

FINAL REPORT

Site Preparation for Longleaf Pine Restoration on Hydric Sites:
Stand Development and Ground Layer Responses 15 Years
Following Planting

SERDP Project RC-2706

NOVEMBER 2021

Benjamin Knapp
Connor Crouch
Geoff Wang
Joan Walker
University of Missouri System

Distribution Statement A

This document has been cleared for public release



This report was prepared under contract to the Department of Defense Strategic Environmental Research and Development Program (SERDP). The publication of this report does not indicate endorsement by the Department of Defense, nor should the contents be construed as reflecting the official policy or position of the Department of Defense. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the Department of Defense.

REPORT DOCUMENTATION PAGE				Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.					
1. REPORT DATE (DD-MM-YYYY) 18-06-2021		2. REPORT TYPE SERDP Final Report		3. DATES COVERED (From - To) 07-20-2017 - 07-21-2021	
4. TITLE AND SUBTITLE Site Preparation for Longleaf Pine Restoration on Hydric Sites: Stand Development and Ground Layer Responses 15 Years following Planting				5a. CONTRACT NUMBER W912HQ-17-C-022	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Knapp, Benjamin O.; Wang, G. Geoff; Walker, Joan L.; Crouch, Connor D.				5d. PROJECT NUMBER RC-2706	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Missouri System 316 University Hall Columbia MO 65211-3020				8. PERFORMING ORGANIZATION REPORT NUMBER RC-2706	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) SERDP 4800 Mark Center Drive Suite 17D08 Alexandria, VA 22350-3600				10. SPONSOR/MONITOR'S ACRONYM(S) SERDP	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S) RC-2706	
12. DISTRIBUTION / AVAILABILITY STATEMENT Distribution A: Approved for public release					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Longleaf pine restoration is especially challenging on hydric soils of the Atlantic Coastal Plain. Site preparation is commonly used to improve establishment of planted trees but may have negative impacts on the ground layer plant communities. This project provides results through 15 years from a manipulative field experiment that evaluates alternative site preparation treatment effects on planted trees and ground layer plant communities. More intensive site preparation treatments, including bedding and broadcast herbicide, provided the greatest benefit to planted longleaf pine trees. The herbicide treatment provided early control over woody vegetation, which recovered after 15 years to result in dominance of woody plants on those treatments. Herbicides also resulted in lasting reductions in wiregrass. In contrast, chopping as site preparation generally resulted in no different from the untreated control. Decisions on site preparation must be relative to management objectives, but our study shows long-term effects of these treatments on stand development.					
15. SUBJECT TERMS bedding; chopping; ecosystem restoration; herbicide; grass stage; ground layer vegetation; mounding; <i>Pinus palustris</i> ; savanna; site preparation; wiregrass					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT SAR	18. NUMBER OF PAGES 103	19a. NAME OF RESPONSIBLE PERSON Knapp, Benjamin O.
a. REPORT UNCLASS	b. ABSTRACT UNCLASS	c. THIS PAGE UNCLASS			19b. TELEPHONE NUMBER (include area code) 573-882-0867

Table of Contents

Table of Contents	i
List of Tables	iii
List of Figures	iv
List of Acronyms	vii
Keywords	vii
Acknowledgements.....	vii
Abstract.....	1
Introduction and Objectives	1
Technical Approach	1
Results.....	1
Benefits	1
Executive Summary	2
Introduction.....	2
Objectives	2
Technical Approach	3
Results and Discussion	5
Implications for Future Research and Benefits.....	8
Objective.....	9
Technical Objective 1 (O1).....	10
Technical Objective 2 (O2).....	10
Background.....	12
Longleaf Pine Restoration.....	13
Site Preparation.....	14
Longleaf Pine Plantation Development	16
Overview of SERDP SI-1303	17
Materials and Methods.....	19
Study Area	19
Experimental Design.....	19
Data Collection and Analysis.....	27
Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation	27
Objective 2 – Ground-Layer Vegetation Response to Site Preparation.....	29

Results.....	32
Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation	32
Stand-level Responses	32
Individual Tree-level Responses.....	36
Objective 2 – Ground-Layer Vegetation Response to Site Preparation.....	40
Response of Vegetation Cover.....	40
Plant Diversity in Year 15.....	46
Vegetation Composition through Time.....	46
Light Transmittance through the Canopy	49
Local Effects of Bedding	51
Discussion.....	53
Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation	53
Objective 2 – Ground-Layer Vegetation Response to Site Preparation.....	56
Management Implications.....	58
Conclusions and Implications for Future Research/Implementation	60
Response to IPR Action Items	63
Literature Cited	65
Appendices.....	75

List of Tables

Table 1. Summary of treatments used in the study.	20
Table 2. Least square means of longleaf pine stand-level responses to individual site preparation treatment levels 15 years after application.....	34
Table 3. Least square means of tree-level responses to individual site preparation treatment levels 15 years after application.....	37
Table 4. Responses to individual soil manipulation and vegetation control treatments 15 years after application, based on analysis using a 2×3 factorial structure.	45
Table 5. Means and standard errors for measures of diversity, percent cover, and light transmittance on bedded rows and interbed areas 15 years after treatment application.	52
Table 6. Summary of estimated costs for site preparation treatments used in the study.	64

List of Figures

Figure 1. Layout of Block 1, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).	21
Figure 2. Layout of Block 2, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).	22
Figure 3. Layout of Block 3, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).	23
Figure 4. Layout of Block 5, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).	24
Figure 5. Layout of Block 7, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).	25
Figure 6. Images from treatment application or shortly after, depicting A) application of chopping treatment; B) broadcast application of herbicide; C) regrowth near end of one growing season on CF treatment; D) regrowth near end of one growing season on CB treatment, with beds apparent; E) mounds scattered throughout treatment area; F) planted longleaf pine seedling during first growing season after treatment.	26
Figure 7. Example of longleaf pine tree cut into sections at specific intervals for reconstructing height growth patterns through time.	27
Figure 8. Longleaf pine stand-level responses to site preparation study treatments 15 years after application. Treatments are defined in Table 1. Bars show treatment means and standard errors; points show means for each experimental unit. Same letters indicate no significant difference among treatments within a response variable based upon Tukey-adjusted pairwise comparisons; p-values are from one-way ANOVA tests.	33
Figure 9. Mean survival for each study treatment (defined in Table 1) at three points in time: 8 months, 20 months, and 15 years after planting.	35

Figure 10. Diameter (means and standard error) of each study treatment (defined in Table 1) relative to the control (F) at five points in time, from seedling establishment into stand development.	35
Figure 11. Tree-level responses (means and standard error) to site preparation study treatments 15 years after application. Treatments are defined in Table 1. Same letters indicate no significant difference among treatments within a response variable based upon Tukey-adjusted pairwise comparisons; p-values are from one-way ANOVA tests.....	37
Figure 12. Reconstruction of mean height growth after grass stage emergence for each study treatment (defined in Table 1). Individual treatment panels include a line for the treatment mean and an envelope of the 95% confidence interval. The plot with all eight treatments shows only treatment means.	38
Figure 13. Mean annual height growth after grass stage emergence for each study treatment (defined in Table 1). Individual treatment panels include a line for the treatment mean and an envelope of the 95% confidence interval. The plot with all eight treatments shows only treatment means. Lines are smoothed on a three-year mean to reduce noise. Treatment lines are cut off after all five experimental units are no longer represented in the data.	39
Figure 14. Cover (means and standard errors) of (a) woody and (b) herbaceous plants through 15 years after site preparation in a restored longleaf pine plantation. Same letters indicate no significant difference among treatments for a given measurement year. Treatments are defined in Table 1. Intensive site preparation reduced woody plant cover through three years after treatment without reducing herbaceous cover, but by year 15 all treatments had similar woody and herbaceous cover.....	41
Figure 15. Cover (means and standard errors) of (a) shrubs and (b) graminoids through 15 years after site preparation in a restored longleaf pine plantation. Same letters indicate no significant difference among treatments for a given measurement year. Treatments are defined in Table 1. Intensive site preparation reduced shrub cover through three years after treatment without reducing herbaceous cover. However, by year 15 all treatments had similar shrub cover, and treatments that combined herbicide and bedding had reduced graminoid cover compared to CF.	42
Figure 16. Cover (means and standard errors) of bunchgrasses, wiregrass, and forbs through 15 years after site preparation and among eight study treatments (defined in Table 1). Same letters indicate no significant difference among measurement periods or treatments within each panel. Wiregrass recovered more slowly than bunchgrasses or forbs, and intensive site preparation resulted in lasting reductions in bunchgrass and wiregrass cover.	44
Figure 17. Three measures of plant species diversity (means and standard errors) across eight site preparation treatments (defined in Table 1). Same letters indicate no significant difference among treatments within each panel. There were no significant differences in richness, but CF had significantly higher diversity and evenness than HF.	47

Figure 18. Results from NMDS ordination in which the centroid of each treatment in each year is shown. PERMANOVA indicated that all treatments significantly differed except for the following treatments that were similar: CM and CB; CHB, HB, and HM.	48
Figure 19. Estimates of A) gap light index and B) gap fraction (means and standard errors) obtained from hemispherical photos taken 15 years after site preparation. Treatments are defined in Table 1. The p-values are from one-way ANOVA tests for treatment effects; same letters indicate no significant difference ($\alpha = 0.05$) among treatments within a response variable.	49
Figure 20. Hemispherical photographs to contrast the canopy closure and light transmittance of the CF treatment (top) and the HB treatment (bottom).....	50
Figure 21. Images to contrast the stand structure and development of A) an untreated control (F); B) chop-bedding treatment (CB); and C) herbicide-bedding treatment (HB).	53
Figure 22. Bedded site immediately following treatment application, illustrating differences in plant cover on beds and interbed areas.	57

List of Acronyms

ANOVA: analysis of variance
CB: chop-bed (site preparation treatment used in study)
CF: chop-flat (site preparation treatment used in study)
CHB: chop-herbicide-bed (site preparation treatment used in study)
CM: chop-mound (site preparation treatment used in study)
DBH: diameter at breast height, measured at 1.37 m
DoD: Department of Defense
F: flat-plant (untreated control treatment used in study)
HB: herbicide-bed (site preparation treatment used in study)
HF: herbicide-flat (site preparation treatment used in study)
HM: herbicide-mound (site preparation treatment used in study)
MCBCL: Marine Corps Base Camp Lejeune
NMDS: non-metric multidimensional scaling
RCD: root collar diameter
RCW: red-cockaded woodpecker

Keywords

bedding; chopping; ecosystem restoration; herbicide; grass stage; ground layer vegetation; mounding; *Pinus palustris*; savanna; site preparation; wiregrass

Acknowledgements

RC-2706 was built upon efforts of invested into SI-1303, the original study established to better understand effects of site preparation on longleaf pine ecosystems during restoration. This work was instigated by land managers in the Environmental Conservation Branch at Marine Corps Base Camp Lejeune, including John Townsend, Danny Becker, and Danny Marshburn. Numerous individuals within the Environmental Conservation Branch provided logistical support in establishing this study, from identification of potential study sites to the implementation of treatments. Critically, the sites were maintained through time with the study design uncompromised, either accidentally or though other land use. We are grateful for the forethought that allowed opportunity to remeasure the study areas 15 years after original establishment.

The Environmental Conservation Branch has continued to support this work, with particular thanks to Martin Korenek, Austin Powell, and Peter Steponkus for logistical assistance and maintenance of the sites with prescribed fire. Susan Cohen deserves recognition for her extensive efforts to make this project happen (both as SI-1303 and as RC-2706) and making sure the research team had safe access to the site locations when needed. The hard work of Connor Crouch, who completed his Master of Science degree working on this project, was largely responsible for the completion of data collection and analyses. Finally, we thank the SERDP program for funding support and for interest in this work, creating the mechanism for valuable scientific-practitioner exchange that results in management action.

Abstract

Introduction and Objectives:

Longleaf pine restoration is a major objective of land managers throughout the southeastern United States, including across Department of Defense installations. Within the Atlantic Coastal Plain, hydric conditions are common on flatwoods and savanna sites. These site types can support highly diverse plant communities, but establishing longleaf pine trees on such sites is difficult due to the wet soil conditions and the abundant competition from other vegetation. Site preparation is commonly used to improve establishment of planted trees but may have negative impacts on the ground layer plant communities. Short-term results from site preparation studies are useful but do not provide information on trajectories of plantation establishment. This study remeasured a previously established field experiment (SI-1303) to understand effects of alternative site preparation treatments on plantation development through 15 years after planting.

Technical Approach

This project used a manipulative field experiment to contrast eight site preparation treatments that included a factorial combination of two treatments that controlled competing vegetation (chopping and herbicide) with three treatments that manipulated soil conditions (bedding, mounding, and untreated/flat). In addition to the six factorial combinations, the study included an untreated control and a more intense treatment that combined bedding, chopping, and herbicide. Data collected in SI-1303 during the first three years after study initiation (2004-2006) were combined with data collected in 2017/2018 to report on longer-term responses of the planted trees and the ground layer plant community.

Results

The site preparation treatments had long-term effects on longleaf pine establishment and plantation development. The untreated control and the chopping treatment (without the addition of bedding and mounding) resulted in the lowest survival of planted trees and the poorest tree growth. In contrast, the higher intensity treatments of bedding or mounding combined with herbicide resulted in the greatest tree survival and the largest longleaf pine trees. In general, successful trees emerged from the grass stage within the first 3 years of the study, with the chopping treatment having later average grass stage emergence. The site preparation treatments resulted in sustained growth increases across the 15-year period. While resulting in the greatest benefit to planted longleaf pine trees, the site preparation treatments of greater intensity (bedding, mounding, herbicide) generally also had greater impacts on the ground layer plant community. Herbicide in particular had long-term effects. The herbicide treatment was designed to control woody vegetation and was effective in short-term control, but woody plant abundance had recovered and dominated these treatments after 15 years. The herbicide treatment also reduced the abundance of bunchgrasses, and wiregrass in particular, compared to chopping. The mechanical disturbances of bedding and mounding also reduced wiregrass, but this effect was locally variable in the bedded treatments.

Benefits

Understanding long-term patterns in plantation development is critical for management for restoration objectives. Maximizing the rate of plantation establishment will be achieved with more intensive site preparation, including bedding and herbicide. These treatments will also result in greater dominance of woody vegetation and shifts in plant community composition, including reductions in wiregrass. Site preparation decisions can be made based on specific restoration objectives.

Executive Summary

Introduction

Longleaf pine restoration is a major objective of land managers throughout the southeastern United States, including across Department of Defense installations. Within the Atlantic Coastal Plain, hydric conditions are common on flatwoods and savanna sites. These site types can support highly diverse plant communities, but establishing longleaf pine trees on such sites is difficult due to the wet soil conditions and the abundant competition from other vegetation. Site preparation is commonly used to improve establishment (survival and growth) of planted trees but may have negative impacts on the ground layer plant communities. The specific site preparation treatments used depend on the limiting conditions. On wet sites, soil manipulation treatments are commonly used to improve soil drainage around the root systems of newly planted trees. Bedding is often used in the southern United States, and mounding is an alternative site preparation treatment that yields similar benefit, more often used in the northern United States. Other treatments are used to reduce competition vegetation. Chopping is a surface disturbance that crushes standing vegetation and is also common in the south, whereas herbicide treatments can be used to target mortality of specific types of plants.

Short-term results from site preparation studies are useful but do not provide information on trajectories of plantation establishment. This is particularly relevant to longleaf pine plantations due to the early growth morphology of longleaf pine. As seedlings, longleaf pines remain in the ‘grass stage’ without height growth, for several years. If growing in unfavorable conditions, longleaf pine seedlings may never emerge from the grass stage and instead succumb to mortality. While emergence from the grass stage is a critical step in plantation development, it is not known if site preparation treatments will 1) accelerate the time to emergence, 2) increase growth rates after emergence, or 3) both. Moreover, short-term studies often report dramatic effects of site preparation on the ground layer plant community, with treatments often designed to reduce abundance of these plants. Through time, the plant community recovers, although it is not known if alternative site preparation treatments alter the course of that recovery. This study remeasured a previously established field experiment (SI-1303) to understand effects of alternative site preparation treatments on plantation development through 15 years after planting.

Objectives

Project RC-2706 addresses Research Needs 1, 2, and 4 from Statement of Need RCSON-17-02, “Long-term Ecological Studies: Testing Previous Hypotheses and Conclusions”. The restoration and management of longleaf pine ecosystems are of high priority for land managers throughout the southeastern United States, including several large Department of Defense (DoD) installations. The overall goal of this project was to determine the effects of site preparation treatments on the development of longleaf pine (*Pinus palustris*) plantations at a temporal scale appropriate for evaluating restoration outcomes and informing management decisions on wet Coastal Plain sites. This project re-measured study plots established for SERDP Project SI-1303, “Regenerating Longleaf Pine on Hydric Soils: Short- and Long-term Effects on Native Ground-layer Vegetation,” which provided information about site preparation treatment effects through three years following artificial regeneration.

The project includes two objectives: 1) determine the effects of site preparation treatments on the establishment of longleaf pine stands (e.g., tree survival, emergence from the grass stage, and tree size) 15 years after plantation establishment; and 2) determine the effects of site preparation treatments on ground layer and mid-story vegetation abundance, species richness, and composition 15 years after plantation establishment. Site preparation treatments are typically prescribed to improve conditions for planted trees, and Objective 1 will provide long-term information on treatment efficacy. Objective 2 is motivated by the ecological value of the ground flora within longleaf pine ecosystems, with recognition that tree growth during plantation development generally reduces the abundance of ground flora, and site preparation may directly and indirectly affect the rate and magnitude of those impacts.

Technical Approach

This study was conducted on Marine Corps Base Camp Lejeune (34°36'N 77°24'W) in Onslow County, North Carolina. The study sites were located primarily (90%) on Leon sand (sandy, siliceous, thermic Aeric Alaquod), a poorly drained Spodosol with a fluctuating water table that may be at or near the surface, with smaller areas of drier Kureb soils (8%) and trace amounts of other savanna soils. Spodosols are the primary soil order of flatwoods and are characterized by their sandy, acidic, and infertile nature. The study sites spanned an elevation gradient from 13.5 m to 19.7 m. Corresponding to these elevation differences, fairly distinct vegetation gradients were evident, ranging from pocosin-like habitats dominated by Cyperaceae and pocosin indicator shrubs to mesic flatwoods containing *Pteridium latiusculum*, *Gaylussacia dumosa*, *Lyonia mariana*, *Eupatorium rotundifolium*, *Sericocarpus tortifolius*, and other mesic species. These natural communities include longleaf pine as the overstory species, with density varying along the hydrologic gradient, and common grasses including wiregrass (*Aristida* spp.), bluestems (*Andropogon* spp., *Schizachyrium* spp.), and panic grasses (*Panicum* spp., *Dichanthelium* spp.). The sites selected for this study were previously used for slash and loblolly pine (*Pinus taeda*) plantations, which were harvested 6 months to 2 years prior to treatment installation.

