) kg C/m<sup>2</sup>. The largest litter carbon stock, 0.20 ( $\pm 0.06$ ) kg C/m<sup>2</sup>, occurred in unburned restored longleaf pine flatwoods. The litter carbon stock of recently burned restored longleaf pine flatwoods was 52% less than that of unburned restored longleaf pine flatwoods at 0.10 ( $\pm 0.03$ ) kg C/m<sup>2</sup>.

### Soil Carbon Stock

My data did not indicate significant differences for mean soil carbon stock in a core to a depth of 90cm in different stages of restoration ( $P = 0.361$ ) or at different times since prescribed fire management ( $P = 0.064$ ). Also, no significant interaction was found between stage of restoration and time since prescribed fire management ( $P = 0.095$ ). Mean soil carbon stock of unburned un-restored pasture was 16.8 ( $\pm 2.1$ ) kg C/m<sup>2</sup>, and mean soil carbon stock of burned un-restored pasture was 9.3 ( $\pm 3.3$ ) kg C/m<sup>2</sup> (Figure 8). Unburned pasture in restoration for longleaf pine flatwoods had a mean soil carbon stock of 15.7 ( $\pm 3.9$ ) kg C/m<sup>2</sup>, and pasture in restoration for longleaf pine flatwoods that was recently burned had a mean soil carbon stock

of 8.9 ( $\pm 1.6$ ) kg C/m<sup>2</sup>. Unburned restored longleaf pine flatwoods had a mean soil carbon stock of 14.9 ( $\pm 3.5$ ) kg C/m<sup>2</sup>, and burned restored longleaf pine flatwoods had a mean soil carbon stock of 17.1 ( $\pm 2.6$ ) kg C/m<sup>2</sup>.

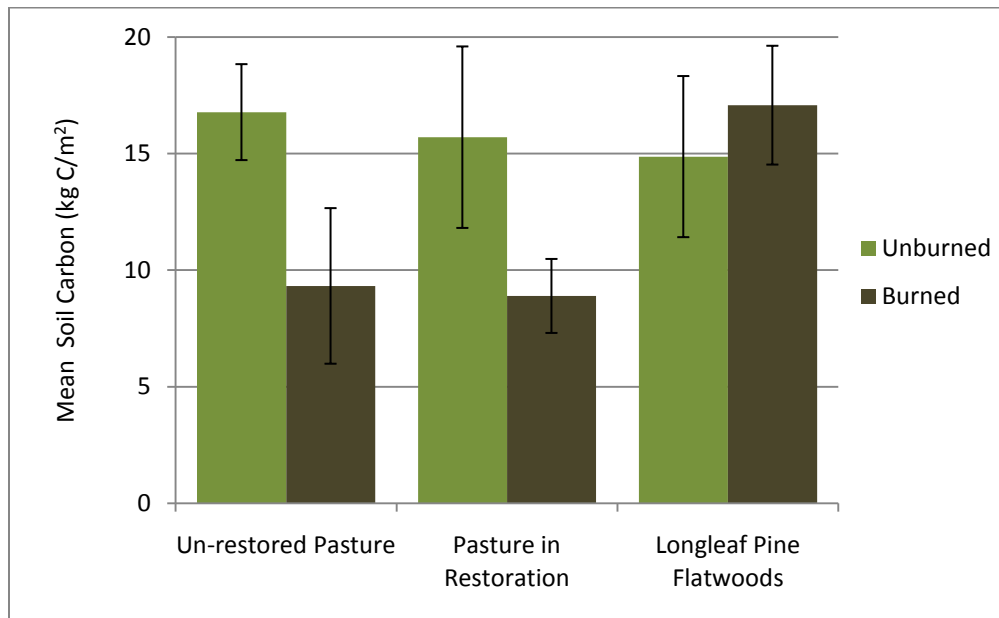


Figure 8. Mean carbon stock (kg C/m<sup>2</sup>) to a depth of 90 cm in soil of un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means (n=5).

### Charcoal and Organic Matter in Soil

Mean content of charcoal > 2 mm in a soil core to a depth of 90 cm was significantly different in different stages of restoration ( $P = 0.012$ ), but my data did not indicate a significant difference in mean charcoal content at different times since prescribed fire management ( $P = 0.924$ ). A significant interaction was found between stage of restoration and time since prescribed fire

management ( $P = 0.038$ ). Tukey's HSD test indicated that the charcoal content in the soil of un-restored pasture was not significantly different than the charcoal content in the soil of restored longleaf pine flatwoods ( $P = 0.106$ ) or pasture in restoration for longleaf pine flatwoods ( $P = 0.533$ ), but the charcoal content in the soil of restored longleaf pine flatwoods was significantly higher than the charcoal content in the soil of pasture in restoration for longleaf pine flatwoods ( $P = 0.010$ ).

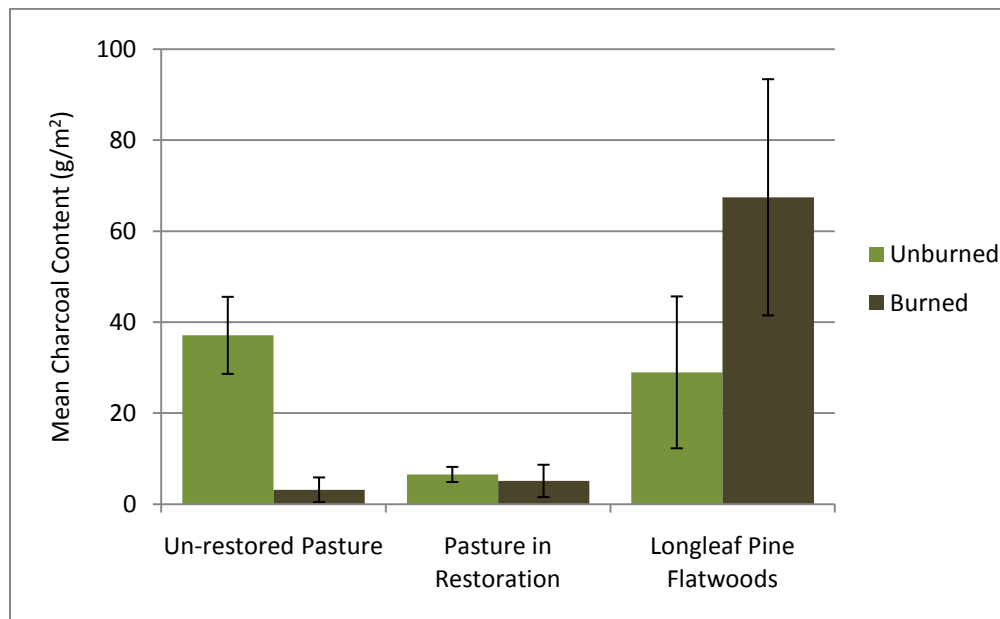


Figure 9. Mean charcoal content ( $\text{g/m}^2$ ) to a depth of 90 cm in soil of un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

Mean charcoal content varied greatly in the soil of unburned un-restored pasture compared to the soil of burned un-restored pasture which had mean charcoal contents of  $37.1 (\pm 8.5) \text{ g/m}^2$  and  $3.2 (\pm 2.7) \text{ g/m}^2$  respectively (Figure 9). In unburned pasture in restoration for longleaf pine flatwoods mean charcoal content of the soil was  $6.5 (\pm 1.7) \text{ g/m}^2$ , and in burned pasture in

restoration for longleaf pine flatwoods mean charcoal content was  $5.1 (\pm 3.6) \text{ g/m}^2$ . Unburned restored longleaf pine flatwoods had a mean soil charcoal content of  $29.0 (\pm 16.7) \text{ g/m}^2$ , while burned restored longleaf pine flatwoods had a mean soil charcoal content of  $67.4 (\pm 26.0) \text{ g/m}^2$ , which was the largest mean charcoal content.

Mean content of un-charred organic matter > 2 mm (OM) in a soil core to a depth of 90 cm was found to be significantly different in different stages of restoration ( $P = 0.000$ ), but my data did not indicate that mean OM content was significantly different at different times since prescribed fire management ( $P = 0.153$ ). A significant interaction was found between stage of restoration and time since prescribed fire management ( $P = 0.022$ ). Tukey's HSD test shows that the soil of restored longleaf pine flatwoods had a significantly higher mean OM content than the soil of un-restored pasture ( $P = 0.000$ ) and pasture in restoration for longleaf pine flatwoods ( $P = 0.000$ ), but the mean OM content in the soil of un-restored pasture and pasture in restoration for longleaf pine flatwoods was not significantly different ( $P = 0.147$ ).

Un-restored pasture had a mean soil OM content of  $1005.8 (\pm 125.7) \text{ g/m}^2$  and  $856.4 (\pm 180.9) \text{ g/m}^2$  in unburned and burned respectively (Figure 10). Pasture in restoration for longleaf pine flatwoods had the least mean soil OM with  $433.2 (\pm 124.1) \text{ g/m}^2$  in unburned plots and  $273.3 (\pm 88.3) \text{ g/m}^2$  in burned plots. The highest mean soil OM was found in restored longleaf pine flatwoods which had a mean soil OM of  $3610.2 (\pm 467.6) \text{ g/m}^2$  in unburned plots and  $4992.4 (\pm 486.8) \text{ g/m}^2$  in burned plots.