The study uses a randomized complete block design with 5 blocks. Each block includes eight experimental units measuring approximately 0.6 ha each, with 15 m buffers between experimental units (i.e., plots). Plots are rectangular (100 x 60 m) and oriented according to spatial constraints associated with each block. The eight study treatments (Table ES1) are made up of combinations of site preparation treatments that control competing vegetation (herbicide and chopping) and site preparation treatments that manipulate the planting site (flat-planting, mounding, and bedding). The resulting treatments include: F (flat planting; control), HF (herbicide and flat-planting), CF (chopping and flat-planting), HM (herbicide and mounding), CM (chopping and mounding), HB (herbicide and bedding), CB (chopping and bedding), and CHB (chopping, herbicide, and bedding). The design creates a 2 x 3 factorial structure for evaluating vegetation control treatments and planting site manipulation treatments, plus an untreated control and the more intensive CHB treatment. For each block, the overstory of the previous stands had been harvested within two years prior to treatment application, and remaining vegetation was removed by shearing with a KG blade. Site preparation treatments were applied in summer 2003, with vegetation control treatments applied first and followed by the mechanical treatments. All study blocks were burned prior to planting in 2003, then again in 2006. Two blocks were burned in 2009, then all blocks were burned in 2011/2012 and 2016/2017.

Planted longleaf pine seedlings were tagged and growth was measured repeatedly during the first three years (2004-2006). In 2018, the study plots were sampled again to determine the number of trees per hectare, tree size (height and diameter), and stand density (basal area). A complete census of each plot was

Table ES1. Summary of site preparation treatments

Treatment		<u>Vegetation Control</u>		<u>Soil Manipulation</u>		
		Chop	Herbicide	Flat	Mound	Bed
Flat [control]	(F)			X		
Chop/flat	(CF)	X		X		
Herbicide/flat	(HF)		X	X		
Chop/mound	(CM)	X			X	
Herbicide/mound	(HM)		X		X	
Chop/bed	(CB)	X				X
Herbicide/bed	(HB)		X			X
Chop/herbicide/bed	(CHB)	X	X			X

conducted at planting (2003), in 2006, and in 2018 to determine survival. The timing of grass stage emergence and individual tree growth rates were determined using stem analysis, a destructive sampling technique in which trees are cut down, sectioned at known heights, and aged through ring counting.

The ground layer plant community was sampled in the first three years following site preparation treatment application (2004, 2005, 2006) using a stratified random sampling approach. Within each study plot, four equally spaced transects were located at consistent intervals. Along each transect, three 1 x 1 m quadrats were randomly located (determined by a randomly generated number representing the distance along the transect), resulting in a total of 12 sampling quadrats per plot. The same quadrat locations were remeasured during the 2004, 2005, and 2006 sampling efforts of SI-1303. In 2018, we resampled the ground layer vegetation using the same sampling methodology. However, the quadrat locations could not be re-located from the earlier sampling periods, so we used the same approach to randomly locate three quadrats along each of four transects located at consistent intervals across each experimental unit. In summer 2018, each quadrat in bedded treatments was paired with an adjacent quadrat to determine the local effects of bedding on the ground layer vegetation community. Within each quadrat, every vascular plant was identified to species, and percent cover of each species was recorded.

We determined response variables measured consistently between the two study periods (SI-1303 in 2004-2006 and RC-2706 in 2017/2018) and joined the datasets for longitudinal data analyses. For tree data, this included survival and a measure of diameter relative to the untreated controls. For ground layer plant data, this included cover species or functional group. For these datasets, we used generalized linear mixed models to determine effects of treatments on response variables through time, using a repeated measures structure in a randomized complete block design. The sub-sampling approaches during sampling were incorporated into the models through random intercept terms. In addition, the plant community composition datasets from all years were combined, and non-metric multidimensional scaling (NMDS) was used to visualize compositional differences by treatment and through time. Permutational multivariate analysis of variance using distance matrices was used to determine significant effects among treatments through time.

Some response variables were relevant only to data collected in 2018. For tree data, this included diameter at breast height (DBH), because the seedlings measured in SI-1303 were not large enough for DBH measurements, as well as height and basal area. For the ground layer plant data, this included species richness, diversity, and evenness. Although these metrics could be calculated for the data from SI-1303, the taxonomic resolution differed in the two sampling periods such that the data were not comparable in that way. Finally, we used the stem analysis data to reconstruct height growth patterns through time. For each treatment, we calculated the mean and confidence intervals of tree height by age and annual height growth following grass stage emergence.

Results and Discussion

Site preparation had lasting impacts on longleaf plantation development through fifteen years after planting. The untreated control and the chop-only treatment were similar for all metrics and resulted in the smallest trees, the fewest number of trees per hectare, and the greatest number of trees remaining in the grass stage (Figure ES1). High intensity treatments, including both bedding with herbicide, resulted in the largest trees and the greatest stand densities. Survival was lowest on F and CF, each of which was below 25% survival. For all other treatments, survival ranged from around 30% to 45% and did not differ among the site preparation treatments.

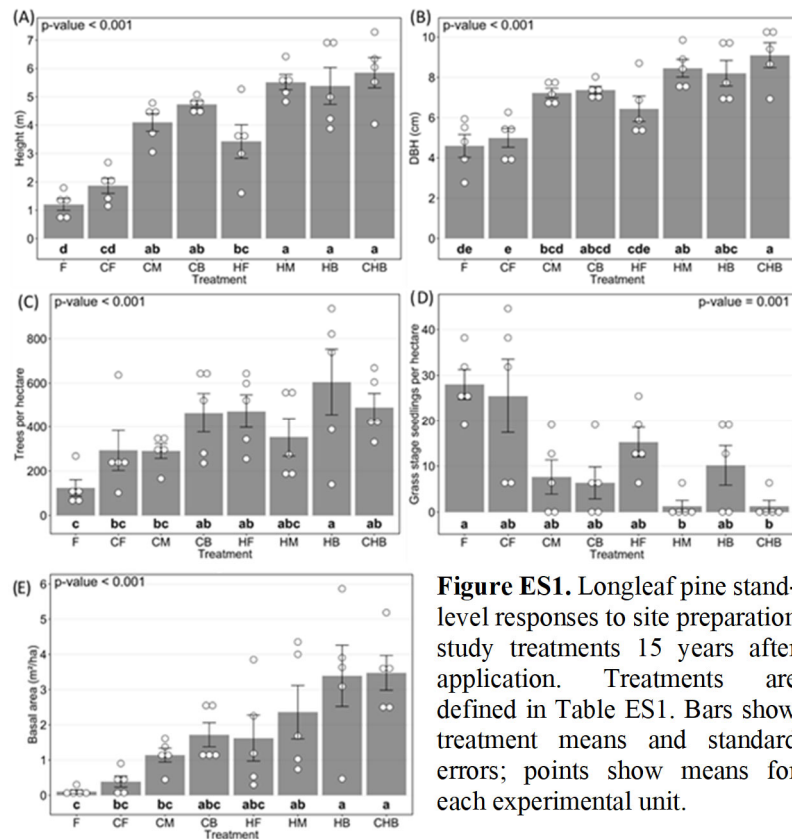


Figure ES1. Longleaf pine stand-level responses to site preparation study treatments 15 years after application. Treatments are defined in Table ES1. Bars show treatment means and standard errors; points show means for each experimental unit.

Stem analysis allowed us to reconstruct height growth of each sampled longleaf pine through time. Because identifying tree rings of grass stage longleaf pine seedlings is challenging, and may be impossible, we used a stem height of 25 cm as the starting point for grass stage emergence. All treatments were significantly taller through time than CF, due to that treatment's late emergence from the grass stage and slow growth following emergence. During the most recent four years, HB was significantly taller than F, and during that same period, HM approached significantly greater height than F. In general, the patterns of total tree height observed in the sub-sample used for the stem analysis dataset were similar to those from the stand-level analysis.

Annual height growth was standardized as ‘years after grass stage emergence’ to account for variation in the length in the grass stage. Trees are expected to rapidly increase growth during early years and then level off or decrease growth through time. For years 1-3, HB had significantly greater height growth than F, while HM had significantly greater height growth than F during years 2-5 (Figure ES2). Annual height growth continued to increase slightly over time for F, while growth generally levelled off after 4-5 years for the other treatments. Because the height growth represents annual growth, differences among treatments maintained through time will result in increased differences in total height through time. Therefore, these results suggest that most of the gains in total height for the treatments with the tallest trees occurred between years 2 and 6 following site preparation, although HB continues to show rapid annual height growth.

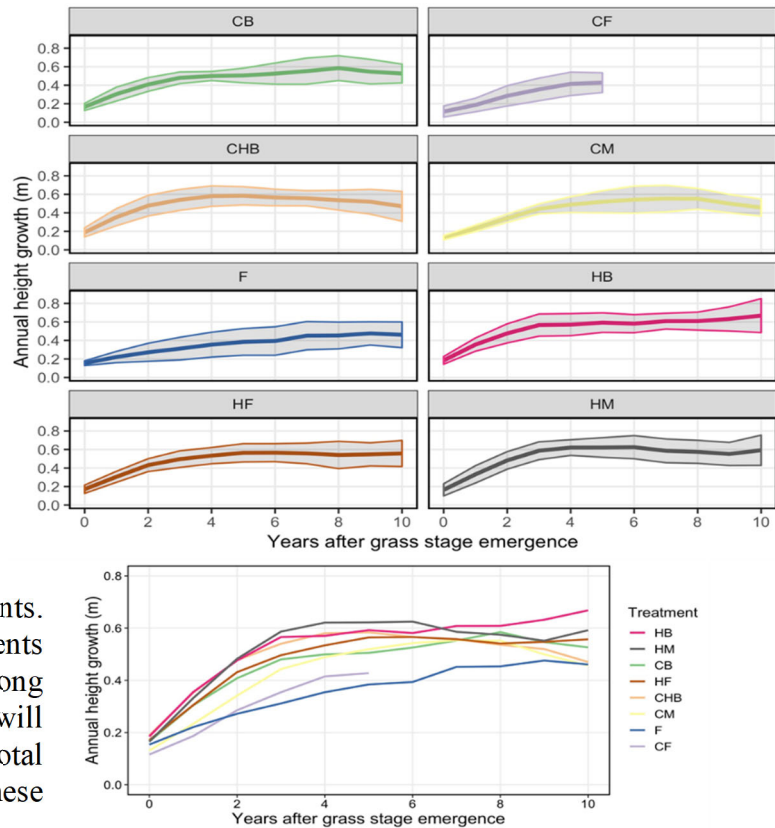


Figure ES2. Mean annual height growth after grass stage emergence for each study treatment (defined in Table 1). Individual treatment panels include a line for the treatment mean and an envelope of the 95% confidence interval. The plot with all eight treatments shows only treatment means. Lines are smoothed on a three-year mean to reduce noise.

All site preparation treatments that included herbicide resulted in reductions in woody vegetation, when compared to the untreated control, during the first year following treatment, whereas the cover of herbaceous plants did not differ among the treatments (Figure ES3). These results were initially promising for encouraging development of the herbaceous species, which include several species of high conservation value and harbor most of the plant diversity. Through time, however, the abundance of woody vegetation generally increased, with dramatic increases in the cover of woody vegetation by 2018 in treatments with herbicide. The cover of herbaceous vegetation did not differ among the treatments in 2018 (Figure ES3). The patterns observed for all woody vegetation was similar to that for the shrub group, due to the dominance of shrub species on these sites. Among the herbaceous species functional groups, graminoids were significantly lower on the herbicide and bed treatments (HB, CHB) than on the chopping treatment (CF) by 2018. Results further showed that the treatments with herbicide resulted in significantly lower bunchgrasses, and wiregrass (*Aristida stricta*) specifically, compared to treatments without herbicides. The sensitivity of wiregrass to mechanical disturbance has been previously reported, and is also supported by our results, but the effect of the herbicide treatment on wiregrass may be problematic for future fire management on these sites.

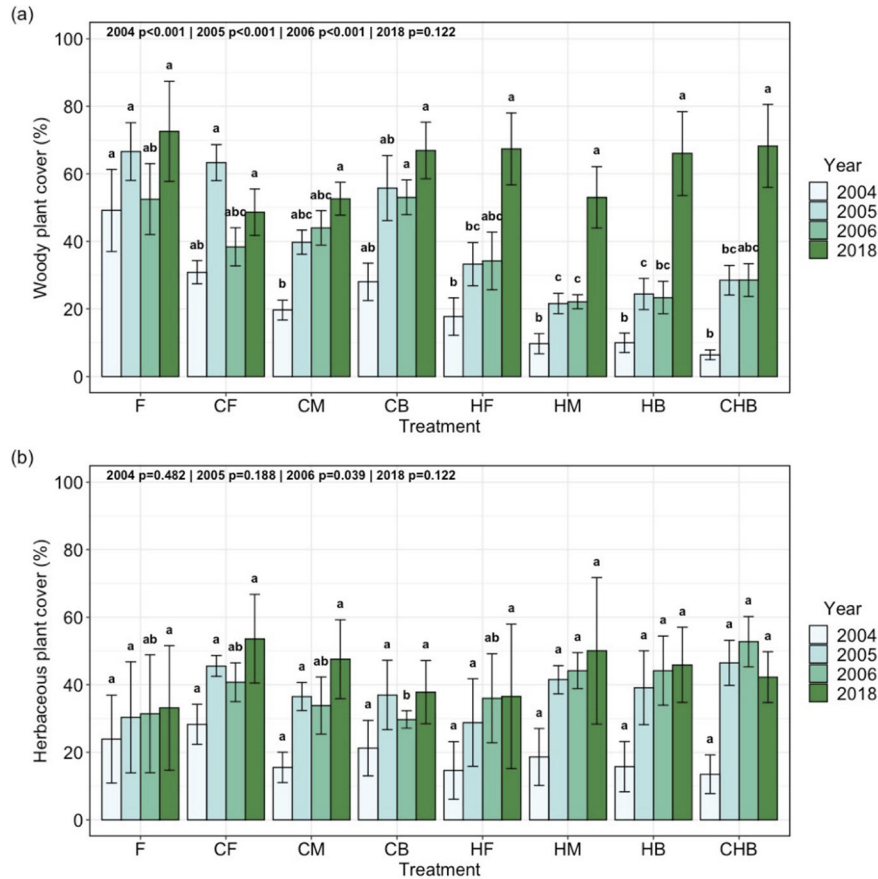


Figure ES3. Cover (mean and standard error) of (a) woody and (b) herbaceous plants through 15 years after site preparation in a restored longleaf pine plantation. Same letters indicate no significant difference among treatments for a given measurement year. Treatments are defined in Table 1. Intensive site preparation reduced woody plant cover through three years after treatment without reducing herbaceous cover, but by year 15 all treatments had similar woody and herbaceous cover.

Fifteen years after site preparation, plant species richness did not significantly differ among the eight study treatments. Across all treatments, mean richness was 16.1 species per 1 m². In contrast, diversity was significantly greater on CF, which had a mean of 8.22, than F, HF, and HB, which had mean diversities of slightly greater than 6. Evenness was significantly greater on CF, which had a mean of 0.71, than HF, which had a mean of 0.63. The herbicide treatment resulted in lower diversity and evenness than the chopping treatment.

The composition of the ground layer plant community was significantly affected by the site preparation treatments, with strong separation in ordination space for treatments that included herbicide in 2004, the first year after treatment (Figure ES4). The plant communities showed similar trajectories through time among the site preparation treatments, with movement mostly along the first and third axes over the 15 year study period. There was no significant interaction between site preparation treatment and time for the composition analysis, but the communities did change through time and differed among treatments. The only treatments that did not differ significantly were comparisons of CM and CB and comparisons of CHB, HB, and HM.

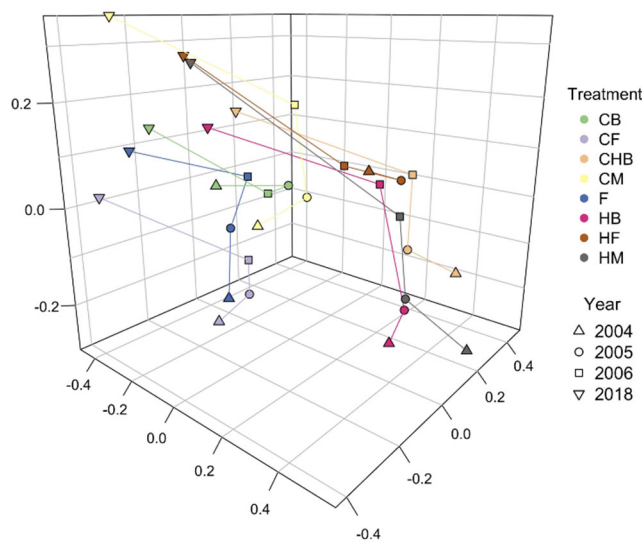


Figure ES4. Results from NMDS ordination in which the centroid of each treatment in each year is shown. PERMANOVA indicated that all treatments significantly differed except for the following treatments that were similar: CM and CB; CHB, HB, and HM.

Implications for Future Research and Benefits

Site preparation has lasting, long-term impacts on stand development. More intensive treatments, including bedding and herbicide in combination, maximized longleaf pine seedling survival and growth. However, these treatments also reduced the abundance of wiregrass and resulted in a dominance of woody vegetation 15 years after treatment. Although species richness, diversity, and evenness had few differences among treatments, with no site preparation treatments reducing these when compared to the control, the composition of the ground layer plant community differed among treatments, showing lasting effects of the herbicide treatment on community composition. The untreated control and the chopping-only treatments resulted in few trees established at the sites and the slowest tree growth. On the untreated control sites, mean tree height after 15 years was only 1.2 m, with many seedlings remaining in the grass stage. It is likely that these trees will not survive. Although it is possible to establish savanna structure through time on the untreated control, our results support that some form of site preparation is needed for confidence in longleaf pine establishment on these hydric sites.

Long-term responses to site preparation can be complicated by additional management action post-site preparation as well as indirect effects of site preparation on other management actions. For longleaf pine restoration, frequent fire is critical to maintaining and improving understory conditions through time. The study sites were maintained with prescribed burning at frequencies that ranged from 3- to 6-year fire return intervals. Abundant woody vegetation on these sites generally needs more frequent fire to keep in check, so it is likely the effects of the site preparation treatments on the ground layer plant communities would differ with more aggressive burning. Further, it is likely the site preparation treatments affected fire behavior, although we were not able to quantify interactions of site preparation and fire in this study.

Objective

Project RC-2706 addresses Research Needs 1, 2, and 4 from Statement of Need RCSON-17-02, “Long-term Ecological Studies: Testing Previous Hypotheses and Conclusions”. The restoration and management of longleaf pine ecosystems are of high priority for land managers throughout the southeastern United States, including several large Department of Defense (DoD) installations. The overall goal of this project was to determine the effects of site preparation treatments on the development of longleaf pine (*Pinus palustris*) plantations at a temporal scale appropriate for evaluating restoration outcomes and informing management decisions on wet Coastal Plain sites. This project re-measured study plots established for SERDP Project SI-1303, “Regenerating Longleaf Pine on Hydric Soils: Short- and Long-term Effects on Native Ground-layer Vegetation,” which provided information about site preparation treatment effects through three years following artificial regeneration.

The project addressed Research Need 1 (“Facilitate confirmation, modification, or abandonment of initial hypotheses and conclusions regarding treatment effects, mechanistic understanding of system processes, or management implications”) by adjusting early conclusions from SI-1303 through evaluation of site preparation effects at year 15. The need for extending inference from the initial findings was recognized at the completion of SI-1303 and addressed at that time by applying existing growth and yield models to data collected during the first three growing seasons of the original project (Knapp and Walker 2013). However, none of the existing models was developed for application in plantations younger than 20 years old on wet sites, so projections using existing models were not deemed accurate. Empirical data describing effects of site preparation treatments on tree and ground layer vegetation beyond the first few years after establishment on wet Coastal Plain sites did not exist prior to this project. Previous studies suggest that early site preparation effects may not persist through time, demonstrating the need for re-evaluating previous findings.

The project addressed Research Need 2 (“Extend the degree of inference of results from previous studies in statistically relevant ways”) by incorporating data from SI-1303 with newly measured data to evaluate treatment effects through time using a variety of statistical approaches, including generalized linear mixed models, repeated measures analysis of variance, and multivariate ordinations. Analyses linked data from SI-1303 to data from RC-2706 by sampling in consistent ways through time or standardizing data for analyses. In addition, data will be collected on variables that were not possible during the measurement of SI-1303, such as diameter at breast height (DBH), because seedlings were not yet tall enough for DBH measurement in the first few years following planting.

The project addressed Research Need 4 (“Translate findings from additional data collection and analysis into actionable information for DoD resource managers”) by evaluating success/failure of plantation establishment on wet Coastal Plain sites using alternative site preparation treatments in the context of longleaf pine ecosystem restoration objectives. Results inform possible management scenarios following initial site preparation decisions, including no need for additional management, post-planting management required, or possible plantation establishment failure. This research also contributes to the general understanding of longleaf pine tree growth and plantation development by contributing new information regarding height-age patterns of

individual trees grown under different conditions and patterns in the development of ground layer and mid-story vegetation through time.

The proposed work satisfied the six specified requirements of the SON: 1) previous and proposed work was conducted at Marine Corps Base Camp Lejeune (MCBCL), NC; 2) previous data were collected by members of the project team and were accessible for the project; 3) the proposed work did not require new study treatments; 4) the study sites had been maintained with minimal alteration; 5) prior data collection was completed in 2006; and 6) managers at MCBCL need scientifically-informed guidance on site preparation effects on plantation establishment and ground layer response, with particular interest in bedding treatments, which are commonly used in plantation establishment.

Technical Objective 1 (O1)

Determine the effects of site preparation treatments on the establishment of longleaf pine stands (e.g., tree survival, emergence from the grass stage, and tree size) 15 years after plantation establishment. Within this objective, we are interested in addressing the following two research questions:

Do early effects of site preparation treatments persist through year 15?

Are patterns in tree size and density at year 15 driven by rates of grass stage emergence or sustained differences in growth rates of trees?

Hypothesis 1 (O1H1): Site preparation treatments improve total survival of planted trees at age 15.

Hypothesis 2 (O1H2): Site preparation results in earlier grass stage emergence, leading to a significant increase in the number of saplings at age 15.

Hypothesis 3 (O1H3): Site preparation treatments affect mean tree size at year 15 but do not affect annual height growth rates after emergence from the grass stage.

Hypothesis 4 (O1H4): Site preparation treatments that impact planting site (e.g., bedding and mounding) have more persistent effects on tree responses than those that impact competing vegetation (e.g., chopping and herbicide).

Technical Objective 2 (O2)

Determine the effects of site preparation treatments on ground layer and mid-story vegetation abundance, species richness, and composition 15 years after plantation establishment. This objective is motivated by the ecological value of the ground flora within longleaf pine ecosystems, with recognition that tree growth during plantation development generally reduces the abundance of ground flora, and site preparation may directly and indirectly affect the rate and magnitude of those impacts. Study questions include:

Do early effects of site preparation treatments persist through year 15?

Do site preparation treatments result in greater stand density or earlier canopy closure and thus reduce ground flora abundance or richness?

How does bedding affect within-stand variation in ground layer vegetation?

Hypothesis 1 (O2H1): Early effects of herbicides on woody species richness and abundance diminish through time and are no longer significant 15 years following establishment, resulting in long-term reductions in herbaceous vegetation abundance.

Hypothesis 2 (O2H2): Greater tree size and density from improved plantation establishment correlate to locally reduced ground layer vegetation abundance but do not affect species richness at the stand-level.

Hypothesis 3 (O2H3): Bedding treatments increase within-stand variation of ground layer abundance due to differences between treated (on-bed) and untreated (between-bed) locations.

Background

The distribution, extent, and character of longleaf pine (*Pinus palustris*) ecosystems have changed drastically over the past few centuries. At the time of European settlement, longleaf pine is estimated to have dominated 23 million hectares and occurred in mixed-species composition throughout an additional 14 million hectares of the southeastern United States, representing 85% of upland areas and 59% of the entire region (Frost 2006). Land conversion for agriculture and settlement, utilization by timber and naval stores industries, and management decisions such as fire exclusion each contributed to the loss or degradation of longleaf pine ecosystems.

Longleaf pine ecosystems provide critical habitat for numerous plant and animal species. The historical decline of longleaf pine habitats has reduced abundance of associated species, with several species listed as endangered or threatened. The red-cockaded woodpecker (RCW; *Picoides borealis*) is a federally-endangered species that uses frequently burned longleaf pine ecosystems as its preferred habitat (USFWS 2003). The restoration and maintenance of high-quality longleaf pine habitats, in part for the protection of RCWs, are of high priority on several DoD installations (Fort Benning 2006; Fort Bragg 2011; Marine Corps Base Camp Lejeune 2015). In addition, the southeastern Coastal Plain has high levels of floristic endemism (Sorrie and Weakley 1999), and the numbers of rare, threatened, or endangered plant species have increased in recent decades (Walker 1993; Glitzenstein et al. 2001). In particular, the productive flatwoods/savanna sites of the Atlantic Coastal Plain harbor among the highest levels of plant species richness observed within North America (Walker and Peet 1984; Peet 2006), contributing to the high conservation value of longleaf pine ecosystems (Van Lear et al. 2005).

Several key structural and compositional attributes are important to maintaining function of longleaf pine ecosystems. Longleaf pines, in contrast to sympatric tree species, are capable of regenerating and recruiting under a frequent fire regime (Chapman 1932; O'Brien et al. 2008). Longleaf pines contribute long, resinous needles as an important fine fuel for maintaining frequent fire (Mitchell et al. 2009). Pine needles become intermixed with herbaceous, ground layer vegetation to create well-aerated fuelbeds that ignite easily and burn quickly. Frequent fire eliminates the development of other woody vegetation (Brockway and Lewis 1997; Addington et al. 2015), maintaining the species-rich herbaceous ground flora and the open vertical structure preferred by RCWs. The herbaceous ground layer vegetation is an important component of quality RCW habitat, supporting arthropod species important to RCW diets (Hanula and Engstrom 2000; James et al. 2001).

In the wet, productive sites within the flatwoods/savannas of the Atlantic Coastal Plain, land managers are challenged to reach restoration objectives of establishing longleaf pine trees and maintaining or improving ground layer vegetation. Site preparation treatments are commonly used on such sites to improve pine tree establishment by changing the hydrology of the site and by controlling competing vegetation. Although a few studies have reported effects of alternative site preparation treatments on longleaf pine seedling establishment and ground layer vegetation responses within flatwoods sites from the Atlantic Coastal Plain, none has provided information on the development of these critical ecosystem components through time. It is not known if early benefits to planted trees persist through stand development or if early responses of ground layer

vegetation change through time. This information is critical for developing successful restoration prescriptions, while filling gaps in our basic understand of longleaf pine plantation development.

Longleaf Pine Restoration

Restoration and conservation of longleaf pine ecosystems are common objectives throughout the historical range of the species (Brockway et al. 2005; Lavoie et al. 2011). In spite of this, the extent of longleaf pine ecosystems has continued to decrease over the past few decades (Outcalt and Sheffield 1996; Oswalt et al. 2012), primarily due to losses on privately owned lands. In contrast, public and other federal lands represent a disproportionate amount of remaining, intact longleaf pine ecosystems (Oswalt et al. 2012). Efforts to expand the extent of longleaf pine ecosystems, through federal cost-share programs and as explicit management objectives on state and federal ownerships, have resulted in increased acreage of small-diameter longleaf pine stands, primarily through plantation establishment throughout the Southeast (Brockway et al. 2005; Oswalt et al. 2012).

Given the broad range of ecological conditions encompassed by the longleaf region, including xeric conditions in the Fall-line Sandhills and hydric conditions within poorly drained flatwoods along the Coastal Plain (Peet 2006), restoration prescriptions are not necessarily transferable across the range of site conditions and starting conditions targeted for restoration (Johnson and Gjerstad 2006). However, there are often commonalities in desired restoration objectives, including establishing longleaf pine regeneration, reducing the abundance of other woody species within the mid-story, increasing the abundance and diversity of herbaceous ground layer vegetation, and re-introducing a frequent surface fire regime (Brockway et al. 1998; Freeman and Jose 2009; Martin and Kirkman 2009; Aschenbach et al. 2010).

Reaching restoration objectives may require diverse treatment approaches, depending upon starting conditions and site-specific challenges. In practice, treatments are commonly implemented to target specific needs, such as establishing longleaf pine trees on sites on which they are absent (Ramsey et al. 2003) or reducing the abundance of hardwood trees that have developed in the mid-story (Brockway et al. 2009). Balancing multiple restoration objectives requires strategies for reaching specific restoration targets (e.g., establishing longleaf pine regeneration) without creating additional barriers to reaching other restoration targets (e.g., increasing the abundance and diversity of herbaceous ground layer vegetation) (Kirkman et al. 2007). At the same time, determining the salient limitations to restoration success is critical. Intensive treatments that overcome restoration thresholds may be required to initiate a trajectory towards restoration success (Martin and Kirkman 2009).

Restoring longleaf pine on wet, spodic flatwoods/savannas of the Atlantic Coastal Plain presents unique challenges that have not been widely studied. Characterized by poor drainage and a high water table, these sites have productive plant communities comprised of abundant shrub species and diverse herbaceous plants (Glitzenstein et al. 2003; Peet 2006). Many such sites do not have longleaf pine trees present to provide natural regeneration, and planted seedlings are difficult to establish due to excessive soil moisture and the rapid growth rates of competing vegetation (Knapp et al. 2008). Site preparation treatments designed to improve the survival or growth rates of planted pine seedlings on these sites are often intensive, with concern regarding negative effects on ground

layer vegetation. However, some degree of site preparation is likely required to overcome site limitations for longleaf pine seedling establishment.

Site Preparation

Site preparation treatments are commonly used in conjunction with artificial regeneration of tree seedlings. In general, site preparation treatments are used for two purposes: 1) to ease operational constraints of forest management such as site access or mobility (i.e., dispose of slash following harvest); and 2) to improve growing conditions for planted seedlings. The mechanisms for improving growing conditions can be further categorized into treatments that affect soil conditions and treatments that control competing vegetation (Morris and Lowery 1988). In many cases, treatments are designed for a primary objective but may also affect other limitations to seedling growth, particularly with soil manipulation treatments that also result in competition control.

The primary methods of site preparation include prescribed burning, chemical treatments, and mechanical treatments (Morris and Lowery 1988; Fox et al. 2007). While prescribed burning is commonly used to reduce slash and consume forest floor material prior to planting of southern pine species (e.g., Carter and Foster 2004; Zhao et al. 2009), the importance of fire in maintaining longleaf pine ecosystem function results in prescribed fire often being considered a necessary treatment at planting (e.g., Freeman and Jose 2009; Hu et al. 2012; Knapp et al. 2013). Fire is critical for perpetuating the ground layer vegetation of longleaf pine ecosystems and is generally understood to promote herbaceous vegetation (Brockway and Lewis 1997; Haywood et al. 2001). In wet sites, however, shrub species are commonly well-adapted to fire and co-exist with herbaceous vegetation even with frequent return intervals (Drewa et al. 2002; Glitzenstein et al. 2003). Thus, shrub development during periods of fire exclusion may be difficult to control with the reintroduction of only prescribed burning.

Herbicides are widely used for competition control throughout the southeastern United States and are generally more effective than mechanical treatments or prescribed burning treatments that may result in resprouting of woody vegetation (Fox et al. 2007). Successful control of undesirable vegetation with herbicides is largely dependent on prescribing the appropriate chemical with the proper application method, rate, and time of year (Litt et al. 2001). In plantation forestry, herbicide treatments are commonly applied prior to planting (as site preparation) but also throughout the rotation to achieve repeated, complete competition control (Fox et al. 2007; Zhao et al. 2009). Herbicides vary in their mode of action and selectivity, creating some flexibility for forest managers interested in targeting some species and not others.

The role of herbicides in longleaf pine restoration has been studied extensively, with applications for improving seedling establishment (Ramsey et al. 2003; Haywood 2005; Knapp et al. 2008), shifting dominance from woody to herbaceous vegetation (Brockway et al. 1998; Freeman and Jose 2009; Jose et al. 2010), and increasing the effectiveness of fire management (Brockway and Outcalt 2000; Martin and Kirkman 2009). Several types of herbicide are effective at controlling hardwoods with few effects on pine. As a site preparation treatment, herbicides are often broadcast throughout the entire planting area to provide complete control, which can increase the risk of negative impacts to desirable vegetation (Litt et al. 2001; Kaeser and Kirkman 2010). For example, Brockway et al. (1998) reported that broadcast application of hexazinone for woody control

resulted in decreased cover, diversity, and richness of forb species while a localized spot application resulted in an increase in herbaceous cover in a longleaf pine sandhills in Florida.

Mechanical site preparation treatments may be designed for competition control, to change characteristics of the soil or hydrology, or combinations of both. Chopping is intended to control competing vegetation by running a roller-drum along the ground surface to crush and chop standing vegetation (Miyata et al. 1982; Fox et al. 2007). Shearing uses a blade at the ground surface to cut through standing vegetation and is employed to clear a site prior to planting. In both cases, hardwood vegetation is often able to resprout following these practices. Mechanical treatments such as bedding and mounding alter physical characteristics of the planting site to ameliorate additional growth constraints. Bedding is designed to create rows of continuous, raised beds and is commonly used for poorly drained sites. Planting seedlings along the beds increases aeration at the root system and has consistently resulted in improved growth of southern pine species (Pritchett 1979; McKee and Wilhite 1986). Mounding is commonly used in northern latitudes to similarly modify water availability at the planting site, as well as incorporating organic matter into the rooting zone (Sutton 1993).

Incorporating mechanical site preparation treatments into longleaf pine restoration prescriptions has been less common, largely due to concern with impacts on ground layer vegetation. Several studies indicate that mechanical treatments result in an initial reduction in the abundance of woody vegetation and an increase in herbaceous vegetation (Schultz and Wilhite 1974; Swindel et al. 1983; Lauer and Zutter 2001), although the composition of the vegetation may shift from desirable species to ruderals (Conde et al. 1983a). Further, disturbance from mechanical treatments may disrupt the continuity of ground layer species. Wiregrass (*Aristida* spp.) species are important bunchgrasses associated with much of the longleaf pine range but are reported to spread slowly to recolonize an area once disturbed (Clewell 1989; Outcalt et al. 1999). Thus, it is not known if intensive site preparation treatments would result in long-term reductions in wiregrass abundance.

The majority of previous studies about site preparation report responses from relatively short periods of time following treatment application. However, the effects of these treatments on long-term stand conditions are arguably the true interest. Morris and Lowery (1988) discuss three possible patterns for site preparation effects through stand development: A) site preparation improves the site and results in increasing differences between treated and untreated sites through time; B) early beneficial effects of site preparation are maintained but differences between treated and untreated sites do not increase after the first few years; and C) early improvements/benefits are transient and untreated sites ‘catch up’ with treated sites through time. These trajectories could apply to different response variables, including the number of trees per hectare (survival), tree size (growth), and ground layer responses. Thus, questions remain regarding long-term responses to site preparation treatments, even with positive short-term effects.

Studies that follow southern pine plantation development over longer time periods have reported variable results. Zutter and Miller (1998) reported that loblolly pine growth was increased through 11 years after treatment by combining bedding with herbicide control of herbaceous and woody vegetation. However, Outcalt (1984) found that bedding increased slash pine growth on a flatwoods spodosol soil through 10 years of growth, but there were no differences among treatments after 15 years. In contrast, Nilsson and Allen (2003) found that treatments combining

bedding with shearing and chopping increased growth of loblolly pine through 18 years, primarily due to persistent reductions in competing hardwoods. In a longer study, Zhao et al. (2009) found that early effects of bedding and prescribed burning on slash pine growth diminished through 26 years after planting, with long-term increases in growth associated with sustained vegetation control rather than initial site preparation treatments. As commonly observed with site preparation studies, outcomes are related to how strongly limiting the unfavorable site conditions are and the ability of the site preparation treatments to ameliorate those limitations.

Longleaf Pine Plantation Development

Extensive research has contributed to a general understanding of plantation development, with particular interest in southern pine species grown for timber production. Following initial stand establishment, patterns of plantation development (growth, mortality, and productivity) are largely driven by site quality and relationships between the number and the size of the trees present (Dean and Baldwin 1993). Several growth and yield models have been developed for even-aged longleaf pine stands (Lohrey and Bailey 1977; Farrar 1981; Gonzalez-Benecke et al. 2014) but using them to determine outcomes of restoration treatments can be challenging for several reasons (Knapp and Walker 2014). First, growth and yield models are generally designed for stands that have already progressed through the early stages of stand development, often initiating projections at ages greater than 10. The only existing models for early longleaf pine plantation development (ages 2 to 24) were developed in southwestern Georgia (Brooks and Jack 2006; 2014) and may not be applicable to other regions because of inherent limitations associated with empirical models.

Second, the unique grass stage morphology of longleaf pine introduces uncertainty to modeling the early establishment of longleaf pine plantations. For other southern pine species, the number of trees at the start of model projections is a function of planting density and early survival, with stochastic processes of density-independent mortality having largely been determined prior to the start of growth and yield models. For longleaf pine, however, the number of trees moving through stand development is dependent on initial seedling survival and subsequent emergence from the grass stage. Under favorable conditions, longleaf pine seedlings may initiate height growth in one or two years but are capable of remaining in the grass stage for several years or decades (Pessin 1944). As a result, the potential delays in grass stage emergence increase variability regarding temporal patterns of individual tree and stand-level development and may increase error in early growth and yield projections that do not account for the timing of grass stage emergence.

Site preparation treatments have been found to increase early growth of planted longleaf pine seedlings, resulting in earlier grass stage emergence (Boyer 1983, Knapp et al. 2006). However, rates of seedling emergence are not well-understood. It is possible that site preparation treatments may be required for grass stage emergence on highly productive sites with intense vegetation competition. Due to the need for longer monitoring time, effects of site preparation treatments on height growth rates after grass stage emergence are largely unknown. If growth rates after grass stage emergence are consistent regardless of site preparation, long-term differences in tree size may be related only to the amount of time out of the grass stage.

Finally, traditional applications of growth and yield models are primarily production-oriented and may not be relevant to restoration objectives. Maximizing total or merchantable volume is likely a low priority for plantations established for conservation or restoration objectives, with desirable

characteristics instead related to individual tree size (e.g., large trees for RCW habitat) and stand structure (e.g., relatively low-density stands with variable tree distribution). However, traditional models of stand development, based on density-dependent stand trajectories, may be applied to restoration objectives by adjusting structural targets (Shaw and Long 2007). For such applications, understanding early stand establishment processes (e.g., rates of grass stage emergence and subsequent growth patterns) is critical to predicting starting conditions for model use.

Site preparation treatments can have direct and indirect effects on ground layer vegetation through time. Direct effects include the initial responses and subsequent patterns of recovery following treatment. Several studies suggest that ground layer vegetation recovers in abundance and richness following site preparation treatments (Conde et al 1983b, Lewis et al. 1988, Hedman et al. 2000), although negative effects on individual species, such as wiregrass, have been reported (Moore et al. 1982). Indirect effects of site preparation on ground layer vegetation relate to processes of stand development; as plantations reach crown closure and competition among trees increases for light and other resources, the abundance and diversity of the ground layer vegetation decreases (Harrington 2011). Relationships among the developmental rates of planted pines, canopy closure, and reductions in herbaceous ground flora have not been well-described.