Figure 10. Mean OM content ( $\text{g/m}^2$ ) to a depth of 90 cm in soil of un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

### Elemental and Isotopic Analysis of Soil Carbon

Mean percent carbon (% C) of litter varied significantly with stage of restoration ( $P = 0.000$ ) and time since prescribed fire management ( $P = 0.018$ ), but no significant interaction was found between stage of restoration and time since prescribed fire management ( $P = 0.149$ ). Tukey's HSD test found that the % C in litter of restored longleaf pine flatwoods was significantly higher than that of un-restored pasture ( $P = 0.000$ ) and pasture in restoration for longleaf pine flatwoods ( $P = 0.000$ ), while % C in litter of un-restored pasture and pasture in restoration for longleaf pine flatwoods was not significantly different ( $P = 0.580$ ). Mean % C of litter in unburned plots ranged from 44.2 ( $\pm 0.5$ ) % to 48.5 ( $\pm 1.2$ ) %, and mean % C of burned plots



ranged from 44.3 ( $\pm 1.3$ ) % to 52.0 ( $\pm 0.7$ ) %, with mean % C in litter of restored longleaf pine flatwoods as the highest in both unburned and burned (Table 4).

Table 4. Mean % C in litter and sampled soil depths for un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire.

Depth (cm)	Mean % C					
	Un-restored Pasture		Pasture in Restoration		Longleaf Pine Flatwoods	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Litter	44.3 ( $\pm 0.3$ )	44.3 ( $\pm 1.3$ )	44.2 ( $\pm 0.5$ )	46.1 ( $\pm 0.9$ )	48.5 ( $\pm 1.2$ )	52.0 ( $\pm 0.7$ )
0-30	3.1 ( $\pm 0.6$ )	2.3 ( $\pm 1.2$ )	2.5 ( $\pm 1.2$ )	2.5 ( $\pm 0.5$ )	1.7 ( $\pm 0.2$ )	2.9 ( $\pm 0.4$ )
30-60	1.4 ( $\pm 0.4$ )	0.3 ( $\pm 0.1$ )	0.9 ( $\pm 0.3$ )	0.3 ( $\pm 0.1$ )	1.8 ( $\pm 0.9$ )	1.8 ( $\pm 0.6$ )
60-90	0.5 ( $\pm 0.2$ )	0.2 ( $\pm 0.1$ )	0.9 ( $\pm 0.2$ )	0.2 ( $\pm 0.0$ )	0.7 ( $\pm 0.2$ )	0.8 ( $\pm 0.3$ )

The values in parentheses are the standard error of the means (n=5).

My data did not indicate significant differences in mean % C of sampled soil depths, for any depth, at different stages of restoration (0-30 cm,  $P = 0.850$ ; 30-60 cm,  $P = 0.055$ ; 60-90 cm,  $P = 0.125$ ) or at different times since prescribed fire management (0-30 cm,  $P = 0.833$ ; 30-60 cm,  $P = 0.155$ ; 60-90 cm,  $P = 0.055$ ), and no significant interaction was found between stage of restoration and time since prescribed fire management (0-30 cm,  $P = 0.465$ ; 30-60 cm,  $P = 0.577$ ; 60-90 cm,  $P = 0.119$ ). Mean % C in soil 0-30 cm deep ranged from 1.7 ( $\pm 0.2$ ) % to 3.1 ( $\pm 0.6$ ) %, while mean % C in soil 30-60 cm deep ranged from 0.3 ( $\pm 0.1$ ) % to 1.8 ( $\pm 0.9$ ) %, and mean % C in soil 60-90 cm deep ranged from 0.2 ( $\pm 0.1$ ) % to 0.9 ( $\pm 0.2$ ) % (Table 4).

Mean  $\delta^{13}\text{C}$  of litter was found to vary significantly with stage of restoration ( $P = 0.000$ ), but my data did not indicate that mean  $\delta^{13}\text{C}$  of litter was significantly different a different times since prescribed fire management ( $P = 0.145$ ), and no significant interaction was found between

stage of restoration and time since prescribed fire management ( $P = 0.659$ ). Tukey's HSD indicated that the mean  $\delta^{13}\text{C}$  of litter in restored longleaf pine flatwoods was significantly less than the mean  $\delta^{13}\text{C}$  of litter in un-restored pasture ( $P = 0.000$ ) and pasture in restoration for longleaf pine flatwoods ( $P = 0.000$ ), but that the mean  $\delta^{13}\text{C}$  of litter in un-restored pasture and pasture in restoration for longleaf pine flatwoods did not vary significantly ( $P = 0.221$ ). Mean  $\delta^{13}\text{C}$  of litter in un-restored pasture and pasture in restoration for longleaf pine flatwoods ranged from  $-19.9 (\pm 2.0) \text{‰}$  to  $-14.8 (\pm 0.7) \text{‰}$ , and mean  $\delta^{13}\text{C}$  of litter in restored longleaf pine flatwoods was  $-27.2 (\pm 0.4) \text{‰}$  and  $-27.5 (\pm 0.4) \text{‰}$  in burned and unburned respectively (Table 5).

Table 5. Mean  $\delta^{13}\text{C}$  distribution in litter and sampled soil depths for un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire.

Depth (cm)	Mean $\delta^{13}\text{C}$ (‰)					
	Un-restored Pasture		Pasture in Restoration		Longleaf Pine Flatwoods	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Litter	-14.8 ( $\pm 0.7$ )	-17.8 ( $\pm 2.2$ )	-17.7 ( $\pm 1.7$ )	-19.9 ( $\pm 2.0$ )	-27.2 ( $\pm 0.4$ )	-27.5 ( $\pm 0.4$ )
0-30	-19.5 ( $\pm 0.5$ )	-19.1 ( $\pm 0.6$ )	-21.0 ( $\pm 0.3$ )	-18.4 ( $\pm 0.9$ )	-24.4 ( $\pm 0.3$ )	-24.8 ( $\pm 0.4$ )
30-60	-21.3 ( $\pm 0.2$ )	-20.3 ( $\pm 1.3$ )	-23.5 ( $\pm 0.2$ )	-21.6 ( $\pm 0.7$ )	-24.4 ( $\pm 0.3$ )	-24.5 ( $\pm 0.4$ )
60-90	-21.9 ( $\pm 0.3$ )	-20.7 ( $\pm 1.0$ )	-23.7 ( $\pm 0.1$ )	-22.3 ( $\pm 0.9$ )	-24.1 ( $\pm 0.2$ )	-24.4 ( $\pm 0.3$ )

The values in parentheses are the standard error of the means ( $n=5$ ).

Mean  $\delta^{13}\text{C}$  at sampled soil depths was found to vary significantly with stage of restoration at all depths (0-30 cm,  $P = 0.000$ ; 30-60 cm,  $P = 0.000$ ; 60-90 cm,  $P = 0.000$ ). Mean  $\delta^{13}\text{C}$  was significantly different at different times since fire management at the depth of 0-30 cm ( $P = 0.046$ ), but was not significantly different at the depth of 30-60 cm ( $P = 0.097$ ) or 60-90 cm ( $P = 0.121$ ). Also, a significant interaction was found between stage of restoration and time since

prescribed fire management at the depth of 0-30 cm ( $P = 0.025$ ), but no significant interaction was found at the depth of 30-60 cm ( $P = 0.309$ ) or 60-90 cm ( $P = 0.307$ ). Tukey's HSD test shows that at all soil depths, mean  $\delta^{13}\text{C}$  was significantly less in restored longleaf pine flatwoods compared to mean  $\delta^{13}\text{C}$  in the soil of un-restored pasture (0-30 cm,  $P = 0.000$ ; 30-60 cm,  $P = 0.000$ ; 60-90 cm,  $P = 0.000$ ). Mean  $\delta^{13}\text{C}$  of restored longleaf pine flatwoods was only significantly less than mean  $\delta^{13}\text{C}$  of pasture in restoration for longleaf pine flatwoods in the 0-30 cm ( $P = 0.000$ ) and 30-60 cm ( $P = 0.016$ ) depths; in the 60-90 cm depth no significant difference was found between the two ( $P = 0.094$ ). Also, Tukey's HSD test shows that mean  $\delta^{13}\text{C}$  of un-restored pasture and pasture in restoration for longleaf pine flatwoods was not significantly different at the 0-30 cm depth ( $P = 0.532$ ), but at the 30-60 and 60-90 cm depths, the mean  $\delta^{13}\text{C}$  of un-restored pasture was significantly higher than the mean  $\delta^{13}\text{C}$  of pasture in restoration for longleaf pine flatwoods (30-60 cm,  $P = 0.036$ ; 60-90 cm,  $P = 0.020$ ).