Overview of SERDP SI-1303

To evaluate the effects of site preparation on longleaf pine plantations on poorly drained flatwoods/savanna sites of the Atlantic Coastal Plain, SERDP project SI-1303 was implemented in 2003 at MCBCL, NC. The study used a randomized complete block design, originally with seven blocks, although one block (Block 4) was dropped from analyses in the original study due to impacts from a wildfire, and treatments were misapplied in another block (Block 6). Each block includes eight study treatments, each made up of combinations of site preparation treatments that control competing vegetation (herbicide and chopping) and site preparation treatments that manipulate the planting site (flat-planting, mounding, and bedding). The resulting treatments include: F (flat planting; control), HF (herbicide and flat-planting), CF (chopping and flat-planting), HM (herbicide and mounding), CM (chopping and mounding), HB (herbicide and bedding), CB (chopping and bedding), and CHB (chopping, herbicide, and bedding). Site preparation treatments were applied in summer 2003, and container-grown longleaf pine seedlings were planted in December 2003.

The original study measured seedling response through two growing seasons and reported findings that were generally consistent with other studies. Although site preparation treatments did not affect seedling survival, with around 60% seedling survival after two years, seedling growth was significantly improved with site preparation (Knapp et al. 2006). Chopping had little apparent effect on seedling growth, whereas bedding and mounding similarly increased seedling growth compared to flat-planting. Soil moisture was negatively related to seedling growth, with results suggesting that excessive soil moisture and competition each limit seedling growth (Knapp et al. 2008). Site preparations affected the number of seedlings that had emerged from the grass stage, but after two growing seasons only 25% of seedlings had emerged on the treatment with the best growth (bedding, chopping, and herbicide). The duration of SI-1303 was insufficient for quantifying grass stage emergence, as the process had just begun when the study ended.

Ground layer vegetation was affected by the mechanical and chemical treatments in the first year after application, with reductions in cover and richness following site preparation. In general, woody vegetation was affected more strongly than herbaceous vegetation, with lasting effects of herbicides on woody vegetation through three years but effects of mechanical treatments diminishing after the first year. However, herbicides resulted in apparent reductions in wiregrass (*Aristida stricta*) and increases in chalky bluestem (*Andropogon capillipes*). Similar results have been reported for effects of mechanical site preparation on wiregrass and chalky bluestem (Conde et al. 1983a). It is not known if these changes persisted through stand development, but reductions in wiregrass could potentially affect future fire management. Effects of site preparation on species richness were similar to those on woody cover. Given the potential abundance of shrubs on these sites, it is not known if site preparation treatments would affect the developmental patterns of ground layer vegetation through time, particularly as planted trees increase in size and reach canopy closure.

Materials and Methods

Study Area

This study was conducted on MCBCL (34°36'N 77°24'W) in Onslow County, North Carolina. MCBCL is located within the Atlantic Coastal Flatwoods section of the Outer Coastal Plain Mixed Forest ecological province (McNab et al., 2007). The climate is classified as warm humid temperate with an average annual temperature of 17.4 °C and an average precipitation of 145 cm (National Climate Data Center, Hofmann Forest Station, 34°5' N, 77°2' W). The study sites were located primarily (90%) on Leon sand (sandy, siliceous, thermic Aeric Alaquod), a poorly drained Spodosol with a fluctuating water table that may be at or near the surface (Barnhill, 1992; NRCS, 2014), with smaller areas of drier Kureb soils (8%) and trace amounts of other savanna soils. Spodosols are the primary soil order of flatwoods and are characterized by their sandy, acidic, and infertile nature (Peet, 2006). Leon sand is one of the most extensive soil series on Camp Lejeune (Barnhill, 1992; Frost, 2001) and is of large extent throughout the Southeast (NRCS, 2014). These soils are characterized by an A horizon of salt and pepper appearance and albic E horizons of light-gray to white sand, underlain by dark (Bh) spodic horizons. The spodic horizons, cemented by organic and iron compounds, are present in varying thickness of 15 to 25 cm and tend to be strongly cemented when dry (Barnhill, 1992).

The study sites spanned an elevation gradient from 13.5 m to 19.7 m. Corresponding to these elevation differences, fairly distinct vegetation gradients were evident, ranging from pocosin-like habitats dominated by Cyperaceae and pocosin indicator shrubs (Mitchell et al. 2015) to mesic flatwoods containing *Pteridium latiusculum*, *Gaylussacia dumosa*, *Lyonia mariana*, *Eupatorium rotundifolium*, *Sericocarpus tortifolius*, and other mesic species. Between these extremes were wet savannas containing *Xyris* spp., *Carphephorus tomentosus*, *Eupatorium pilosum*, *Sporobolus pinetorum*, and many other herbaceous species. These natural communities include longleaf pine as the overstory species, with density varying along the hydrologic gradient, and common grasses including wiregrass (*Aristida* spp.), bluestems (*Andropogon* spp., *Schizachyrium* spp.), and panic grasses (*Panicum* spp., *Dichanthelium* spp.). Vegetation gradients encompassing pocosin and longleaf pine communities, including planted sites, on Camp Lejeune are discussed further in Mitchell et al. (2015).

The sites selected for this study were previously used for slash and loblolly pine (*Pinus taeda*) plantations, which were harvested 6 months to 2 years prior to treatment installation. Condition of the understory community prior to overstory removal is unknown because pre-treatment data were not collected. However, the condition of the understory community is not assumed to be that of a reference community for these site types due to the history of pine plantation on the sites.

Experimental Design

The study uses a randomized complete block design, originally implemented with 7 blocks (referred to as Blocks 1 – 7). Following initial treatment application, one block was impacted by a wildfire (Block 4) and subsequently dropped from the study. A second block had treatments misapplied (Block 6). Therefore, we focused the study on Blocks 1, 2, 3, 5, and 7, dropping Blocks 4 and 6 from re-measurement. Each block includes eight experimental units measuring approximately 0.6 ha each, with 15 m buffers between experimental units (i.e., plots). Plots are

rectangular (100 x 60 m) and oriented according to spatial constraints associated with each block (Figures 1-5).

The eight study treatments (Table 1) are made up of combinations of site preparation treatments that control competing vegetation (herbicide and chopping) and site preparation treatments that manipulate the planting site (flat-planting, mounding, and bedding). The resulting treatments include: F (flat

planting; control), HF (herbicide and flat-planting), CF (chopping and flat-planting), HM (herbicide and mounding), CM (chopping and mounding), HB (herbicide and bedding), CB (chopping and bedding), and CHB (chopping, herbicide, and bedding). The design creates a 2 x 3 factorial structure for evaluating vegetation control treatments and planting site manipulation treatments, plus an untreated control and the more intensive CHB treatment. For each block, the overstory of the previous stands had been harvested within two years prior to treatment application, and remaining vegetation was removed by shearing with a KG blade. Site preparation treatments were applied in summer 2003, with vegetation control treatments applied first and followed by the mechanical treatments.

Table 1. Summary of treatments used in the study.

Treatment		Vegetation Control		Soil Manipulation		
		Chop	Herbicide	Flat	Mound	Bed
Flat [control]	(F)			X		
Chop/flat	(CF)	X		X		
Herbicide/flat	(HF)		X	X		
Chop/mound	(CM)	X			X	
Herbicide/mound	(HM)		X		X	
Chop/bed	(CB)	X				X
Herbicide/bed	(HB)		X			X
Chop/herbicide/bed	(CHB)	X	X			X

Chopping was applied with a 2.4 m Lucas Drum Chopper pulled by a TD15 Dresser crawler tractor. The herbicide treatment was 0.70 kg/ha of imazapyr and 0.56 kg/ha of triclopyr mixed and broadcast at a rate of 280 liters/ha. These chemicals control woody vegetation, with triclopyr effective on waxy shrubs common to these sites. The mounding treatment was applied with a custom mounding bucket on a Caterpillar 320BL excavator, creating mounds approximately 1.2 m wide and oriented in rows. Rows of continuous beds were created 2.1 to 2.4 m wide with a Rome 6 disc Bedding Harrow with three discs per side. Following treatment application, all sites were prescribed burned in October-November 2013. Treatment plots were hand-planted with container-growth longleaf pine seedlings produced from locally collected seed. Plots were planted at an average density of 930 trees per hectare (standard deviation of 203 trees per hectare), and average root collar diameter (RCD) at planting was 6.6 mm with a standard deviation of 1.2 mm. Various images from treatment application and the first year following planting are provided in Figure 6.

All study blocks were burned using strip-head fires ignited by drip torches on either March 2, 7, or 14 2006. Two of blocks (Block 3 and Block 5) also burned in 2009, with the entirety of these two blocks, so there are no confounding effects of fire on treatments within each block. One block (Block 5) was burned by a wildfire in 2011, and all other study blocks were prescribed burned in March 2012. All blocks were burned most recently in 2016-2017.



Figure 1. Layout of Block 1, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).



Figure 2. Layout of Block 2, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).



Figure 3. Layout of Block 3, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).



Figure 4. Layout of Block 5, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).



Figure 5. Layout of Block 7, with eight experimental units representing eight different site preparation treatments. Treatments are combinations of two vegetation control methods ('H' = herbicide; 'C' = chopping) and three soil manipulation methods ('B' = bedding; 'F' = flat-planting; 'M' = mounding).

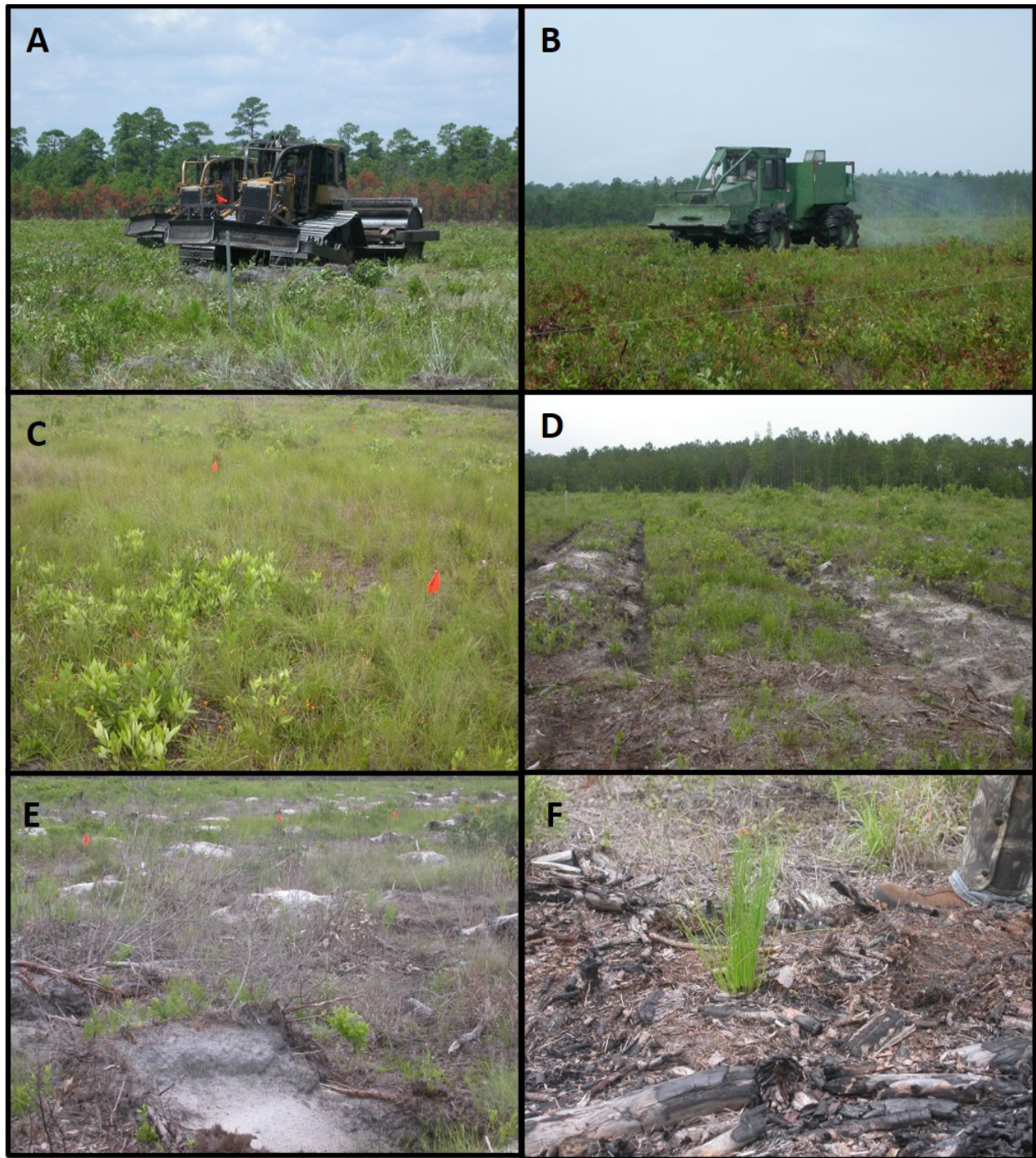


Figure 6. Images from treatment application or shortly after, depicting A) application of chopping treatment; B) broadcast application of herbicide; C) regrowth near end of one growing season on CF treatment; D) regrowth near end of one growing season on CB treatment, with beds apparent; E) mounds scattered throughout treatment area; F) planted longleaf pine seedling during first growing season after treatment.

Data Collection and Analysis

Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation

After planting in 2003, a full census was conducted to record the number of seedlings planted in each experimental unit. A sub-sample of 45 seedlings from each experimental unit was then randomly selected for repeated growth and survival measurement. Survival was measured through two years after planting, with two measurements periods in August 2004 and 2005. Root collar diameter (RCD) was measured through three years after planting, with four measurement periods occurring in June and December 2004, December 2005, and December 2006 (see Knapp et al., 2006, 2008 for more details). Although the study plots were maintained without disruption through time, the individual seedlings measured in SI-1303 (2004-2006) were not permanently monumented, so these trees could not be tracked through time.

To evaluate stand-level development of longleaf pine plantations in response to site preparation treatments, we resampled the plantations in summer 2018. A sample population of the planted longleaf pine trees was selected from each experimental unit to quantify stand density and tree size. Five circular subplots were systematically located within each experimental unit, with one subplot located at the experimental unit center and each of the other four subplots spaced between each respective corner and the center. Each subplot had a radius of 10 m, resulting in approximately a third of each experimental unit being sampled. Within each subplot, every tree was identified to species and measured for height and diameter at breast height (DBH). There were some volunteer seedlings of longleaf, slash, and pond pine (*Pinus serotina* Michx.) present on the edges of a few of the experimental units. However, this was always a minor component of the developing stands, apparently present mostly in plot buffers, and our analyses considered only the planted longleaf pine trees from the sampled areas.

To quantify patterns in individual-tree growth responses to site preparation treatments, we conducted stem analysis on a random sample of longleaf pine trees from each experimental unit. In summer 2017, we destructively sampled longleaf pine trees from each experimental unit to reconstruct the annual height growth of individual trees. We established a transect running longways across the middle of each rectangular experimental unit and located six equally spaced points along the transect. We then selected the longleaf pine nearest each point for destructive sampling for stem analysis. Each selected tree was felled in the field and cut into sections at 10 cm intervals from the ground line to 1.5 m and every 25 cm thereafter. These intervals gave us a fine resolution toward the base of the tree, where transitions between growth years were closer. Each sample was prepared by sanding with progressively finer grit, and tree age was determined for each section (at known heights) using a stereo microscope to count annual rings.



Figure 7. Example of longleaf pine tree cut into sections at specific intervals for reconstructing height growth patterns through time.

We fit linear mixed models to each stand-level response variable of interest (i.e., total height, DBH, trees per hectare, grass stage seedlings per hectare, and basal area), with treatment as a fixed effect and the hierarchical, nested sampling design (i.e., subplots within experimental units within blocks) as a random effect. We used a height of 15 cm (from ground line to terminal bud) as the threshold to classify grass stage emergence (Boyer, 1988; Wang et al., 2016). We conducted one-way analysis of variance (ANOVA) on each of the fitted mixed models to test for differences among the eight study treatments for each response variable of interest. When one-way ANOVA indicated that treatments significantly differed ($\alpha = 0.05$), post-hoc Tukey-adjusted pairwise comparisons were used to determine which treatments significantly differed. Additionally, we used a 2 x 3 factorial ANOVA, excluding the F and CHB treatments, to determine the effect of individual factors (e.g., chopping vs. herbicide) on each response variable of interest and to test for interactions between the vegetation control treatments and soil manipulation treatments. With significant treatment or interaction effects, we used post-hoc Tukey-adjusted pairwise comparisons to determine which individual factors significantly differed, as appropriate.

To understand how longleaf pine responses to site preparation changed through time, we integrated the stand-level measurements taken 15 years after planting with data collected in the original study, which measured seedlings through three years after planting (from 2003 through 2006). We computed two response variables of interest: survival and relative diameter. Survival in the initial two-year measurement period was calculated by applying the survival rates from sub-sampled seedlings to the number of planted seedlings recorded in the initial census. Survival at 15 years was calculated by scaling longleaf pine density within the subplots up to the experimental unit level and then dividing those estimates by the number of seedlings planted in each experimental unit. To integrate root collar diameter measurements (RCD) from 2003-2006 with DBH measurements recorded in 2018, we calculated the respective diameter (RCD or DBH) for each treatment relative to the untreated control (F). Relative diameter was calculated for each measurement period using the following equation:

$$\text{relative diameter} = \frac{\text{treatment mean} - \text{control mean}}{\text{control mean}}$$

Therefore, a treatment mean diameter that is twice as large as the control mean diameter results in a relative diameter value of one. We fit linear mixed models to both survival and relative diameter and then used repeated measures ANOVA to test for differences among treatments and through time and to test for interactions between treatment and time. Significant main effect and interaction terms ($\alpha = 0.05$) from repeated measures ANOVA were followed by post-hoc Tukey-adjusted pairwise comparisons to determine which treatments and time periods significantly differed.

For the stem analysis data, we determined the age at each height level by counting annual rings and adjusting internode tree heights using the Carmean method (Carmean, 1972; Dyer and Bailey, 1987). Tree height growth through time was reconstructed for the six sampled trees per experimental unit. Patterns of mean height and mean annual height growth through time were determined for each treatment, with non-overlapping 95% confidence intervals indicating statistically significant differences in growth patterns. We computed two additional response variables of interest: age of emergence from the grass stage and mean rate of height growth after emergence. Age of emergence from the grass stage was determined by the number of years a tree

took to reach 15 cm in height (Boyer, 1988; Wang et al., 2016). The rate of growth after emergence was determined by computing the mean annual height growth after the tree exceeded 15 cm in height. We fit linear mixed models for both age of emergence and post-emergence growth rate. We then conducted one-way ANOVA, followed by Tukey-adjusted pairwise comparisons, on both mixed models to test for differences among the eight study treatments. We also conducted a 2 x 3 factorial ANOVA, excluding the F and CHB treatments and followed by Tukey-adjusted pairwise comparisons, to determine the effects of individual treatment levels on the response variables.