Mean  $\delta^{13}\text{C}$  of soil in restored longleaf pine flatwoods ranged from  $-24.8 (\pm 0.4) \text{‰}$  to  $-24.1 (\pm 0.2) \text{‰}$ , mean  $\delta^{13}\text{C}$  of soil in un-restored pasture ranged from  $-21.9 (\pm 0.3) \text{‰}$  to  $-19.1 (\pm 0.6) \text{‰}$ , and mean  $\delta^{13}\text{C}$  of soil in pasture in restoration for longleaf pine flatwoods ranged from  $-23.7 (\pm 0.1) \text{‰}$  to  $-18.4 (\pm 0.9) \text{‰}$  (Table 5). Overall, restored longleaf pine flatwoods experienced a trend of enrichment in  $^{13}\text{C}$  between litter inputs and soil depths, while un-restored pasture and pasture in restoration for longleaf pine flatwoods experience a trend of depletion in  $^{13}\text{C}$  between litter inputs and soil depths (Figure 11).

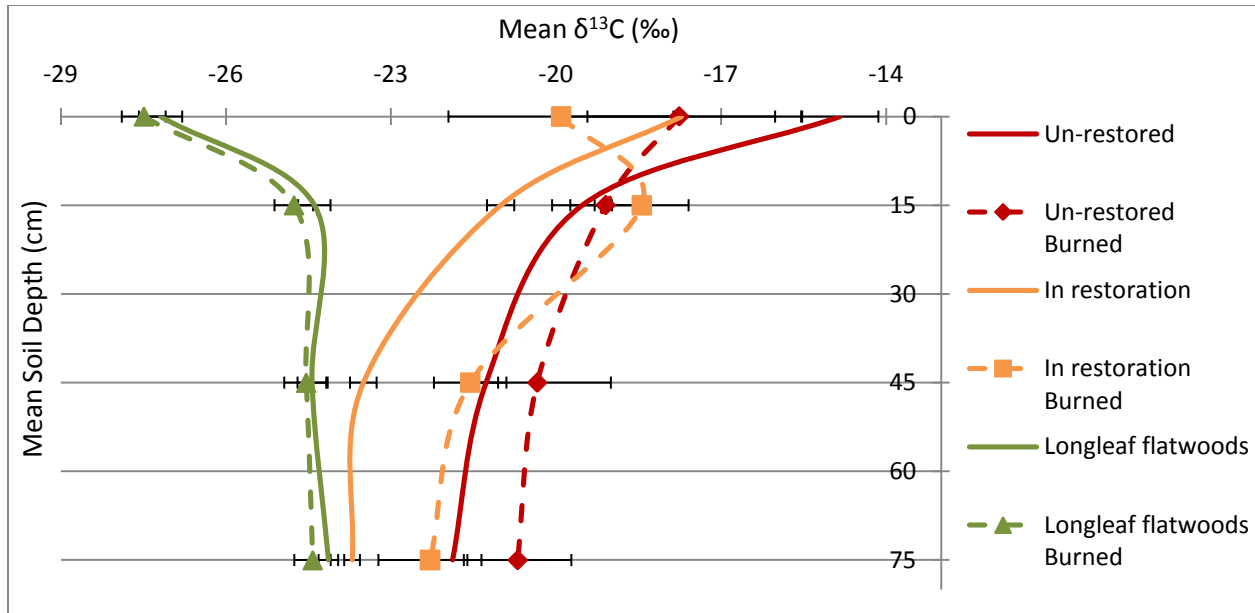


Figure 11. Mean  $\delta^{13}\text{C}$  distribution in litter and mean sampled soil depths for un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

The trend of depletion in  $^{13}\text{C}$  with increasing soil depth, seen in both the burned and unburned sites of the un-restored pasture and pasture in restoration, can be related to the increasing percent of total carbon derived from C3 vegetation with increasing soil depth found in each of these sites. When comparing the burned and unburned sites of the un-restored pasture and pasture in restoration, the soil profile most depleted in  $^{13}\text{C}$  is that of the unburned pasture in restoration (Figure 11). Accordingly, the soil profile of the unburned pasture in restoration was calculated to have the highest mean percent of total carbon derived from C3 vegetation, ranging from 35 ( $\pm 3$ ) % to 63 ( $\pm 1$ ) % (Table 6).

The mean percent of total carbon derived from C3 vegetation was significantly higher at all soil depths in the unburned pasture in restoration when compared to C3 derived carbon in the

unburned un-restored pasture (0-30 cm,  $P = 0.038$ ; 30-60 cm,  $P = 0.000$ ; 60-90 cm,  $P = 0.000$ ) and burned un-restored pasture (0-30 cm,  $P = 0.024$ ; 30-60 cm,  $P = 0.049$ ; 60-90 cm,  $P = 0.016$ ). The percent of carbon derived from C3 vegetation in the unburned pasture in restoration was also significantly higher than the C3 derived carbon in the 0-30 cm ( $P = 0.015$ ) and 30-60 cm ( $P = 0.024$ ) soil depths of the burned pasture in restoration, but was not significantly higher than percent of C3 carbon in the 60-90 cm depth ( $P = 0.173$ ) of the burned pasture in restoration. The percent of total carbon derived from C3 vegetation was not significantly different at any soil depth between the burned pasture in restoration, unburned un-restored pasture, and burned un-restored pasture. Un-restored pasture, both unburned and burned, had a mean percent of total carbon derived from C3 vegetation ranging from 16 ( $\pm 7$ ) % to 44 ( $\pm 3$ ) %, and the burned pasture in restoration had a mean percent of total carbon derived from C3 vegetation ranging from 9 ( $\pm 9$ ) % to 48 ( $\pm 10$ ) % (Table 6).

Table 6. Mean percent of total carbon contributed by C4 and C3 vegetation in sampled soil depths of un-restored pasture and pasture in restoration for longleaf pine flatwoods.

Depth (cm)	Un-restored Pasture				Pasture in Restoration			
	Unburned		Burned		Unburned		Burned	
	% C4	% C3	% C4	% C3	% C4	% C3	% C4	% C3
0-30	80 ( $\pm 6$ )	20 ( $\pm 6$ )	84 ( $\pm 7$ )	16 ( $\pm 7$ )	65 ( $\pm 3$ )	35 ( $\pm 3$ )	91 ( $\pm 9$ )	9 ( $\pm 9$ )
30-60	62 ( $\pm 2$ )	38 ( $\pm 2$ )	72 ( $\pm 14$ )	28 ( $\pm 14$ )	39 ( $\pm 2$ )	61 ( $\pm 2$ )	59 ( $\pm 7$ )	41 ( $\pm 7$ )
60-90	56 ( $\pm 3$ )	44 ( $\pm 3$ )	68 ( $\pm 10$ )	32 ( $\pm 10$ )	37 ( $\pm 1$ )	63 ( $\pm 1$ )	52 ( $\pm 10$ )	48 ( $\pm 10$ )

The values in parentheses are the standard error of the means (n=5).

## Comparing Flatwoods Communities and Time Since Fire Management

### Aboveground Biomass Carbon Stock

My data did not indicate significant differences in mean aboveground biomass carbon stock among flatwoods community types ( $P = 0.641$ ), but did indicate a significant difference in mean aboveground biomass carbon stock at different times since prescribed fire management ( $P = 0.033$ ). No significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.578$ ).

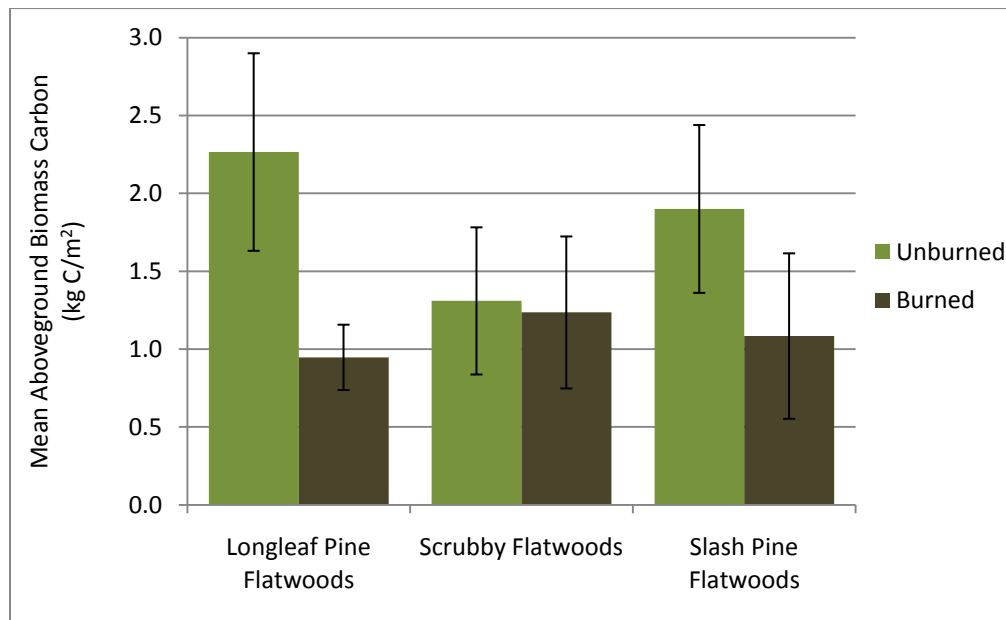


Figure 12. Mean carbon stock (kg C/m<sup>2</sup>) in aboveground biomass of restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means (n=5).