Data were analyzed in R (R Core Team, 2018). The *lme* function in the *nlme* package (Pinheiro et al., 2018) and the *glmer* function in the *lme4* package (Bates et al., 2015) were used for fitting linear mixed models, with the former being used in most cases and the latter being used to specify a Poisson distribution where necessary. The *anova* function in the *stats* package (R Core Team, 2018) was used for running ANOVA tests. The *lsmeans* function from the *emmeans* package (Lenth, 2019) and the *CLD* function from the *multcompView* package (Graves et al., 2015) were used for post-hoc, Tukey-adjusted pairwise comparisons. When factorial or repeated measures ANOVA resulted in a significant ($\alpha = 0.05$) interaction term, we used the *testInteraction* function in the *phia* package (De Rosario-Martinez, 2015) to conduct post-hoc interaction significance tests. The *dplyr* package (Wickham et al., 2019) was used for data manipulation, the *ggsurvplot* function in the *survminer* package (Kassambara and Kosinski, 2018) was used for plotting survival, and the *ggplot2* package (Wickham, 2016) was used to create all other figures.

Objective 2 – Ground-Layer Vegetation Response to Site Preparation

The ground layer vegetation community was sampled in the first three years following site preparation treatment application (2004, 2005, 2006) using a stratified random sampling approach. Within each experimental unit, four equally spaced transects were located at consistent intervals, extending across the entire plot. Along each transect, three 1 x 1 m quadrats were randomly located (determined by a randomly generated number representing the distance along the transect), resulting in a total of 12 sampling quadrats per experimental unit. The same quadrat locations were remeasured during the 2004, 2005, and 2006 sampling efforts of SI-1303. In 2018, we resampled the ground layer vegetation using the same sampling methodology. However, the quadrat locations could not be re-located from the earlier sampling periods, so we used the same approach to randomly locate three quadrats along each of four transects located at consistent intervals across each experimental unit. In summer 2018, each quadrat in bedded treatments was paired with an adjacent quadrat to determine the local effects of bedding on the ground layer vegetation community. The area between bedding rows had unaltered soil and will henceforth be referred to as the interbed area. Each randomly located quadrat occurred within either a bedded or interbed area; the paired quadrat was located within 5 m on the nearest bed or interbed area. The same sampling protocol was used for quadrats on both the beds and the interbed areas.

Within each quadrat, every vascular plant was identified to species, and percent cover of each species was recorded following the Carolina Vegetation Survey cover classes (Peet et al., 2012). For cases in which determination of species was not possible, due to lack of key identifying traits, species were grouped by genera. The botanical survey completed in 2018 had greater taxonomic resolution than that from 2004-2006, with nearly all individuals identified to species in 2018.

To quantify light transmittance to the forest understory, a hemispherical photograph was taken in summer 2018 at the center of each quadrat. We positioned a camera at 1.5 m above the ground with the lens parallel to the ground, and we used a fisheye lens to capture a 180° image. All photographs were taken on cloudy days to reduce direct sun reflection. We used the HemiView Forest Canopy Image Analysis System (Delta-T Devices, Cambridge, UK) to process the photographs and obtain estimates of gap light index and gap fraction for each quadrat. Gap light index estimates the percentage of incident photosynthetically active radiation (PAR) through a gap to a point in the understory (Canham, 1988), while gap fraction estimates the percentage of sky unobstructed by the canopy from a point in the understory (Welles and Cohen, 1996). Both gap light index and gap fraction have been shown to accurately estimate canopy light transmittance in longleaf pine ecosystems (Battaglia et al., 2003).

We obtained percent cover of functional groups by obtaining the mean value of the recorded cover class range (e.g., 7.5% for a range of 5-10%) and summarizing those values for all species within the functional group. We categorized species into six main functional groups: ferns, forbs, graminoids, shrubs, trees, and vines. We also further divided forbs into annuals and perennials, graminoids into bunchgrasses, sedges and rushes, and other graminoids, and shrubs into tall and short shrubs. The only species that we analyzed individually was wiregrass because of its ecological value and functional role in longleaf pine ecosystems. We fit linear mixed models to each response variable of interest (i.e., percent cover of each functional group), treating treatment and time as fixed effects and the hierarchical, nested sampling design (i.e., experimental units within blocks) as a random effect. We then used repeated measures analysis of variance (ANOVA) to test for differences among treatments and through time and to test for interactions between treatment and time. Additionally, for the measurements taken in 2018, we used a 2 x 3 factorial ANOVA, excluding the F and CHB treatments, to determine the effect of individual factors (e.g., chopping vs. herbicide) on each response variable and to test for interactions between the vegetation control treatments and soil manipulation treatments.

We calculated three measures of diversity using the data collected in 2018: species richness, Shannon entropy transformed into a diversity index (or Hill number), and evenness. Unlike for cover, we did not integrate the most recent diversity measurements with those from the first three years because some species were lumped into groups differently during the two time periods. Species richness was calculated by counting the total number of plant species present in each 1 m² quadrat. We used relative cover of each species in each quadrat as a proxy for abundance when calculating Shannon entropy and evenness. Therefore, Shannon entropy (H) was calculated using the following equation, where p_i is the relative cover of a species.

$$H = - \sum_{i=1}^s p_i \ln p_i$$

To convert Shannon entropy into a diversity index, or Hill number, we took the exponent of Shannon entropy (Jost, 2006). To calculate evenness, we divided Shannon entropy by the natural logarithm of species richness, so that each species is assigned a value between 0 and 1, where 1 indicates a completely even distribution of all species. For each of these three measures of diversity, we fit linear mixed models, treating treatment as a fixed effect and block as a random effect. We then conducted one-way ANOVA, followed by post-hoc pairwise comparisons, on each

of the fitted mixed models to test for differences among the eight study treatments for each measure of diversity. We also conducted a 2 x 3 factorial ANOVA, excluding the F and CHB treatments, to determine the effects of individual treatment levels on richness, diversity, and evenness.

We also fit linear mixed models to gap light index and gap fraction estimates obtained from hemispherical photographs following the same approach outlined for vegetation cover and diversity. We conducted one-way ANOVA, followed by pairwise comparisons, to test for differences among the eight study treatments and 2 x 3 factorial ANOVA, excluding F and CB, to determine effects of individual treatment levels on canopy cover and gap light index. To test for differences in vegetation between bedding rows and interbed areas, we conducted paired *t*-tests for all of the cover, diversity, and light transmittance response variables outlined above.

Data were analyzed in R (R Core Team, 2018). The *lme* function in the *nlme* package (Pinheiro et al., 2018) was used for fitting linear mixed models. The *anova* function and the *t.test* function in the *stats* package (R Core Team, 2018) were used for running ANOVA and paired *t*-tests, respectively. The *lsmeans* function from the *emmeans* package (Lenth, 2019) and the *CLD* function from the *multcompView* package (Graves et al., 2015) were used for post-hoc pairwise comparisons. When factorial or repeated measures ANOVA resulted in a significant ($\alpha < 0.05$) interaction term, we used the *testInteraction* function in the *phia* package (De Rosario-Martinez, 2015) to conduct post-hoc interaction significance tests. The *dplyr* package (Wickham et al., 2019) was used for data manipulation and the *ggplot2* package (Wickham, 2016) was used for figure creation.

We conducted non-metric multidimensional scaling (NMDS) ordination to quantify changes to the understory plant community composition using the *metaMDS* function in the *vegan* package (Oksanen et al. 2020). To reconcile differences in the resolution of species identification across the time periods (i.e., more species were identified to genus in years 1-3 (2004-2006) than in year 15 (2018)), we grouped species at the coarsest common level across time periods. We excluded species that occurred in less than two percent of quadrats to reduce the impact of rare species on the analysis. When running the NMDS ordination, we used the Bray-Curtis dissimilarity index and called for 100 iterations. A three-dimensional ordination provided the optimal fit for our data, and the optimal solution converged after 20 iterations. When plotting the three-dimensional solution, we obtained the centroid of each treatment at each point in time. To determine if the plant community composition varied among treatments and through time, we conducted permutational multivariate ANOVA using distance matrices (PERMANOVA) using the *adonis* function in *vegan* (Oksanen et al. 2020). With significant main effects, we used the *pairwise.perm.manova* function in the *RVAideMemoire* package (Hervé, 2020) to conduct post-hoc Holm-adjusted pairwise comparisons.

Results

Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation

Stand-level Responses

Fifteen years after planting, site preparation resulted in significant differences in mean height ($p < 0.001$) and DBH ($p < 0.001$) among the eight study treatments (Figure 8A and 8B). We found that CHB (chop, herbicide, and bed), HM (herbicide and mound), and HB (herbicide and bed) resulted in the greatest tree size at age 15, with mean heights exceeding 5 m and mean DBH near 8 cm. These three treatments resulted in significantly greater heights than HF (herbicide and flat-planting), CF (chop and flat-planting), and F (flat-planting and no vegetation control) and significantly greater DBH than CF and F. In contrast, CF and F resulted in the smallest trees, with mean heights of less than 2 m and mean DBH less than 5 cm. Based on factorial ANOVA, both soil manipulation and vegetation control treatments affected height ($p < 0.001$) and DBH ($p \leq 0.001$) of planted longleaf pine (Table 2), and there was no significant interaction between the treatment types for height ($p = 0.469$) or DBH ($p = 0.773$). Bedding and mounding resulted in greater height and DBH than flat-planting (no soil manipulation), with differences in height and DBH exceeding 2 m and 2 cm, respectively. Herbicide resulted in greater height and DBH than chopping, with differences in height and DBH exceeding 1 m and 1 cm, respectively.

Site preparation treatments also resulted in significant differences in longleaf pine tree density ($p < 0.001$) and grass stage seedling density ($p = 0.001$) at age 15. HB, CHB, CB (chop and bed), and HF (herbicide and flat--planting) had greater than three times the number of trees/ha compared to F (Figure 8C), while F resulted in more grass stage seedlings/ha than CHB and HM (Figure 8D). F averaged 28 grass stage seedlings/ha, while grass stage seedlings were absent from all but one experimental unit for CHB and HM. Based on factorial ANOVA, soil manipulation ($p = 0.010$) and vegetation control treatments ($p = 0.026$) affected the number of trees/ha, and there was no significant interaction between the treatment types ($p = 0.659$). Bedding resulted in greater tree density than mounding, and herbicide resulted in greater tree density than chopping 15 years after planting (Table 2). For grass stage seedling density, differences among soil manipulation treatments were significantly different ($p = 0.006$), but there was no significant difference between vegetation control treatments ($p = 0.264$) and no significant interaction between treatment types ($p = 0.162$). Flat-planting resulted in greater grass stage seedling density than mounding and bedding (Table 2).

Basal area, which integrates tree size and density at the stand level, was also significantly different among the eight study treatments ($p < 0.001$). Mean basal area for CHB and HB, which approached 3.5 m²/ha, was significantly greater than that of CM, CF, and F, which were near or below 1 m²/ha (Figure 8E). Soil manipulation ($p = 0.009$) and vegetation control treatments ($p = 0.001$) affected basal area, and there was no significant interaction between the treatment types ($p = 0.848$). Bedding resulted in significantly greater basal area than flat-planting, and herbicide resulted in significantly greater basal area than chopping 15 years after planting (Table 2).

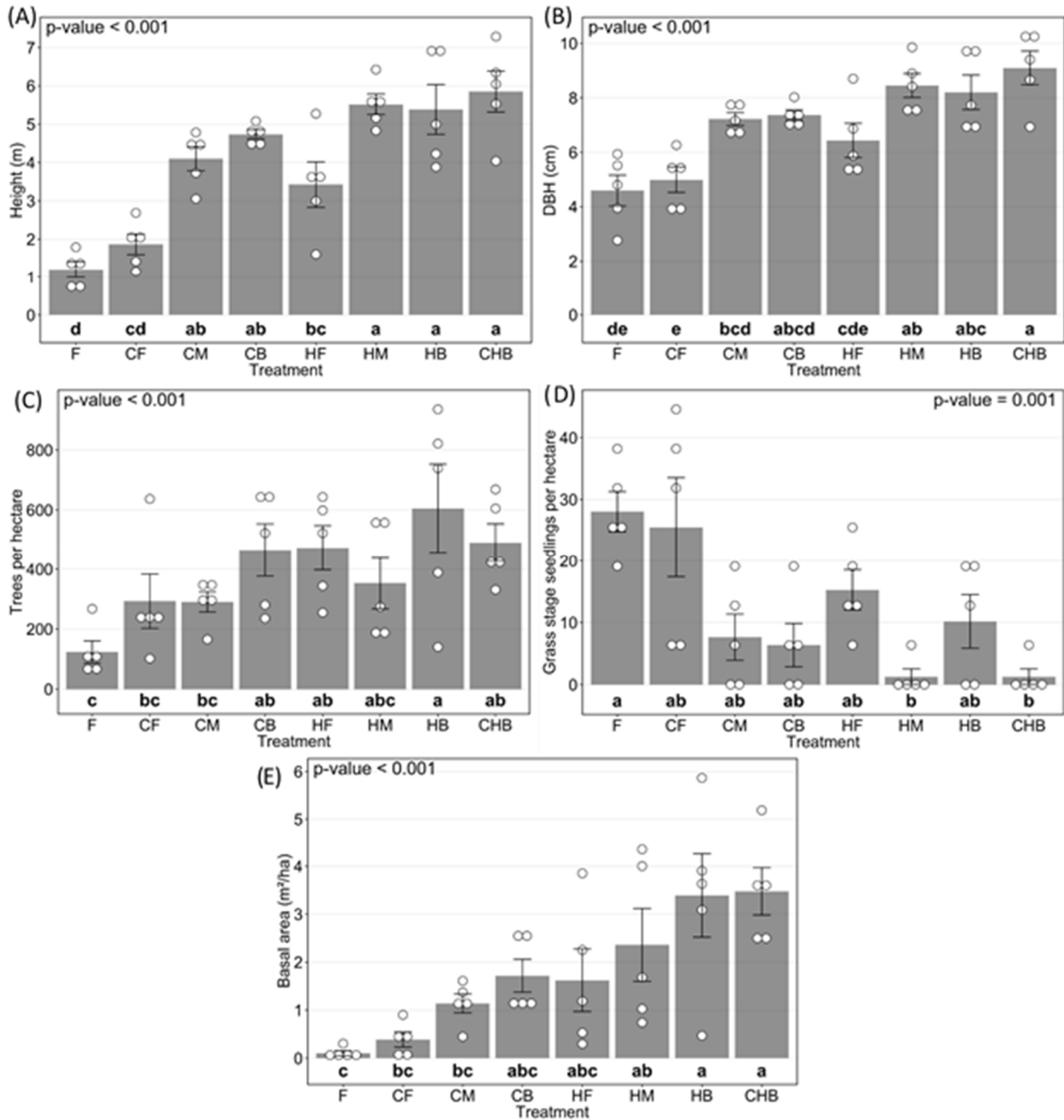


Figure 8. Longleaf pine stand-level responses to site preparation study treatments 15 years after application. Treatments are defined in Table 1. Bars show treatment means and standard errors; points show means for each experimental unit. Same letters indicate no significant difference among treatments within a response variable based upon Tukey-adjusted pairwise comparisons; p-values are from one-way ANOVA tests.

Table 2. Least square means of longleaf pine stand-level responses to individual site preparation treatment levels 15 years after application.

Treatment	Mean height (m)	Mean DBH (cm)	Mean trees per hectare	Mean grass stage seedlings per hectare	Mean basal area (m ² /ha)
Flat	2.59 b	5.77 b	383 ab	20.4 a	1.01 b
Mounding	4.79 a	7.79 a	321 b	8.3 b	1.75 ab
Bedding	5.02 a	7.78 a	535 a	4.5 b	2.55 a
<i>p</i> -value	< 0.001	< 0.001	0.010	0.006	0.009
Chopping	3.50 b	6.46 b	349 b	13.2 a	1.08 b
Herbicide	4.76 a	7.76 a	477 a	8.9 a	2.46 a
<i>p</i> -value	< 0.001	0.001	0.026	0.264	0.001

Same letters indicate no significant difference within a treatment type and response variable based upon Tukey-adjusted pairwise comparisons; *p*-values are from 2 x 3 factorial ANOVA tests.

Effects of Site Preparation through Time

Repeated measures ANOVA indicated that time significantly affected longleaf pine survival ($p < 0.001$) through 15 years after planting, while treatment did not ($p = 0.281$). Survival decreased through time, and mean survival across all treatments was 72.5% at eight months (August 2004) after planting, 59.1% at 20 months (August 2005), and 32.7% at 15 years (August 2018). The interaction between time and treatment approached significance ($p = 0.061$), likely because survival did not significantly differ among treatments after the first and second growing seasons (Knapp et al., 2006) but did differ in year 15 according to one-way ANOVA ($p = 0.002$). At this measurement period, the herbicide treatments – HB, HM, HF, and CHB – resulted in higher survival compared to the untreated control (Figure 9). By the 2018 measurement period, survival was <25% with the chop-only treatment (CF) or with site preparation (F). The other site preparation treatments ranged from around 30% to 45% survival through 15 years after planting (Figure 9).

The interaction between treatment and time was significant ($p < 0.001$) for relative diameter of longleaf pine through 15 years after planting. There were no significant differences among treatments at seven or 12 months after planting ($p \geq 0.855$), but there were differences at two (2005), three (2006), and 15 (2008) years after planting ($p < 0.001$). Relative to the untreated control (F), treatment effects on diameter growth rose sharply by two years after planting (Figure 10). At that point in time, CHB and HB consistently had greater relative diameter than CF and F (Figure 10). By year 15, CHB, HM, HB, CB, and CM had significantly greater relative diameter than CF and F. Notably, there were no significant changes in relative diameter for any individual study treatment after year two ($p = 0.382$). For the first three years of the study, relative diameter was measured as the RCD but by the end of the study period this was measured as DBH. By the end of the study period, CHB, HM, and HB had the greatest relative diameter, ranging from 80% to over 100% larger DBH values than the untreated control (F).

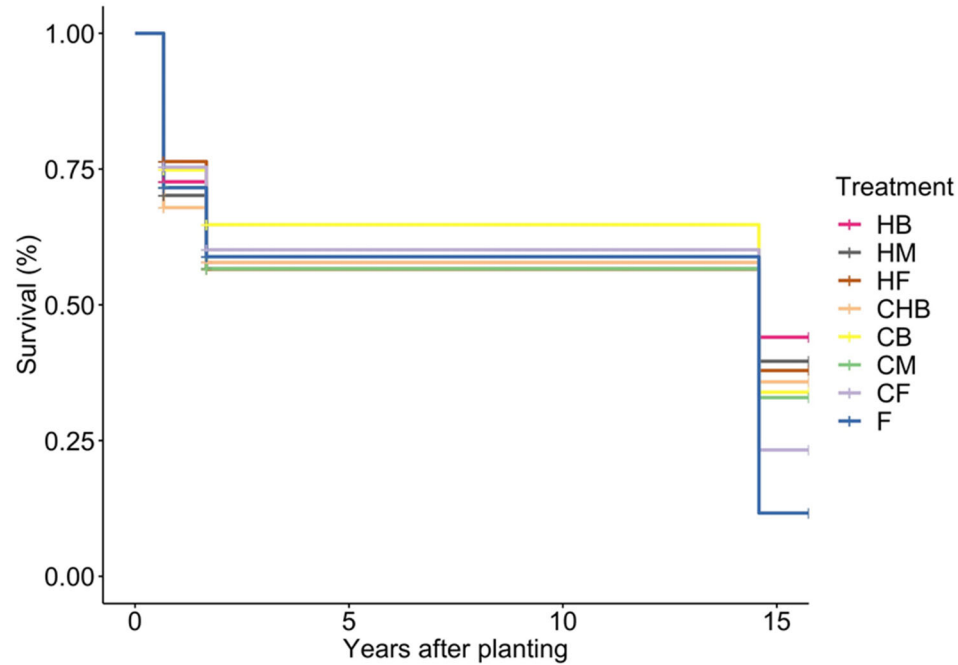


Figure 9. Mean survival for each study treatment (defined in Table 1) at three points in time: 8 months, 20 months, and 15 years after planting.