Among the flatwoods communities, longleaf pine flatwoods had the greatest loss, 58%, of aboveground biomass carbon stock. Unburned longleaf pine flatwoods had a mean aboveground biomass carbon stock at  $2.27 (\pm 0.63)$  kg C/m<sup>2</sup>, and burned longleaf pine flatwoods had a mean aboveground biomass carbon stock of  $0.95 (\pm 0.21)$  kg C/m<sup>2</sup> (Figure 12). Unburned scrubby flatwoods had a mean aboveground biomass carbon stock of  $1.31 (\pm 0.47)$  kg C/m<sup>2</sup> and burned scrubby flatwoods had a mean aboveground biomass carbon stock of  $1.24 (\pm 0.49)$  kg C/m<sup>2</sup>, indicating a loss of only 6% of aboveground biomass carbon stock four months after fire. Mean aboveground biomass carbon stock of unburned slash pine flatwoods was  $1.90 (\pm 0.54)$  kg C/m<sup>2</sup>, and mean aboveground biomass carbon stock of burned slash pine flatwoods was  $1.08 (\pm 0.53)$  kg C/m<sup>2</sup>, showing a loss of 43% of aboveground biomass carbon stock four months after prescribed fire.

### Litter Carbon Stock

My data did not indicate significant differences in mean carbon stock of litter among the different flatwoods communities ( $P = 0.623$ ), or at different times since prescribed fire management ( $P = 0.110$ ), and no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.779$ ). Mean carbon stock of litter in the flatwoods communities ranged between  $0.10 (\pm 0.05)$  kg C/m<sup>2</sup> and  $0.20 (\pm 0.06)$  kg C/m<sup>2</sup> (Figure 13).

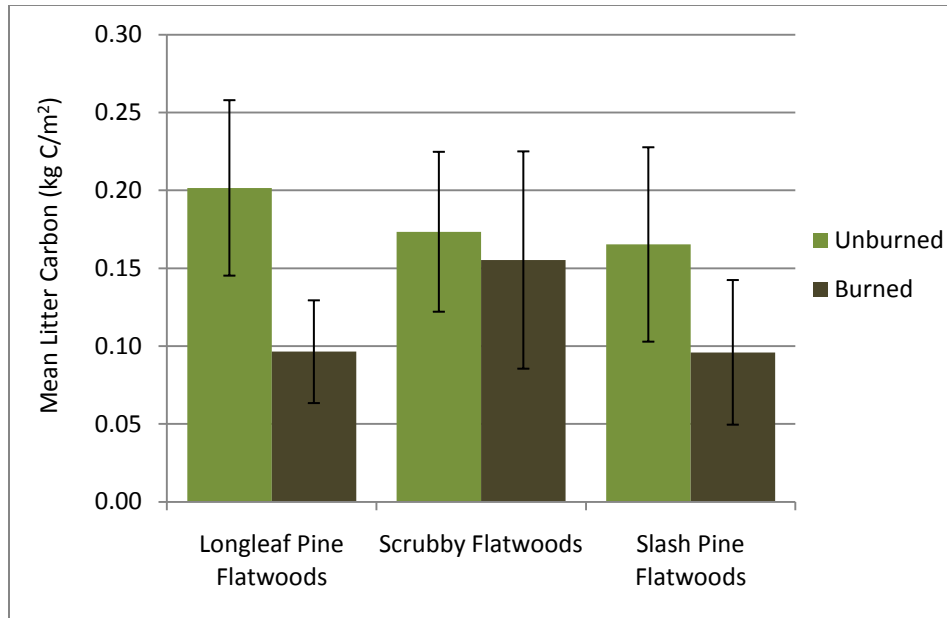


Figure 13. Mean carbon stock (kg C/m<sup>2</sup>) in litter of restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent ± standard error of the means (n=5).

### Soil Carbon Stock

Mean soil carbon stock in a core to a depth of 90 cm varied significantly among the flatwoods communities ( $P = 0.001$ ), but was not indicated to be significantly different at different times since prescribed fire management ( $P = 0.885$ ), and again, no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.154$ ). Tukey's HSD test indicated that the mean soil carbon stock of scrubby flatwoods was significantly less than that of longleaf pine flatwoods ( $P = 0.001$ ) and slash pine flatwoods ( $P = 0.008$ ), and that the mean soil carbon stock of longleaf pine flatwoods and slash pine flatwoods were not significantly different ( $P = 0.735$ ).



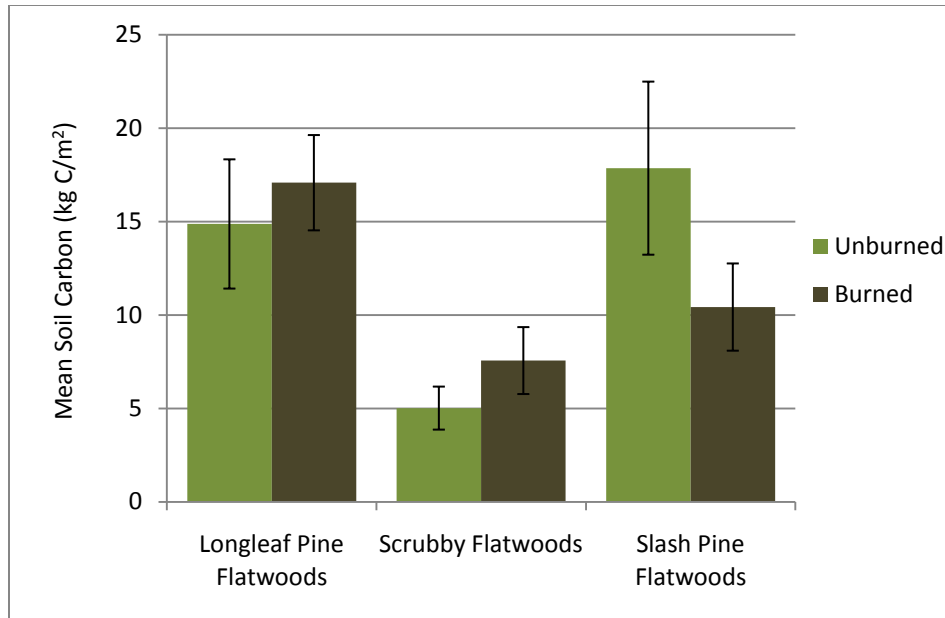


Figure 14. Mean carbon stock (kg C/m<sup>2</sup>) to a depth of 90 cm in soil of restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent ± standard error of the means (n=5).

Mean soil carbon stock of longleaf pine flatwoods was 14.9 (±3.5) kg C/m<sup>2</sup> and 17.1 (±2.6) kg C/m<sup>2</sup> in unburned and burned respectively (Figure 14). Mean soil carbon stock of unburned scrubby flatwoods was 5.0 (±1.2) kg C/m<sup>2</sup>, and 7.6 (±1.8) kg C/m<sup>2</sup> in burned scrubby flatwoods. Unburned slash pine flatwoods had a mean soil carbon stock of 17.9 (±4.6) kg C/m<sup>2</sup>, and burned slash pine flatwoods had a mean soil carbon stock of 10.4 (±2.3) kg C/m<sup>2</sup>.

### Charcoal and Organic Matter in Soil

My data did not indicate a significant difference in mean content of charcoal > 2 mm in a core to a depth of 90cm among the flatwoods communities (*P* = 0.828) or at different times since fire

management ( $P = 0.165$ ). Also, no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.346$ ). Mean charcoal content in the soil of the different flatwoods communities ranged between  $25.9 (\pm 18.6) \text{ g/m}^2$  and  $74.2 (\pm 26.6) \text{ g/m}^2$  (Figure 15).

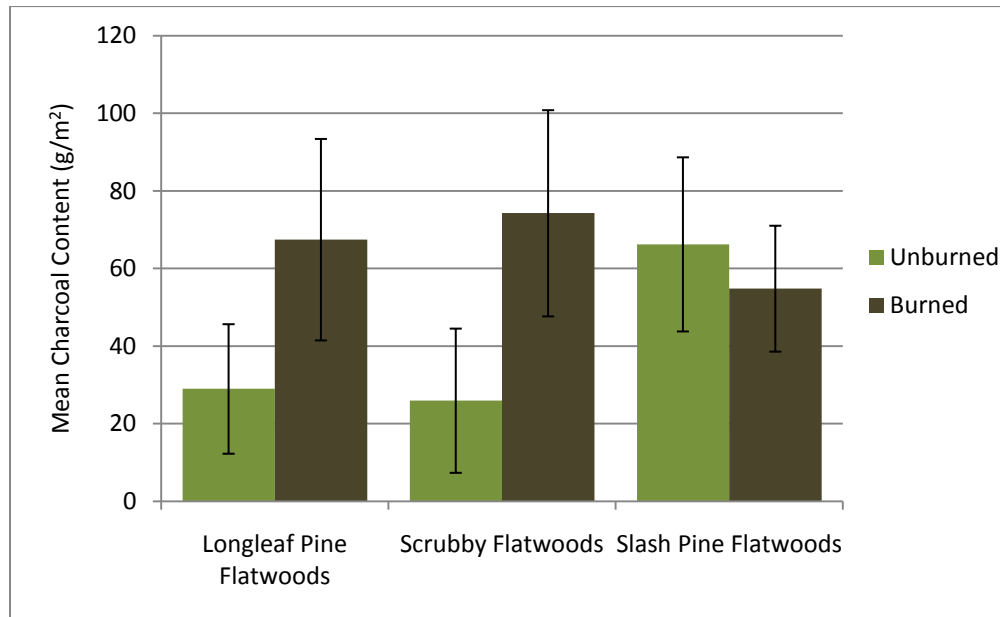


Figure 15. Mean charcoal content ( $\text{g/m}^2$ ) to a depth of 90 cm in soil of restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

Also, my data did not indicate a significant difference in mean content of organic matter (OM) > 2 mm in a core to a depth of 90cm among the flatwoods communities ( $P = 0.185$ ) or at different times since fire management ( $P = 0.067$ ), and no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.087$ ). Mean OM content in the soil of the different flatwoods communities ranged from  $2017.9 (\pm 236.8) \text{ g/m}^2$  to  $4992.4 (\pm 486.8) \text{ g/m}^2$  (Figure 16).

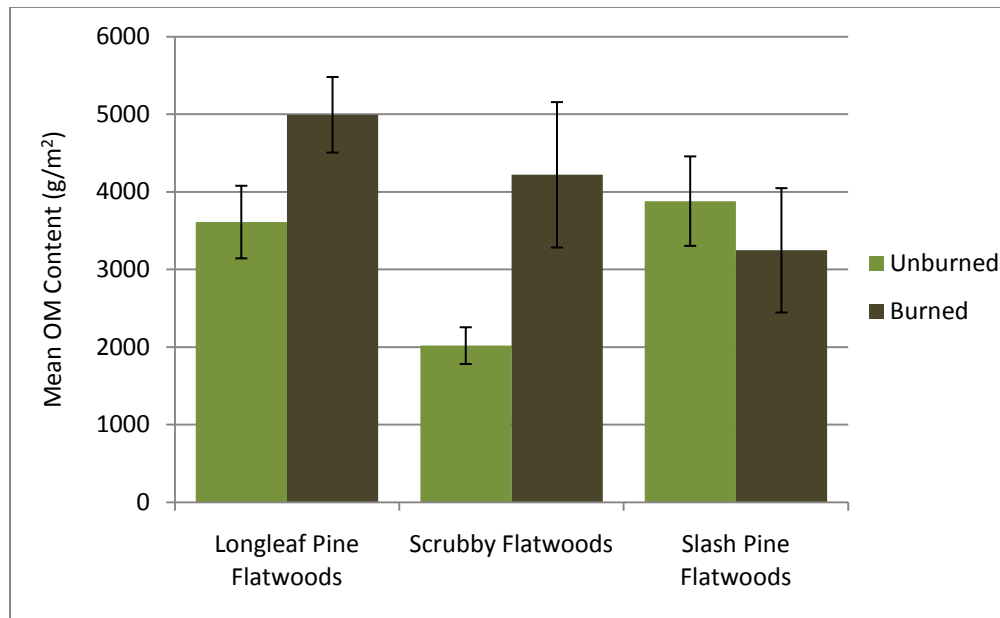


Figure 16. Mean charcoal content ( $\text{g/m}^2$ ) to a depth of 90 cm in soil of restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

### Elemental and Isotopic Analysis of Soil Carbon

Mean percent carbon (% C) in litter was found to vary significantly with flatwoods community type ( $P = 0.018$ ) and time since prescribed fire management ( $P = 0.022$ ), but no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.058$ ). Tukey's HSD test indicated that % C in the litter of slash pine flatwoods was significantly higher than the % C in the litter of scrubby flatwoods ( $P = 0.015$ ), but the % C in the litter of longleaf pine flatwoods was not significantly different than the % C in the litter of slash pine flatwoods ( $P = 0.556$ ) or scrubby flatwoods ( $P = 0.137$ ). The litter of longleaf pine flatwoods contained  $48.5 (\pm 1.2)$  % C, and  $52.0 (\pm 0.7)$  % C in unburned and burned

respectively (Table 7). The only flatwoods community that showed a decrease in % C with recent fire was scrubby flatwoods. Unburned scrubby flatwoods had 48.8 ( $\pm 0.5$ ) % C in the litter layer, and burned scrubby flatwoods had 48.0 ( $\pm 1.2$ ) % C in the litter layer. The highest % C in the litter of both the unburned and burned plots occurred in the slash pine flatwoods, with 49.8 ( $\pm 1.0$ ) % in unburned, and 52.5 ( $\pm 0.7$ ) % in burned.

Table 7. Mean % C in litter and sampled soil depths for restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire.

Depth (cm)	Mean % C					
	Longleaf Pine Flatwoods		Scrubby Flatwoods		Slash Pine Flatwoods	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Litter	48.5 ( $\pm 1.2$ )	52.0 ( $\pm 0.7$ )	48.8 ( $\pm 0.5$ )	48.0 ( $\pm 1.2$ )	49.8 ( $\pm 1.0$ )	52.5 ( $\pm 0.7$ )
0-30	1.7 ( $\pm 0.2$ )	2.9 ( $\pm 0.4$ )	0.8 ( $\pm 0.1$ )	1.2 ( $\pm 0.4$ )	4.2 ( $\pm 1.1$ )	1.9 ( $\pm 0.4$ )
30-60	1.8 ( $\pm 0.9$ )	1.8 ( $\pm 0.6$ )	0.3 ( $\pm 0.2$ )	0.4 ( $\pm 0.1$ )	0.8 ( $\pm 0.3$ )	0.4 ( $\pm 0.2$ )
60-90	0.7 ( $\pm 0.2$ )	0.8 ( $\pm 0.3$ )	0.2 ( $\pm 0.2$ )	0.3 ( $\pm 0.1$ )	0.7 ( $\pm 0.2$ )	0.7 ( $\pm 0.3$ )

The values in parentheses are the standard error of the means (n=5).

In the 0-30 cm and 30-60 cm soil depths, mean % C was found to vary significantly among the flatwoods communities (0-30 cm,  $P = 0.004$ ; 30-60 cm,  $P = 0.011$ ); however, in the 60-90 cm soil depth, it was not indicated that mean % C was significantly different among community types ( $P = 0.060$ ). My data did not indicate that mean % C was significantly different at different times since fire management at any soil depth (0-30 cm,  $P = 0.614$ ; 30-60 cm,  $P = 0.818$ ; 60-90 cm,  $P = 0.691$ ). A significant interaction between stage of restoration and stage of prescribed fire management was found at the depth of 0-30 cm ( $P = 0.010$ ), but no significant interaction was found at the depth of 30-60 cm ( $P = 0.908$ ) or 60-90 cm ( $P = 0.964$ ). Tukey's HSD indicated that in the 0-30 cm soil depth, % C in the soil of scrubby flatwoods is significantly less than that of

slash pine flatwoods ( $P = 0.003$ ), but that the % C in the soil of longleaf pine flatwoods is not significantly different than the % C in scrubby flatwoods ( $P = 0.078$ ) or slash pine flatwoods ( $P = 0.356$ ). Also, in the 30-60 cm soil depth Tukey's HSD indicated that % C in the soil of longleaf pine flatwoods was significantly higher than the % C in the soil of scrubby flatwoods ( $P = 0.014$ ) and slash pine flatwoods ( $P = 0.043$ ), and that the % C in the soil of scrubby flatwoods and slash pine flatwoods was not significantly different ( $P = 0.870$ ).

Mean % C in the 0-30 cm horizon ranged from 1.7 ( $\pm 0.2$ ) % to 4.2 ( $\pm 1.1$ ) % in longleaf pine flatwoods and slash pine flatwoods, and was 0.8 ( $\pm 0.1$ ) % and 1.2 ( $\pm 0.4$ ) % in unburned and burned scrubby flatwoods respectively (Table 7). In longleaf pine flatwoods, mean % C in the 30-60 cm soil depth was 1.8 ( $\pm 0.9$ ) % in unburned and 1.8 ( $\pm 0.6$ ) % in burned, while mean % C in the 30-60 cm soil depth ranged from 0.3 ( $\pm 0.2$ ) % to 0.8 ( $\pm 0.3$ ) % in scrubby flatwoods and slash pine flatwoods.

My data did not indicate a significant difference in mean  $\delta^{13}\text{C}$  in litter samples among the flatwoods communities ( $P = 0.490$ ) or at different times since prescribed fire management ( $P = 0.480$ ), and no significant interaction was found between flatwoods community type and time since prescribed fire management ( $P = 0.747$ ). Mean  $\delta^{13}\text{C}$  in the litter of the different flatwoods communities ranged from -27.6 ( $\pm 0.5$ ) ‰ to -26.8 ( $\pm 0.3$ ) ‰ (Table 8).