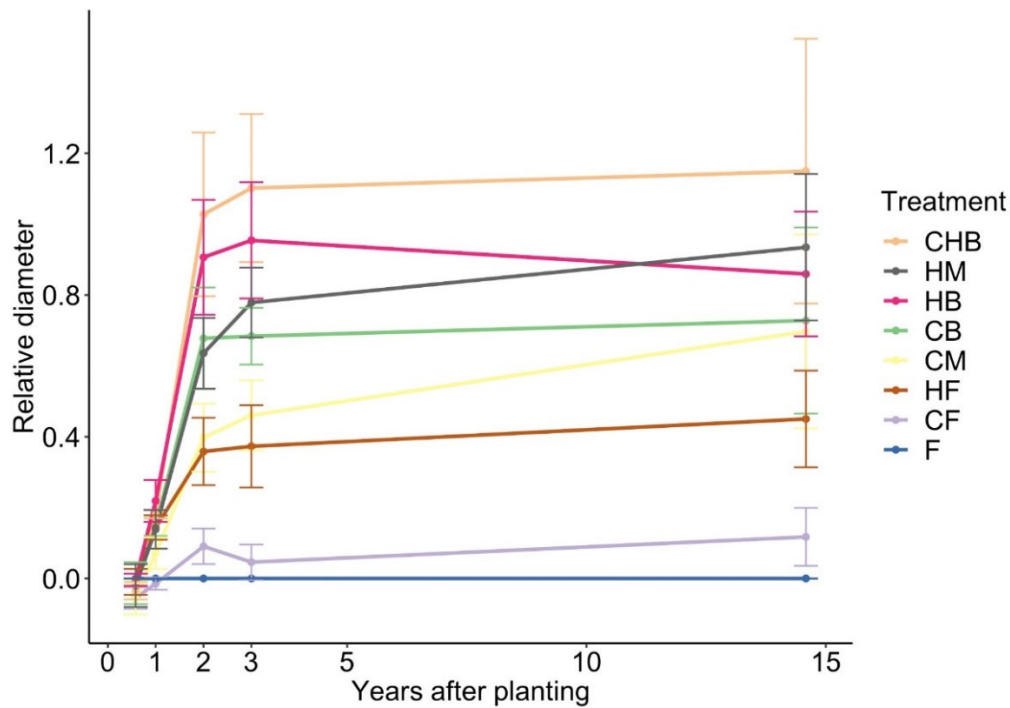


Figure 10. Diameter (mean and standard errors) of each study treatment (defined in Table 1) relative to the control (F) at five points in time, from seedling establishment into stand development.

Individual Tree-level Responses

One-way ANOVA indicated that there were significant differences in age of emergence from the grass stage among the eight study treatments ($p < 0.001$). CF, which had a mean emergence age of 9.25 years after planting, emerged significantly later than the other seven treatments (Figure 11A). The other treatments had emergence ages ranging from 3.72 to 5.29 years and did not significantly differ from each other. Based on factorial ANOVA, soil manipulation ($p < 0.001$) and vegetation control ($p < 0.001$) affected emergence age. The interaction between the treatment types approached significance ($p = 0.054$) because CF resulted in much later emergence than any of the other treatments. Bedding and mounding resulted in earlier grass stage emergence than flat-planting, while herbicide resulted in earlier emergence than chopping (Table 3).

One-way ANOVA also found significant differences in mean annual height growth after grass stage emergence among the eight study treatments ($p < 0.001$). HM and HB, with mean height growth exceeding 0.5 m/year, grew significantly faster than F and CF, which had mean growth rates of 0.33 m/year and 0.23 m/year, respectively (Figure 11B). CF had significantly lower annual height growth than all treatments except for F and CM. Based on factorial ANOVA, soil manipulation ($p < 0.001$) and vegetation control ($p < 0.001$) significantly impacted post-emergence growth, while the interaction between the two treatment types was insignificant ($p = 0.195$). Bedding and mounding resulted in a greater annual growth rates than flat-planting, and herbicide produced greater annual growth than chopping (Table 3).

Stem analysis allowed us to reconstruct height growth of each sampled longleaf pine through time (Figure 12). The year in which each treatment line begins on the x-axis indicates the earliest grass stage emergence age of trees sampled in that treatment. Because identifying tree rings of grass stage longleaf pine seedlings is challenging, and may be impossible, we used a stem height of 25 cm as the starting point for grass stage emergence. All treatments were significantly taller through time than CF, due to that treatment's late emergence from the grass stage and slow growth following emergence. During the most recent four years, HB was significantly taller than F, and during that same period, HM approached significantly greater height than F. In general, the patterns of total tree height observed in the sub-sample used for the stem analysis dataset were similar to those from the stand-level analysis.

Stem analysis also allowed us to reconstruct mean annual height growth after grass stage emergence for each treatment (Figure 13). For this analysis, the annual height growth was standardized as 'years after grass stage emergence' to account for variation in the length in the grass stage. Trees are expected to rapidly increase growth during early years and then level off or decrease growth through time. For years 1-3, HB had significantly greater height growth than F, while HM had significantly greater height growth than F during years 2-5. Annual height growth continued to increase slightly over time for F, while growth generally levelled off after 4-5 years for the other treatments. Because the height growth represents annual growth, differences among treatments maintained through time will result in increased differences in total height through time. Therefore, these results suggest that most of the gains in total height for the treatments with the tallest trees occurred between years 2 and 6 following site preparation, although HB continues to show rapid annual height growth.

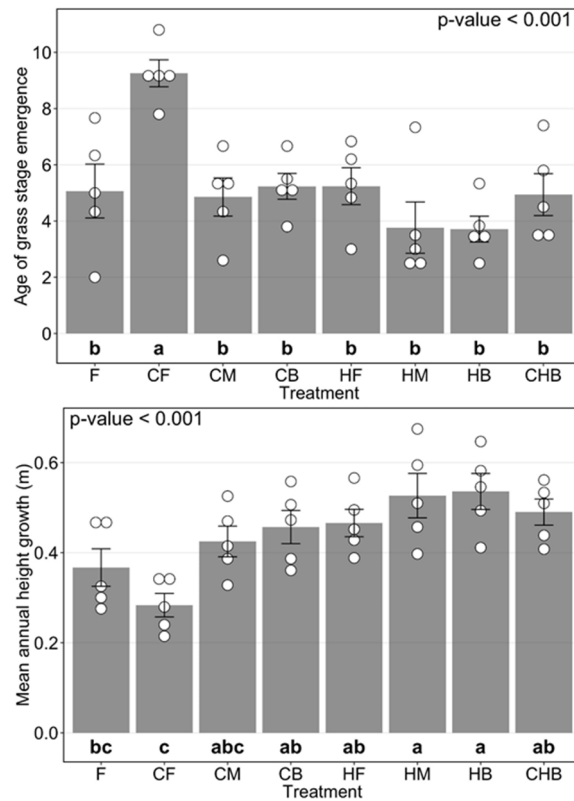


Figure 11. Tree-level responses (means and standard errors) to site preparation study treatments 15 years after application. Treatments are defined in Table 1. Same letters indicate no significant difference among treatments within a response variable based upon Tukey-adjusted pairwise comparisons; p-values are from one-way ANOVA tests.

Table 3. Least square means of tree-level responses to individual site preparation treatment levels 15 years after application.

Treatment	Age of grass stage emergence	Mean annual height growth (m/year)
Flat	7.31 a	0.330 b
Mounding	4.33 b	0.459 a
Bedding	4.48 b	0.486 a
<i>p</i> -value	< 0.001	< 0.001
Chopping	6.47 a	0.357 b
Herbicide	4.27 b	0.493 a
<i>p</i> -value	< 0.001	< 0.001

Same letters indicate no significant difference within a treatment type and response variable based upon Tukey-adjusted pairwise comparisons; *p*-values are from 2 x 3 factorial ANOVA tests.

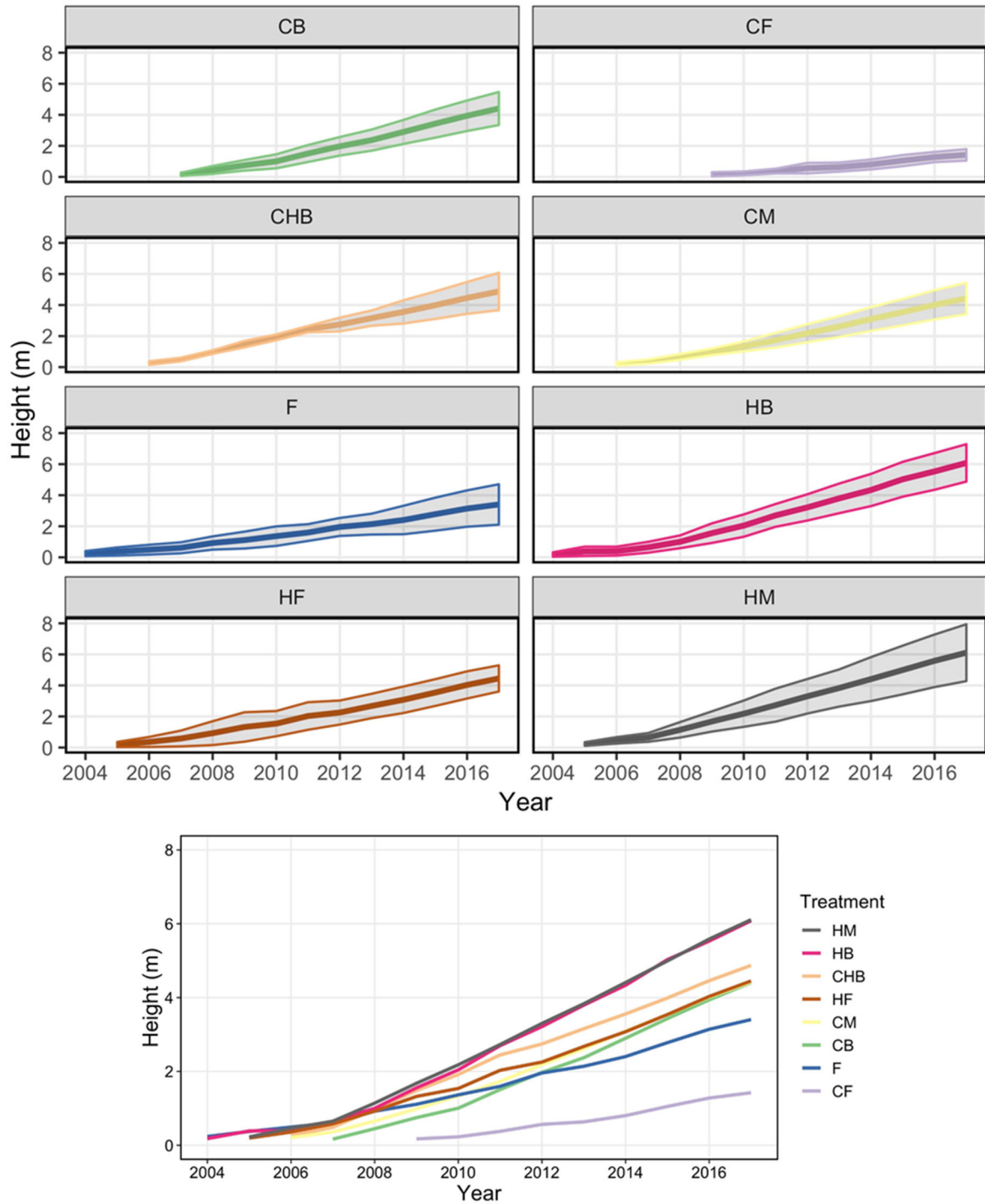


Figure 12. Reconstruction of mean height growth after grass stage emergence for each study treatment (defined in Table 1). Individual treatment panels include a line for the treatment mean and an envelope of the 95% confidence interval. The plot with all eight treatments shows only treatment means.

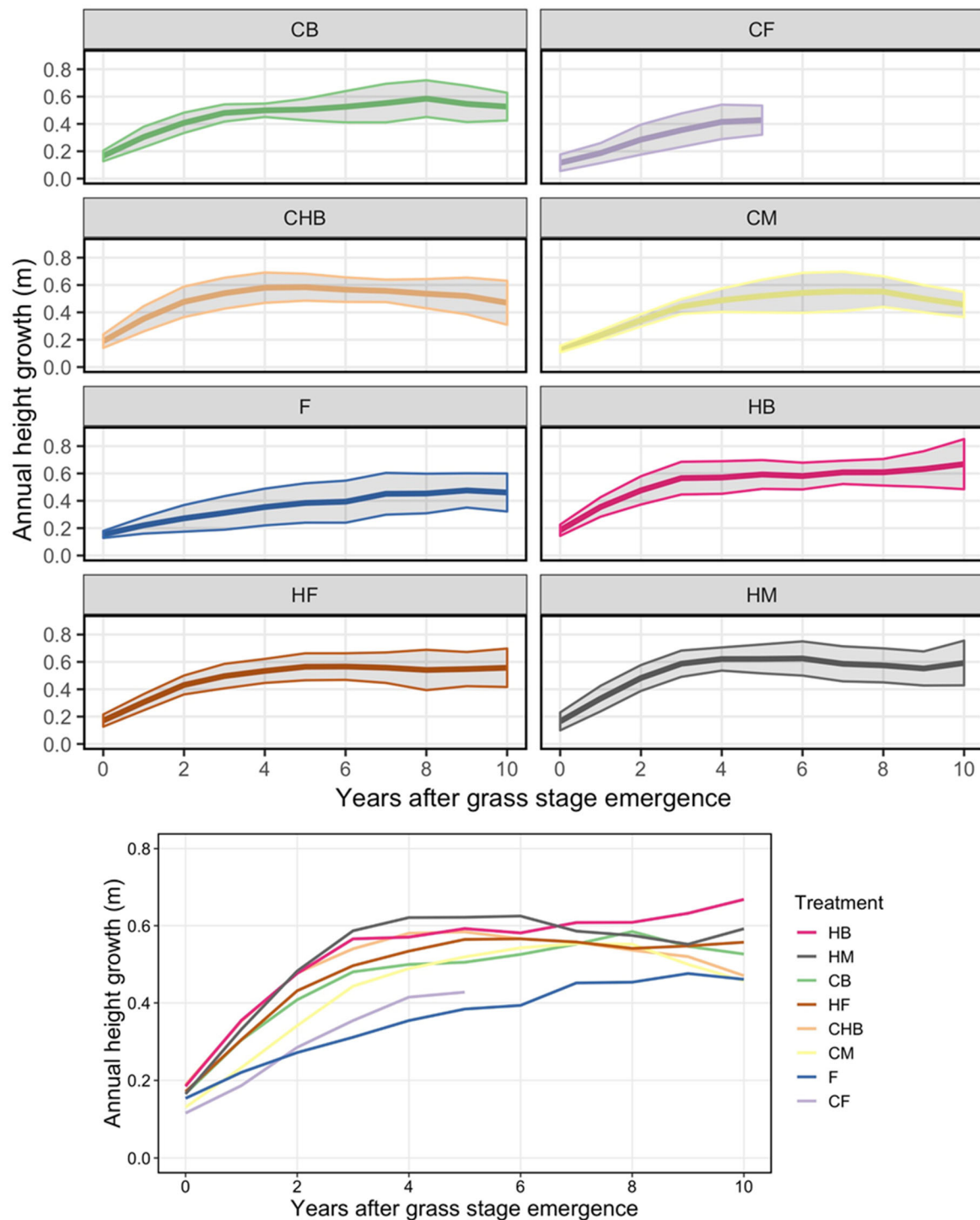


Figure 13. Mean annual height growth after grass stage emergence for each study treatment (defined in Table 1). Individual treatment panels include a line for the treatment mean and an envelope of the 95% confidence interval. The plot with all eight treatments shows only treatment means. Lines are smoothed on a three-year mean to reduce noise. Treatment lines are cut off after all five experimental units are no longer represented in the data.

Objective 2 – Ground-Layer Vegetation Response to Site Preparation

Response of Vegetation Cover

Across all years, a total of 205 plant species were identified in the study plots, with 109 species identified in both sampling periods (SI-1303 sampling in 2004-2006 and RC-2706 sampling in 2018), 74 species identified in only 2018, and 25 species identified in only 2004-2006. Appendix 1 provides a complete species list, including functional group designations for each species and the time period during which it was identified. For functional group analyses through time, any taxonomic differences in species-level identification between time periods will not affect results due to compiling data for analyses at the functional group level.

Repeated measures ANOVA indicated that the interaction between treatment and time was significant ($p < 0.001$) for total vegetation cover through 15 years after treatment application. There were significant differences in total cover among the study treatments for the first 2 years after application ($p < 0.001$) but not in years 3 and 15 ($p \geq 0.246$). In 2004 (the first year after treatment application), F and CF had greater cover than HM, HB, and CHB, and in 2005, F, CF, and CB had greater cover than HF, HM, and HB. Across all treatments, total cover in 2005 and 2018 were significantly greater than in 2004 ($p < 0.001$). Total cover did not significantly increase from 2005 to 2006 likely because there was a prescribed burn on all study blocks prior to sampling in 2006, which resulted in modest increases or, in some treatments, even decreases in total vegetation cover.

The interaction between treatment and time was also significant for cover of woody plants ($p < 0.001$) and shrubs ($p < 0.001$). Because shrubs made up the majority of woody species in this study, patterns of woody vegetation (Figure 14A) and shrub (Figure 15A) cover were similar. Significant differences in cover of both groups ($p < 0.001$) existed among the study treatments during the first 3 years. From 2004 to 2006, the untreated control (F) and CF generally had the greatest woody and shrub cover, particularly compared to the four treatments that included herbicide. However, by year 15, differences in woody and shrub cover among treatments were no longer significant ($p \geq 0.052$). Cover of both groups tended to increase through time, with a few exceptions after the prescribed burn in 2006. The four treatments with herbicide had the greatest increases in woody and shrub cover between year 3 and 15, whereas increases in the non-herbicide treatments were more gradual, with the greatest increases in woody and shrub cover typically occurring in the second year after treatment.

Repeated measures ANOVA also showed that the interaction between treatment and time was significant for cover of herbaceous plants ($p = 0.026$), graminoids ($p = 0.023$), and ferns ($p = 0.014$). For herbaceous cover, the only year in which treatments significantly differed was 2006 ($p = 0.039$), when HM, HB, and CHB had greater cover than CB (Figure 14B). For graminoid cover, the only year in which treatments significantly differed was 2018 ($p = 0.006$), when CF had greater cover than CHB and HB (Figure 15B). Treatments had significantly different fern cover in 2006 ($p = 0.025$) and 2018 ($p < 0.001$). In 2006, fern cover was higher on CHB than F and CF, and in 2018, CHB and HB had greater cover than CB, CM, CF, and F. Both herbaceous and fern cover tended to increase through time, with some exceptions in 2006, but graminoid cover did not follow this trend. Some treatments had reduced graminoid cover in 2006, but HB and CHB experienced significant reductions in graminoid cover between years 3 and 15.