Table 8. Mean  $\delta^{13}\text{C}$  distribution in litter and sampled soil depths for restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire.

Depth (cm)	Mean $\delta^{13}\text{C}$ (‰)					
	Longleaf Pine Flatwoods		Scrubby Flatwoods		Slash Pine Flatwoods	
	Unburned	Burned	Unburned	Burned	Unburned	Burned
Litter	-27.2 ( $\pm 0.4$ )	-27.5 ( $\pm 0.4$ )	-26.8 ( $\pm 0.3$ )	-27.3 ( $\pm 0.3$ )	-27.6 ( $\pm 0.5$ )	-27.5 ( $\pm 0.4$ )
0-30	-24.4 ( $\pm 0.3$ )	-24.8 ( $\pm 0.4$ )	-25.5 ( $\pm 0.2$ )	-25.9 ( $\pm 0.2$ )	-24.9 ( $\pm 0.6$ )	-24.5 ( $\pm 0.5$ )
30-60	-24.4 ( $\pm 0.3$ )	-24.5 ( $\pm 0.4$ )	-25.8 ( $\pm 0.3$ )	-25.6 ( $\pm 0.3$ )	-24.5 ( $\pm 0.8$ )	-24.8 ( $\pm 0.4$ )
60-90	-24.1 ( $\pm 0.2$ )	-24.4 ( $\pm 0.3$ )	-25.5 ( $\pm 0.2$ )	-25.7 ( $\pm 0.4$ )	-23.4 ( $\pm 0.6$ )	-24.1 ( $\pm 0.4$ )

The values in parentheses are the standard error of the means (n=5).

In the soil samples, mean  $\delta^{13}\text{C}$  was found to be significantly different among the flatwoods communities at all sampled depths (0-30 cm,  $P = 0.022$ ; 30-60 cm,  $P = 0.021$ ; 60-90 cm,  $P = 0.000$ ), but my data did not indicate a significant difference in mean  $\delta^{13}\text{C}$  at different times since prescribed fire management (0-30 cm,  $P = 0.746$ ; 30-60 cm,  $P = 0.901$ ; 60-90 cm,  $P = 0.216$ ). No significant interaction was found between flatwoods community type and time since prescribed fire management (0-30 cm,  $P = 0.519$ ; 30-60 cm,  $P = 0.858$ ; 60-90 cm,  $P = 0.693$ ). Tukey's HSD indicated that at all soil depths, mean  $\delta^{13}\text{C}$  was significantly less in scrubby flatwoods compared to mean  $\delta^{13}\text{C}$  in the soil of longleaf pine flatwoods (0-30 cm,  $P = 0.032$ ; 30-60 cm,  $P = 0.028$ ; 60-90 cm,  $P = 0.005$ ). Mean  $\delta^{13}\text{C}$  was significantly less in the soil of scrubby flatwoods compared to mean  $\delta^{13}\text{C}$  in the soil of slash pine flatwoods at the 60-90 cm depth ( $P = 0.000$ ), but was not significantly different between scrubby flatwoods and slash pine flatwoods in the 0-30 cm ( $P = 0.059$ ) or 30-60 cm ( $P = 0.055$ ) depths. Also, mean  $\delta^{13}\text{C}$  in the soil of longleaf pine flatwoods and slash pine flatwoods was not significantly different at any depth (0-30 cm,  $P = 0.951$ ; 30-60 cm,  $P = 0.950$ ; 60-90 cm,  $P = 0.345$ ).

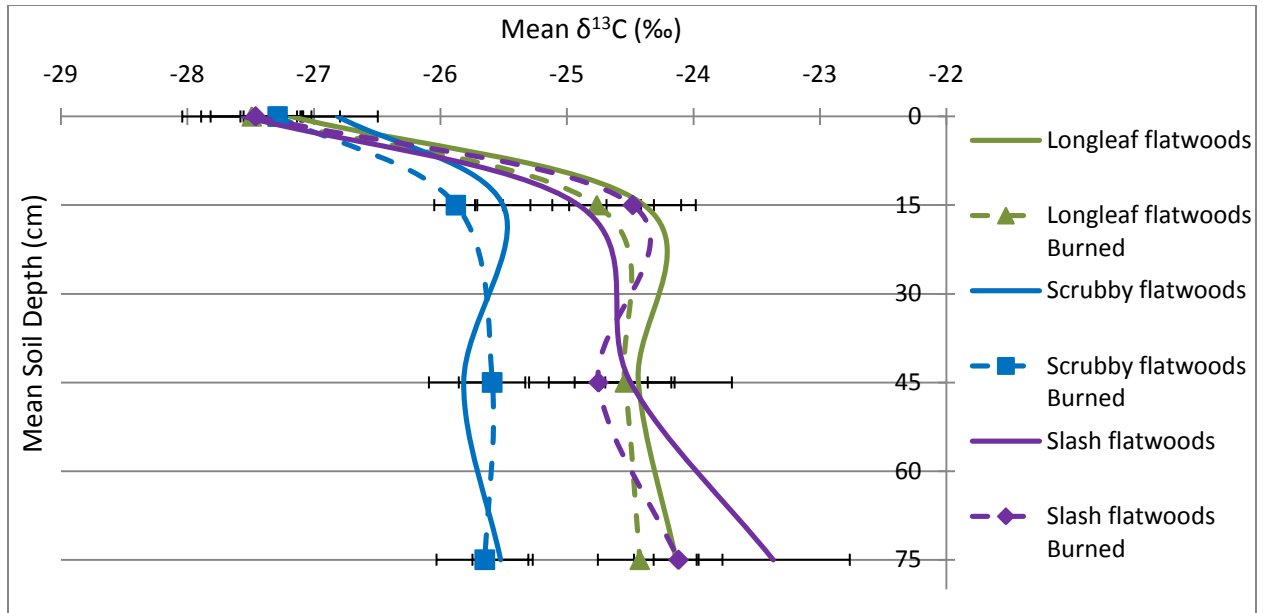


Figure 17. Mean  $\delta^{13}\text{C}$  distribution in litter and mean sampled soil depths for restored longleaf pine flatwoods, restored scrubby flatwoods, and restored slash pine flatwoods; all with and without recent fire. Bars represent  $\pm$  standard error of the means ( $n=5$ ).

In scrubby flatwoods soil, mean  $\delta^{13}\text{C}$  ranged from  $-25.9 (\pm 0.2) \text{‰}$  to  $-25.5 (\pm 0.2) \text{‰}$ , and in longleaf pine flatwoods and slash pine flatwoods mean  $\delta^{13}\text{C}$  ranged from  $-24.8 (\pm 0.4) \text{‰}$  to  $-23.4 (\pm 0.6) \text{‰}$  (Table 8). While each flatwoods community experienced a trend of enrichment in  $^{13}\text{C}$  between litter inputs and soil depths, scrubby flatwoods exhibited the smallest increase (Figure 17).

## DISCUSSION

### Comparing Stages of Restoration and Time Since Fire Management

#### Aboveground Biomass and Litter Carbon Stocks

Aboveground biomass and litter carbon stock were both significantly different in different stages of restoration and at different times since prescribed fire management. Mean aboveground biomass carbon stock increased significantly as the level of restoration increased, with mean aboveground biomass carbon stock for unburned restored longleaf pine flatwoods over 5 times greater than that of unburned pasture in restoration, and 8 times greater than that of unburned un-restored pasture. Additionally, mean litter carbon stock for unburned restored longleaf pine flatwoods was found to be nearly 3 times greater than that of unburned pasture in restoration. Therefore, once the 123 ha of pasture that are currently in restoration are completely restored to longleaf flatwoods, carbon storage on the preserve may increase by as much as 1700 Mg C (1 Mg =  $10^6$  g). Also, if the 470 ha of pasture that are currently un-restored are restored to longleaf pine flatwoods, another 6600 Mg C could be stored on the preserve. Garten (2006) estimated that with prescribed fire management every two to three years, a forest regenerating on sandy soil, such as the soils found at Disney Wilderness Preserve, will reach maximum tree biomass in approximately 100 years. Therefore it can be



expected that aboveground biomass of the pasture in restoration will be comparable to that of the restored longleaf pine flatwoods around the year 2095.

At four months after fire, mean aboveground biomass carbon stock was found to be reduced by approximately 55% in each stage of restoration. These results are similar to the results reported in Lavoie et al. (2010), where the recovery of the biomass carbon pool in a Florida longleaf pine-slash pine forest was found to be 52.8 ( $\pm 6.7$ ) % four months after prescribed fire, and total biomass recovery occurred at three years after fire. In the current study, mean litter carbon stock was found to be reduced by 40%, 69%, and 52% four months after fire in un-restored pasture, pasture in restoration for longleaf pine flatwoods, and restored longleaf pine flatwoods respectively. These results are comparable to those found in Alexis et al. (2007), where litter carbon stock was found to be reduced by 36% one week after a prescribed fire in a Florida scrub oak ecosystem.

### Soil Carbon Stock and Dynamics

The data reported in this study did not provide evidence that soil carbon stock was significantly different in different stages of restoration, or at different times since prescribed fire management. In the literature, consistent patterns of soil carbon loss have been documented when forest or grassland is converted to cropland, but no consistent pattern has been found indicating the reduction of soil carbon stock with conversion of forest to pasture (Houghton and

Hackler 2000; Ahn et al. 2009). For example, in a study on Martha's Vineyard, Peterson and Neill (2003) found that percent carbon in the mineral soil of grasslands that had been converted from forests was higher than the percent carbon in the mineral soil of areas with permanent forest vegetation, and that total soil carbon stock to 10 cm was higher in permanent grasslands than in areas with permanent forest vegetation. However, Rhoades et al. (2000) reported a loss of 15% of soil carbon in the top 15 cm in a site where native Ecuadorian forest vegetation was replaced by pasture grasses. Furthermore, in a study in North Florida, Ahn et al. (2009) found no significant difference in the total organic carbon concentration in the soils of improved pasture, rangeland, and pine plantations. Therefore, the effect of deforestation for pasture on soil carbon stock is likely to vary greatly with ecosystem type, and should be assessed accordingly. On the other hand, similar results have been found by several studies that have assessed the effect of prescribed fire management on soil carbon stock (Johnson and Curtis 2001; Garten 2006; Alexis et al. 2007; Lavoie et al. 2010). As found in the current study, the results of these studies agree that there is not a significant effect of prescribed fire management on soil carbon stock.

Although the results of this study did not provide evidence that overall soil carbon stock is significantly different in different stages of restoration, the profile of  $\delta^{13}\text{C}$  in the soil did vary significantly. The trend of enrichment in  $\delta^{13}\text{C}$  between the litter and mineral soil horizons shown in the restored longleaf pine forest has been found in other ecosystems under persistent C3 vegetation, and has been attributed to the discrimination of the heavier carbon isotope during decomposition and the change in microbial contribution to soil carbon stocks with

increasing soil depth (Buchmann et al. 1997; Ehleringer et al. 2000; Fry 2006). Also, the trend of depletion of  $\delta^{13}\text{C}$  with increasing soil depth in the un-restored pasture and pasture in restoration have been found in other studies where C3 vegetation has been replaced by C4 grasses, often for pasture or cropland (Ehleringer et al. 2000; Haile et al. 2010). This pattern of depletion of  $\delta^{13}\text{C}$  with soil depth indicates that carbon in the upper, more labile soil carbon pool has been derived from the current C4 dominated pasture or native grass and herb vegetation, while carbon in the deeper, more stable carbon pool is a legacy of the historical C3 forest vegetation (Peterson and Neill 2003; Haile et al. 2009). Using similar isotopic techniques, Rhoades et al. (2000) also found that in old growth forests that were converted to pasture, overall changes in soil carbon stock were much less significant than changes in soil turnover rates.

Interestingly, the percent of total carbon derived from C3 vegetation in the unburned pasture in restoration for longleaf pine flatwoods was found to be significantly higher than the percent of total carbon derived from C3 vegetation in the burned pasture in restoration, unburned un-restored pasture, and burned un-restored pasture. This is likely an effect of time since deforestation. The un-burned pasture in restoration was deforested between the 1970s and 1980s, while the other three pasture areas were deforested between the 1940s and 1950s (TNC 1996). Thus, the thirty year gap in time since deforestation has left the older pastures with significantly less forest derived carbon than the newer pasture. A similar result was found by Peterson and Neill (2003), where the isotopic profile of soils in C4 grasslands that had replaced C3 forests were found to become increasingly enriched in  $^{13}\text{C}$  with time. This change in the soil

carbon properties can have implications for soil carbon stability. Wynn and Bird (2007) found that in mixed C4/C3 soils, C4 carbon decomposes faster than C3 carbon. Therefore, the switch from the historical longleaf pine flatwoods vegetation (C3) to the Bahia grass pasture vegetation (C4) may lower the stability of the soil carbon pool. Over time this may lead to a decrease in soil carbon stock if the pastures are left un-restored.

Soil organic matter content and charcoal content of the soil was also found to vary significantly with stage of restoration. The production of charcoal during fire events is an important feature for long-term soil carbon storage in ecosystems under prescribed fire management because charcoal is much less susceptible to degradation than un-charred organic matter (Kuhlbusch 1998; Alexis et al. 2007). It is expected that approximately 2% of pre-fire aboveground biomass will become charcoal during a fire event (Fearnside et al. 2001). Therefore I expected increased woody biomass would result in increased charcoal production, and that the soil of restored longleaf pine flatwoods would have greater charcoal content than the soil of the grass and herb dominated un-restored pasture and pasture in restoration. However, while the burned un-restored pasture and pasture in restoration sites all had less soil charcoal content than the restored longleaf pine sites, the charcoal in the soil of the unburned un-restored pasture did not differ from that of the restored longleaf pine sites. Although this was unexpected, it may be the result of the land use history of the site. Before The Nature Conservancy acquired the Disney Wilderness Preserve this pasture was used for sod production, and as a result was fertilized and burned more frequently than other pasture sites sampled in this study (TNC

1996). Despite this unpredicted result, both charcoal production and soil organic matter content will be likely to increase as the pastures are further restored to longleaf pine flatwoods.

### Comparing Flatwoods Communities and Time Since Fire Management

#### Aboveground Biomass and Litter Carbon Stocks

The data collected in this study did not provide evidence that aboveground biomass carbon stocks are significantly different among the flatwoods communities; however, they were significantly different at different times since fire management. Aboveground biomass carbon stock was lower than I expected for the pine flatwoods communities (1.90 ( $\pm 0.54$ ) kg C/m<sup>2</sup> to 2.27 ( $\pm 0.63$ ) kg C/m<sup>2</sup>, unburned; 0.95 ( $\pm 0.21$ ) kg C/m<sup>2</sup> to 1.08 ( $\pm 0.53$ ) kg C/m<sup>2</sup>, burned). Powell et al. (2008) reported an aboveground biomass carbon stock of 5.91 kg C/m<sup>2</sup> for a longleaf-slash pine flatwoods community that had been burned approximately 3 years prior. It is likely the difference in tree density at these two sites that resulted in the differences in aboveground biomass carbon stock. The tree density for the site studied in Powell et al. (2008) was 363 trees/ha, while the average tree density for the plots in the current study at Disney Wilderness Preserve was 45 trees/ha in the longleaf pine flatwoods and 75 trees/ha in the slash pine flatwoods. However, only trees with DBH > 7.6 cm were included in the mean tree density for Disney Wilderness Preserve, while a DBH minimum was not reported in Powell et al. (2008).

As stated previously, the loss of approximately 50% of aboveground biomass carbon stock four months after prescribed fire management, seen in the longleaf pine and slash pine flatwoods, is similar to the results of other studies (Lavoie et al. 2010). However, the loss of only 6% of aboveground biomass carbon stock four months after fire, as found in the scrubby flatwoods, was not expected. The aboveground biomass carbon stock ( $1.31 (\pm 0.47)$  kg C/m<sup>2</sup>, unburned;  $1.24 (\pm 0.49)$  kg C/m<sup>2</sup>, burned) calculated for the scrubby flatwoods in this study is comparable to the aboveground biomass carbon stock ( $1.08$  kg C/m<sup>2</sup>) calculated 7 years after fire by Powell et al. (2006) in a similar scrub oak ecosystem under prescribed fire management every 5-7 years. However, in a similar scrub oak ecosystem where prescribed fire management had not occurred for 11 years, Alexis et al. (2007) calculated a pre-burn aboveground biomass carbon stock of  $3.46$  kg C/m<sup>2</sup> and a post-fire carbon stock of  $1.11$  kg C/m<sup>2</sup>, indicating a loss of 32% of aboveground biomass carbon stock during prescribed fire. It may be the difference in frequency of prescribed fire events that resulted in the pre-fire aboveground biomass carbon stock reported in Alexis et al. (2007) to be so much higher than that reported in Powell et al. (2006) and the current study. This increased aboveground biomass may have caused the loss of aboveground biomass carbon stock reported in Alexis et al. (2007) to be so much higher than the loss reported in the current study.

The results of this study did not provide evidence that litter carbon stocks are significantly different among the flatwoods communities or at different times since prescribed fire management. The litter carbon stocks calculated for these communities ( $0.10 (\pm 0.05)$  kg C/m<sup>2</sup> to  $0.20 (\pm 0.06)$  kg C/m<sup>2</sup>) were lower than those reported in other studies of similar ecosystems.

For scrub oak ecosystems, Alexis et al. (2007) reported litter carbon stocks of 0.61 kg C/m<sup>2</sup> pre-fire and 0.39 kg C/m<sup>2</sup> post-fire, and Powell et al. (2006) reported a litter carbon stock of 0.34 kg C/m<sup>2</sup> seven years after fire. The variation in the results of these studies and the results of the current study may be due to differences in fire return interval. As stated previously, the study site in Alexis et al. (2010) had not burned for 11 years, while the study sites in Powell et al. (2006) burns every five-seven years, and Disney Wilderness Preserve burns every two-three years.