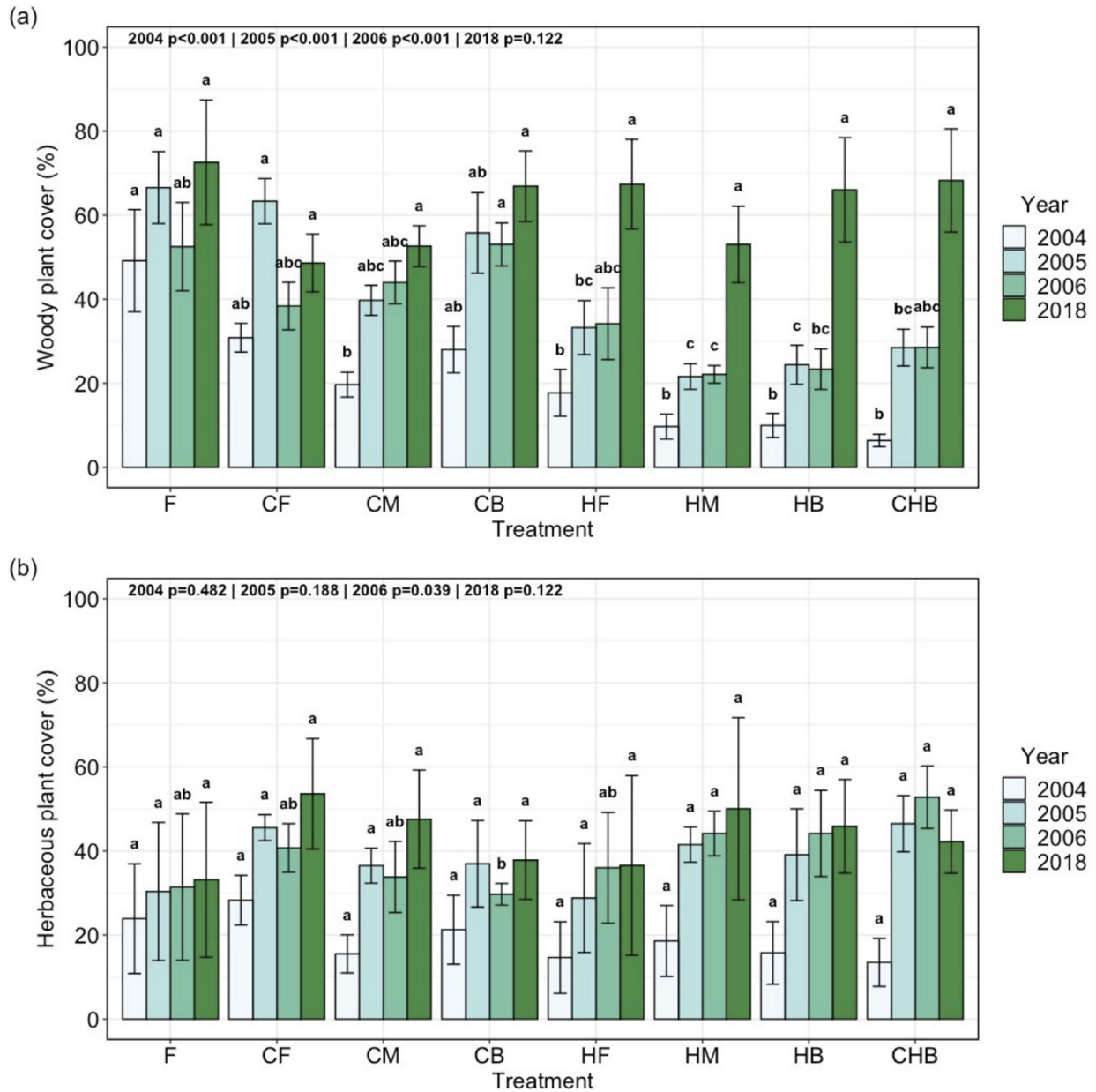


Figure 14. Cover (means and standard errors) of (a) woody and (b) herbaceous plants through 15 years after site preparation in a restored longleaf pine plantation. Same letters indicate no significant difference among treatments for a given measurement year. Treatments are defined in Table 1. Intensive site preparation reduced woody plant cover through three years after treatment without reducing herbaceous cover, but by year 15 all treatments had similar woody and herbaceous cover.

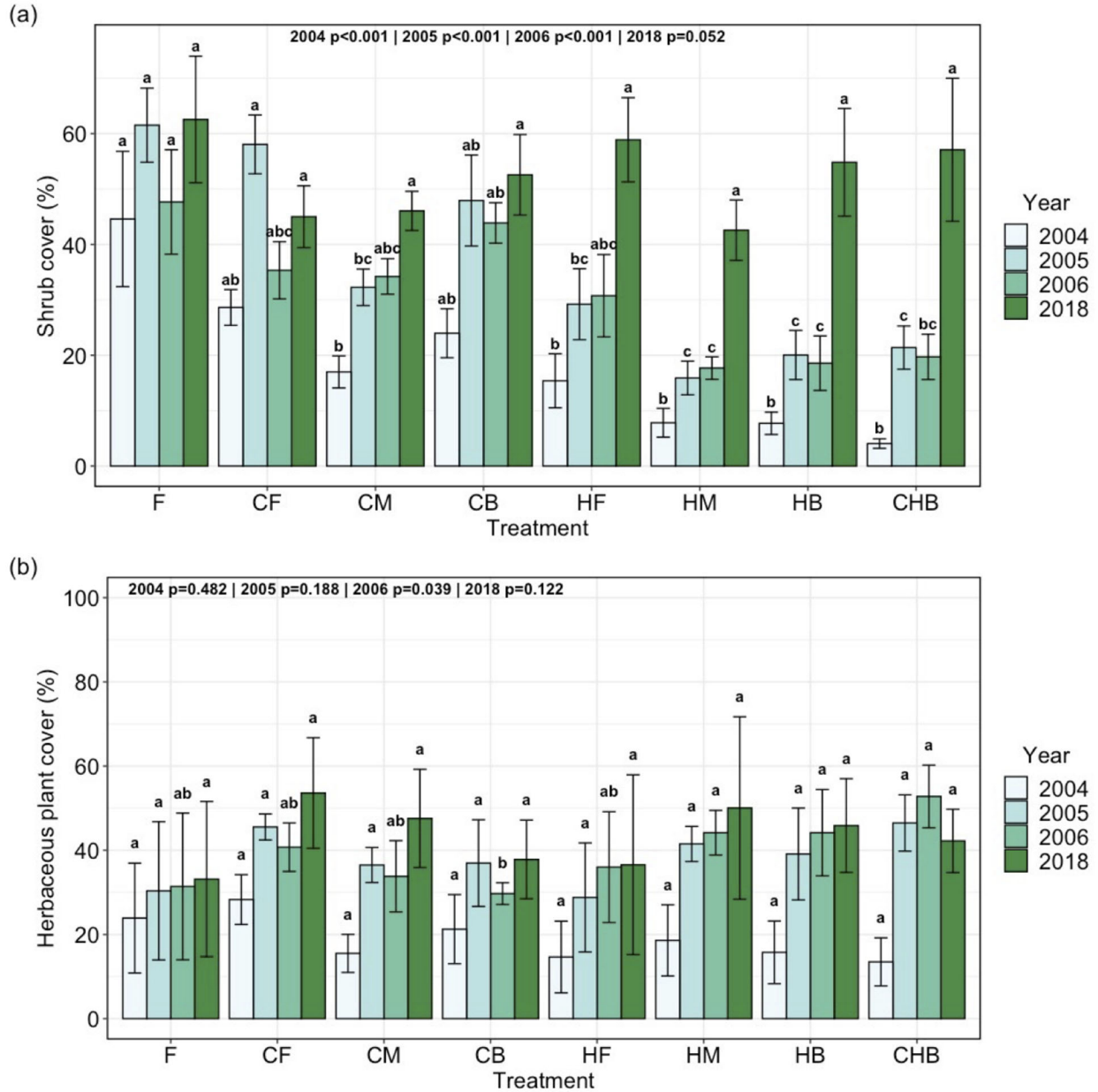


Figure 15. Cover (means and standard errors) of (a) shrubs and (b) graminoids through 15 years after site preparation in a restored longleaf pine plantation. Same letters indicate no significant difference among treatments for a given measurement year. Treatments are defined in Table 1. Intensive site preparation reduced shrub cover through three years after treatment without reducing herbaceous cover. However, by year 15 all treatments had similar shrub cover, and treatments that combined herbicide and bedding had reduced graminoid cover compared to CF.

Repeated measures ANOVA showed that treatment ($p \leq 0.005$) and time ($p < 0.001$) significantly varied for both bunchgrass and *A. stricta* cover, while the interaction between treatment and time was not significant ($p \geq 0.073$). Bunchgrass cover was significantly greater in 2005, 2006, and 2018 than in 2004 (Figure 16A), and *A. stricta* cover was greater in 2018 than during the first 3 years after treatment application (Figure 16C). CF resulted in significantly greater bunchgrass cover than HM, HB, and CHB (Figure 16B), while CF and F resulted in significantly greater *A. stricta* cover than all four herbicide treatments (Figure 16D). Repeated measures ANOVA showed that cover of the other three main functional groups we explored – forbs, trees, and vines – also significantly increased through time ($p < 0.001$). Forb cover increases leveled off after year 2 (Figure 16E), vine cover increases leveled off after year 3, and tree cover increased from year 1 to year 15. Treatment did not significantly affect forb (Figure 16F) or vine cover ($p \geq 0.352$), but there were significant differences among treatments in tree cover ($p = 0.031$), with CB having significantly greater cover than CF. For all three of these functional groups, the interaction between treatment and time was insignificant ($p \geq 0.107$).

Based on the 2 x 3 factorial ANOVA comparing individual vegetation control treatments, herbicide resulted in greater cover of ferns compared to chopping ($p = 0.001$), while chopping resulted in greater cover of forbs ($p = 0.014$) and graminoids ($p = 0.015$) in year 15 after treatment application (Table 4). Both annual and perennial forbs were significantly greater on chopped treatments than herbicide treatments ($p \leq 0.036$). The reduced long-term cover of graminoids from herbicide can be attributed to a significant reduction in bunchgrasses compared to chopping ($p = 0.003$), and much of the bunchgrass reduction is accounted for by a significant reduction in *A. stricta* cover ($p = 0.002$). Cover of all other groups (i.e., total vegetation, herbaceous plants, woody plants, sedges and rushes, other graminoids, shrubs, trees, and vines) did not differ between chopping and herbicide ($p \geq 0.083$).

Factorial ANOVA comparing individual soil manipulation treatments indicated that shrub cover was significantly greater on bedded treatments than mounded treatments ($p = 0.034$) in year 15 after treatment application (Table 4). In addition, *A. stricta* cover was significantly greater on flat-planted treatments than mounded and bedded treatments ($p = 0.024$). Cover of all other groups did not significantly differ among soil manipulation treatments 15 years after application ($p \geq 0.085$). There were no significant interactions between vegetation control and soil manipulation treatments for cover of any group tested ($p \geq 0.238$), although interactions for cover of *A. stricta* ($p = 0.051$), shrubs ($p = 0.099$), and tall shrubs ($p = 0.057$) approached significance. Greater *A. stricta* cover on flat-planted treatments was largely due to high cover on flat-planted treatments crossed with chopping. When flat-planting was crossed with herbicide, *A. stricta* cover was similar to mounding and bedding with herbicide ($p = 0.750$).

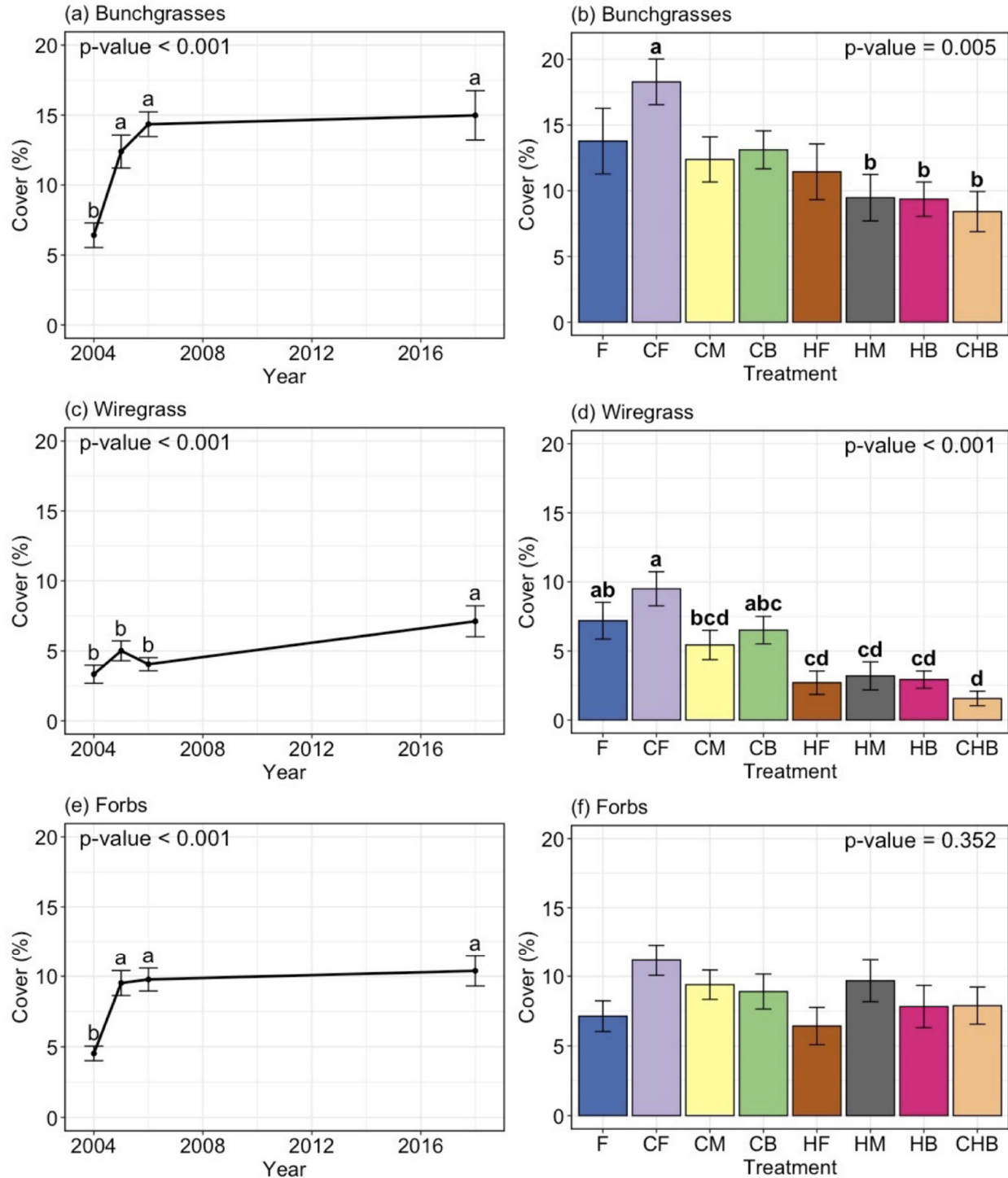


Figure 16. Cover (means and standard errors) of bunchgrasses, wiregrass, and forbs through 15 years after site preparation and among eight study treatments (defined in Table 1). Same letters indicate no significant difference among measurement periods or treatments within each panel. Wiregrass recovered more slowly than bunchgrasses or forbs, and intensive site preparation resulted in lasting reductions in bunchgrass and wiregrass cover.

Table 4. Responses to individual soil manipulation and vegetation control treatments 15 years after application, based on analysis using a 2×3 factorial structure.

	<u>Soil Manipulation</u>			<i>p</i> -value	<u>Vegetation Control</u>		<i>p</i> -value
	Flat	Mound	Bed		Chop	Herbicide	
<u>Cover</u>							
Total	103	102	106	0.775	103	105	0.723
Herbaceous	45.1	48.8	41.1	0.480	47.5	42.5	0.340
Woody	58.0	52.8	65.0	0.144	55.2	62.0	0.171
Ferns	7.4	12.2	12.8	0.085	7.0 b	14.6 a	0.001
Forbs	12.0	13.3	10.4	0.446	14.4 a	9.4 b	0.014
Annual forbs	0.72	0.34	0.31	0.260	0.71 a	0.21 b	0.036
Perennial forbs	10.9	12.6	9.9	0.469	13.4 a	8.9 b	0.020
Graminoids	25.4	21.8	16.6	0.089	25.4 a	17.1 b	0.015
Bunchgrasses	19.5	15.7	12.2	0.067	19.8 a	11.9 b	0.003
Wiregrass	10.6 a	6.2 b	6.3 b	0.024	10.1 a	5.3 b	0.002
Sedges and rushes	3.1	3.6	2.2	0.469	3.1	2.8	0.782
Other graminoid	2.8	2.4	2.1	0.812	2.4	2.4	0.981
graminoids							
Shrubs	51.9 ab	44.3 b	54.8 a	0.034	47.5	53.2	0.083
Tall shrubs	33.1	30.2	36.3	0.425	30.9	35.4	0.236
Short shrubs	18.9	14.1	18.6	0.279	16.6	17.8	0.654
Trees	4.6	7.3	8.7	0.303	5.9	7.8	0.366
Vines	1.8	2.9	2.8	0.477	2.7	2.4	0.707
<u>Diversity</u>							
Richness	17.3	16.5	15.8	0.409	17.3 a	15.7 b	0.079
Diversity (Hill number)	7.18	7.41	6.78	0.376	7.85 a	6.40 b	< 0.001
Evenness	0.67	0.69	0.68	0.260	0.70 a	0.66 b	< 0.001
<u>Light Transmittance</u>							
Gap light index	0.90	0.86	0.86	0.142	0.92 a	0.83 b	< 0.001
Gap fraction	0.74	0.67	0.69	0.058	0.76 a	0.66 b	< 0.001

Same letters indicate no significant difference ($\alpha = 0.05$) within a treatment type and response variable; *p*-values are from 2×3 factorial ANOVA tests.

Plant Diversity in Year 15

Fifteen years after site preparation, plant species richness did not significantly differ among the eight study treatments ($p = 0.259$) (Figure 17A). Across all treatments, mean richness was 16.1 species per 1 m². Similarly, factorial ANOVA indicated that neither soil manipulation ($p = 0.409$) nor vegetation control treatments ($p = 0.079$) significantly impacted species richness (Table 4), and there was no significant interaction between the two treatment types ($p = 0.446$). In contrast, there were significant differences in diversity ($p = 0.006$) and evenness ($p = 0.022$) among the eight study treatments in year 15. Diversity was significantly greater on CF, which had a mean of 8.22, than F, HF, and HB, which had mean diversities of slightly greater than 6 (Figure 17B). Evenness was significantly greater on CF, which had a mean of 0.71, than HF, which had a mean of 0.63 (Figure 17C). Differences in diversity and evenness among vegetation control treatments were significant ($p < 0.001$), with chopping resulting in higher diversity and evenness than herbicide (Table 4). There were no significant differences in soil manipulation treatments ($p \geq 0.260$) and no significant interaction ($p \geq 0.160$) for either diversity or evenness.

Vegetation Composition through Time

For analysis of plant composition through time, we had to reconcile possible disparity in taxonomic identification between the early sampling period (2004-2006) and the recent sampling (2018). In general, the early sampling period used a coarser taxonomic resolution. For example, species in the genus *Dichanthelium* were mostly grouped and identified as “*Dichanthelium* spp.”, whereas in 2018 each individual was identified to the species within *Dichanthelium*. In addition, some species have been taxonomically re-classified in the years between the early sampling and the recent sampling. For example, *Stenanthium densum* was previously known as *Zigadenus densus*, and described as such in the early sampling period. To create a comparable species list through time, we combined the datasets at the coarsest level with consistent taxonomic classification. We have provided summary of the species used for the compositional analyses through time in Appendix 2.