In a longleaf-slash pine flatwoods community that had not burned for six years, Lavoie et al. (2010) reported a pre-fire carbon stock in litter that was approximately 10 times the litter carbon stock calculated for the flatwoods communities in the current study. Again, this discrepancy may be the result of differences in fire return interval. Lavoie et al. (2010) reported a pre-fire litter carbon stock of approximately 1.4 kg C/m<sup>2</sup> and a post-fire litter carbon stock of approximately 0.05 kg C/m<sup>2</sup> four months after fire, and also found that litter carbon stock was only at 53.0 (±11.9) % of pre-fire stock three years after fire. Since Disney Wilderness Preserve has a fire return interval of two-three years, it is possible that the litter carbon stock is not reaching its highest potential level between fire events, and may be lower relative to other locations that have a longer fire return interval.

## Soil Carbon Stock and Dynamics

The highly permeable drier soil of the scrubby flatwoods had significantly less carbon stock when compared to the soils of the pine flatwoods. In addition to differences in soil carbon stock, the soil profile of the scrubby flatwoods was found to be less enriched in  $^{13}\text{C}$  with increasing soil depth than the soils of the longleaf pine flatwoods and slash pine flatwoods. In  $\text{C}_3$  plant communities, enrichment in  $^{13}\text{C}$  with increasing soil depth has been attributed to microbial activity and increased microbial contribution to soil carbon stocks with increasing soil depth (Buchmann et al. 1997; Ehleringer et al. 2000; Fry 2006). Therefore it may be that there is less microbial presence and activity in the soil of the scrubby flatwoods than in the soils of the longleaf pine and slash pine flatwoods; however, further research is necessary to confidently make this conclusion. As in similar studies, this study did not provide evidence that prescribed fire has a significant immediate effect on the soil carbon stocks of these flatwoods communities (Johnson and Curtis 2001; Alexis et al. 2007; Garten 2006; Lavoie et al. 2010); however, additional research is needed to assess the long-term cumulative effect of prescribed fire on soil carbon stock in these communities.



## CONCLUSIONS

Restoring the pastures at Disney Wilderness Preserve to longleaf pine flatwoods will certainly provide both crucial habitat for the species that use this ecosystem and the much desired ecosystem service of carbon storage. In addition to the obvious goal of increased aboveground carbon stock, soil carbon stability could be added as a goal for the restoration efforts currently underway in the pastures at Disney Wilderness Preserve. The isotopic composition of the soil profile in the restored longleaf pine flatwoods community could serve as a reference value for the soil profiles of the restoration sites. This technique provides a means of assessing changes in soil carbon properties as a result of restoration, even if changes in overall soil carbon stock are not apparent.

Additionally, in an effort to increase aboveground biomass in the restored flatwoods communities, the fire management regime in these ecosystems could be modified. While it is important to continue the two to three year fire return cycle while ecosystem structure is still being reestablished, eventually it may be possible to extend the fire return interval to five to six years to allow for increased biomass accumulation between fire events. In addition to increasing litter and aboveground biomass, this could potentially increase charcoal production. Alexis et al. (2007) found that as fire temperature increases, charcoal production increases as well. Therefore, a longer fire return interval for Disney Wilderness Preserve may lead to increased fuel load between fires, resulting in hotter fires and more charcoal production. Future research for the preserve should include studies aimed at assessing the effects of a prolonged fire return interval on the flora, fauna, and carbon stocks in these flatwoods

communities. It will be important to observe the results for each community type as they may respond differently to this treatment.

The soil cores taken during this study accounted for only a small fraction of the fine root biomass present in these ecosystems, and further research is needed to account for the total belowground biomass in each ecosystem. Once total belowground biomass is incorporated, the quantified carbon stock values of these ecosystems will increase greatly. In particular, the scrubby flatwoods will likely have a considerable increase in overall carbon stock with the addition of belowground biomass. As an adaptation to frequent fire, scrub oak species have an extensive root system that allows them to quickly re-sprout after a fire event. Therefore, given the dominant presence of scrub oak species in the scrubby flatwoods, a large portion of the overall biomass of this ecosystem is likely located in the scrub oak root system (Stover et al. 2007).

Currently, the five studied ecosystems at Disney Wilderness Preserve (DWP) encompass 2823 ha of the preserve. The carbon stored in the aboveground biomass, litter, and top 90 cm of soil in this 2823 ha area totals approximately 400,000 Mg C (Figure 18). Restoring the pasture areas to longleaf pine flatwoods will increase carbon storage in these areas by approximately 25%. There are also roughly 1800 ha of wetland areas including marshes, bayheads, and cypress domes that undoubtedly contribute greatly to the carbon stock of the preserve. To gain a more comprehensive estimate of the carbon storage benefits of the restoration and management activities at DWP, future research for the carbon storage objectives on the preserve should be

focused on assessing the carbon stock of these wetland areas and increasing carbon stock monitoring of the areas presented in this study.

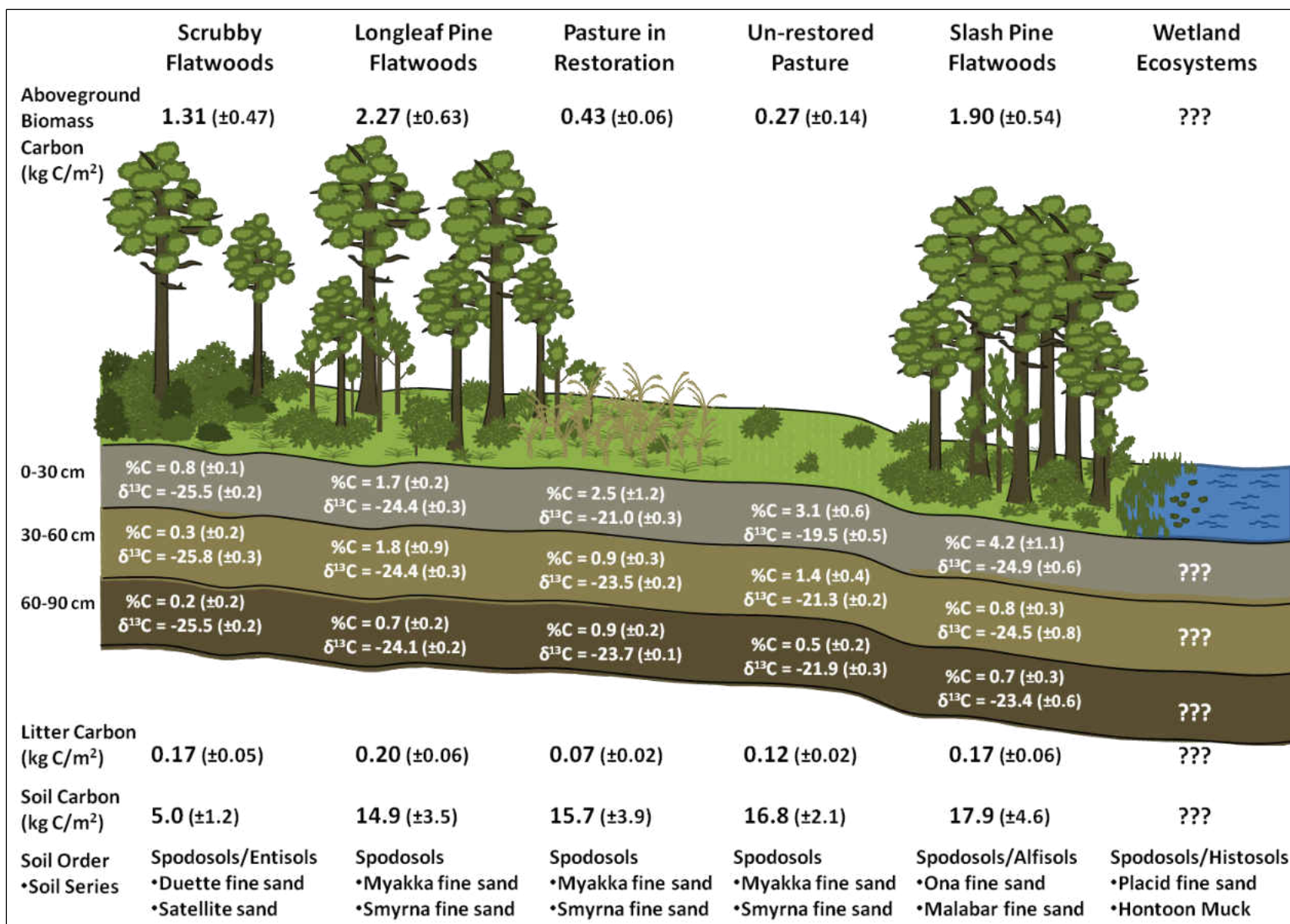


Figure 18. Illustration of the Disney Wilderness Preserve landscape with mean results for unburned ecosystems.

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