The NMDS ordination was fit with a 3-dimensional solution, with a final stress value of 0.189 (Figure 18). In the first year after treatment, the composition of the four herbicide treatments and the four non-herbicide treatments were clustered. Over time, and particularly between 2006 and 2018, treatments moved in the same general direction in the ordination space. Movement was towards lower first axis scores (see Appendix 3 for species scores obtained from NMDS ordination), indicating increases in shrubs and dominant graminoids as well as disappearance of ruderal herbs. In 2018, the four herbicide treatments remained somewhat clustered. PERMANOVA indicated that the plant community composition differed significantly among the eight study treatments ($p < 0.001$) and across the four measurement periods ($p < 0.001$), while the interaction between treatment and time was not significant ($p = 0.820$). All four measurement periods significantly differed ($p \leq 0.013$), and almost all treatments significantly differed ($p \leq 0.042$) with the exceptions of CM and CB ($p = 0.612$) and CHB, HB, and HM ($p \geq 0.612$), which were statistically similar.

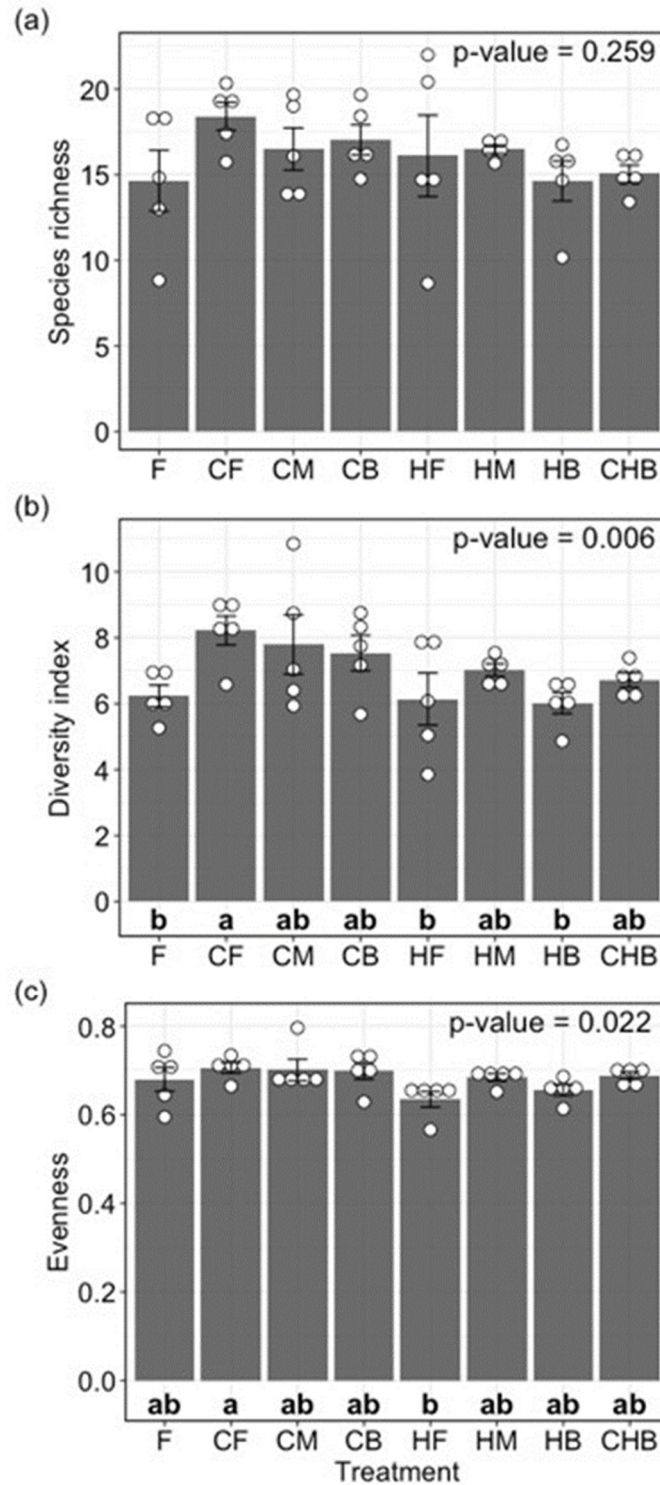


Figure 17. Three measures of plant species diversity (means and standard errors) across eight site preparation treatments (defined in Table 1). Same letters indicate no significant difference among treatments within each panel. There were no significant differences in richness, but CF had significantly higher diversity and evenness than HF.

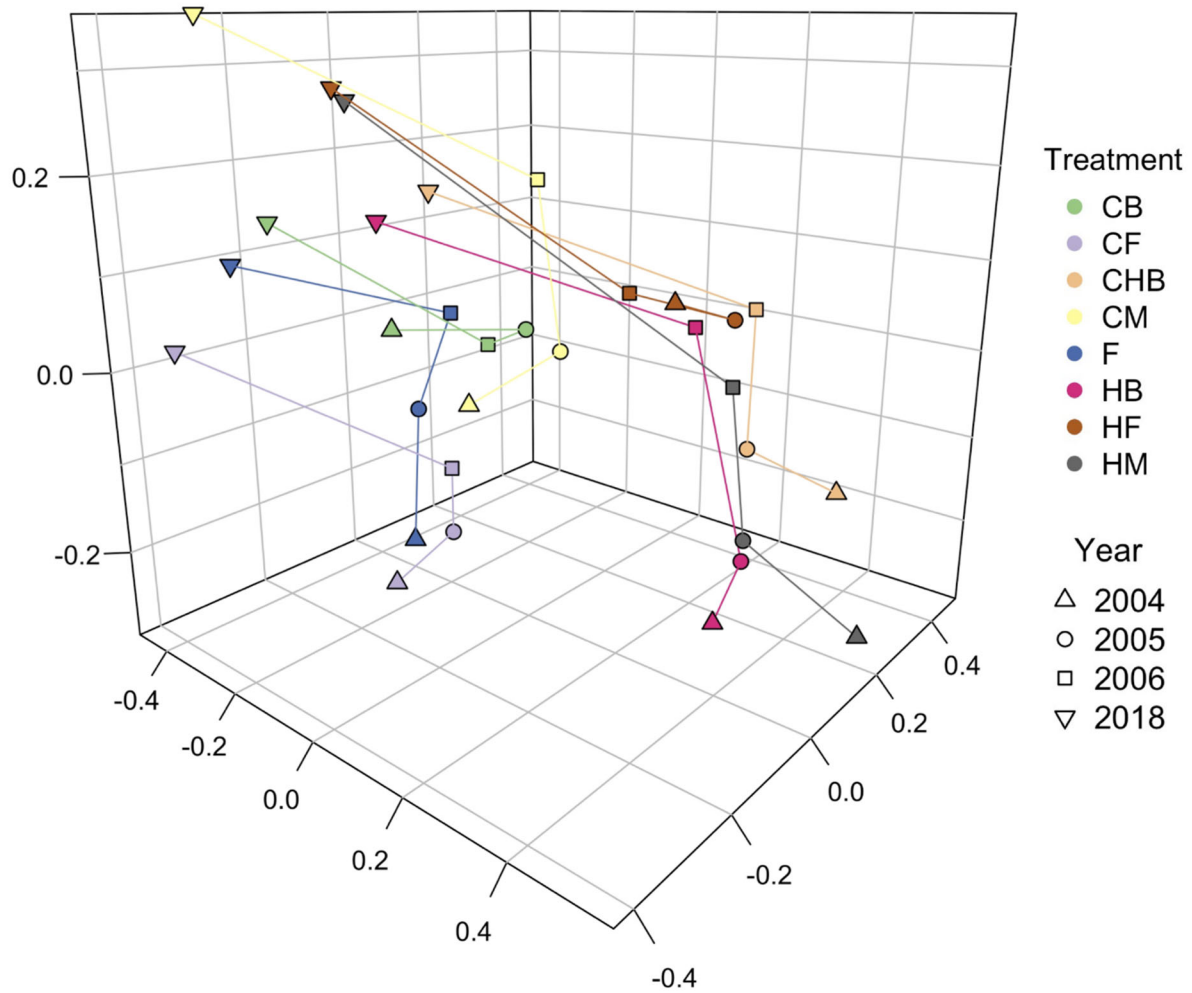


Figure 18. Results from NMDS ordination in which the centroid of each treatment in each year is shown. PERMANOVA indicated that all treatments significantly differed except for the following treatments that were similar: CM and CB; CHB, HB, and HM.

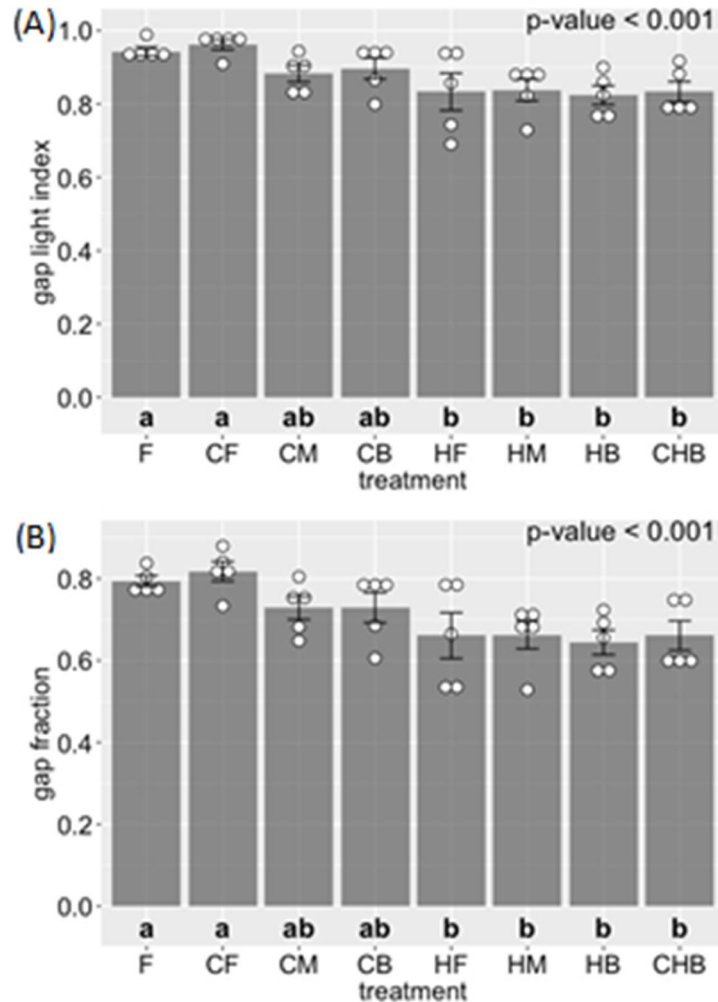


Figure 19. Estimates of A) gap light index and B) gap fraction (means and standard errors) obtained from hemispherical photos taken 15 years after site preparation. Treatments are defined in Table 1. The p-values are from one-way ANOVA tests for treatment effects; same letters indicate no significant difference ($\alpha = 0.05$) among treatments within a response variable.

Light Transmittance through the Canopy

Site preparation treatments also produced significant differences in understory light transmittance 15 years after application ($p < 0.001$). Gap light index and gap fraction were significantly greater on F and CF than on HF, HM, HB, and CHB, with differences in gap light index exceeding 0.10 (Figure 19A) and in gap fraction approaching 0.15 (Figure 19B). The greatest amount of light transmittance was in the CF treatment, and the lowest light transmittance was in HB (Figure 20). Vegetation control treatments significantly affected both measures of light transmittance ($p < 0.001$), with chopping resulting in more light than herbicide (Table 4). Differences in soil manipulation treatments were not significant for either gap light index ($p = 0.142$) or gap fraction ($p = 0.058$), although the latter approached significance. Additionally, the interaction between treatment types was insignificant for both measures of light transmittance ($p > 0.123$).

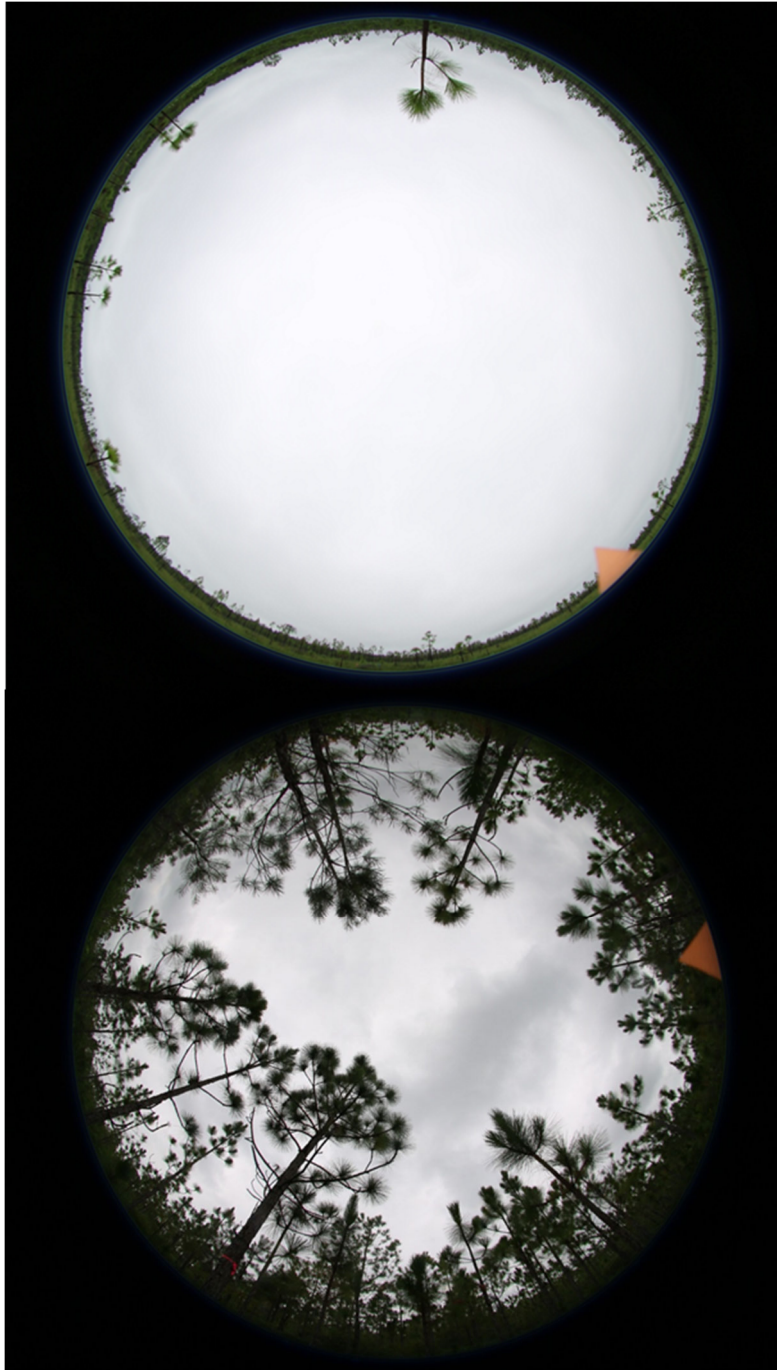


Figure 20. Hemispherical photographs to contrast the canopy closure and light transmittance of the CF treatment (top) and the HB treatment (bottom).

Local Effects of Bedding

Paired *t*-tests indicated that total vegetation cover and cover of some functional groups differed significantly between the bedding rows and interbed areas (Table 5). Total plant cover was almost 10% higher on beds compared to interbed areas ($p = 0.014$). This difference was accounted for by significantly greater cover of woody species on beds (72.6%) compared to interbeds (58.8%) ($p < 0.001$). The difference in cover of herbaceous species between the two locations approached significance ($p = 0.055$), with interbed areas (44.5%) having slightly higher mean cover than beds (39.6%). In terms of the five main functional groups we explored, four of them – ferns, forbs, shrubs, and vines – did have significantly different cover between bedding rows and interbed areas. The difference in shrub cover approached significance ($p = 0.077$), but the other three groups did not ($p \geq 0.193$).

The other two main functional groups – trees and graminoids – did have significantly different cover on beds compared to interbeds (Table 5). Tree cover on beds was over 8% greater compared to interbeds ($p < 0.001$), while graminoid cover was over 3% greater on interbeds compared to beds ($p = 0.023$). The significant difference in graminoid cover can be attributed to bunchgrasses, and wiregrass in particular, as both bunchgrasses as a whole and wiregrass in particular had about 3% higher cover on interbeds compared to beds ($p \leq 0.021$). Annual forbs had significantly higher cover on interbeds compared to beds ($p = 0.011$), although the mean difference was less than 0.2%. There were no significant differences in cover for perennial forbs, tall shrubs, or short shrubs ($p \geq 0.130$).

Paired *t*-tests also indicated that there were no significant differences in plant species richness, diversity, or evenness between bedding rows and interbed areas ($p \geq 0.511$) (Table 5). There were, however, significant differences in understory light transmittance. Gap light index and gap fraction were higher on interbeds than on beds ($p \leq 0.009$), although the mean differences for both estimates were relatively small in magnitude (Table 5).

Table 5. Means and standard errors for measures of diversity, percent cover, and light transmittance on bedded rows and interbed areas 15 years after treatment application.

	<u>Bedding Row</u>		<u>Interbed Area</u>		<i>p</i> -value
	Mean	SE	Mean	SE	
<u>Cover</u>					
Total	112.2	3.6	103.3	2.8	0.014
Herbaceous	39.6	1.9	44.5	2.0	0.055
Woody	72.6	3.5	58.8	2.9	< 0.001
Ferns	15.6	1.4	14.9	1.4	0.629
Forbs	7.3	1.1	9.1	0.9	0.193
Annual Forbs	0.15	0.03	0.34	0.08	0.011
Perennial Forbs	6.8	1.1	8.5	0.9	0.200
Graminoids	15.1	1.2	18.7	1.4	0.023
Bunchgrasses	9.9	1.0	13.3	1.3	0.005
Wiregrass	4.3	0.8	7.2	1.1	0.021
Sedges and rushes	2.3	0.5	2.9	0.6	0.376
Shrubs	56.9	2.6	52.1	2.5	0.077
Tall shrubs	34.0	2.2	31.9	2.1	0.424
Short shrubs	22.9	2.0	20.2	1.8	0.130
Trees	13.8	1.8	5.2	1.2	< 0.001
Vines	3.6	0.5	3.2	0.5	0.427
<u>Diversity</u>					
Richness	15.6	0.3	15.5	0.3	0.708
Diversity (Hill number)	6.54	0.17	6.69	0.20	0.511
Evenness	0.67	0.01	0.67	0.01	0.747
<u>Light transmittance</u>					
Gap light index	0.84	0.01	0.86	0.01	0.007
Gap fraction	0.67	0.01	0.68	0.01	0.009

The *p*-values are from paired *t*-tests between bedding rows and interbed areas; significant differences ($\alpha = 0.05$) are shown in bold.

Discussion

The site preparation treatments used in this study had long-term effects on the developmental trajectory of these ecosystems, which were visually apparent in plantation structure (Figure 21). In some cases, early results from SI-1303 were maintained or magnified through time, whereas other responses shifted in direction or magnitude from the early to recent measurement periods.

Objective 1 – Longleaf Pine Tree- and Stand-Level Responses to Site Preparation

Through 15 years following site preparation treatments, the untreated control (F) generally resulted in the smallest height and DBH, the lowest tree density, and the greatest number of seedlings still in the grass stage, indicating that site preparation of any kind benefits tree establishment on this site type. However, the CF (chopping treatment) did not differ from the untreated control for many of these metrics, indicating that benefits from chopping were minimal. Our results also demonstrate a pattern of increasing magnitude of response with increasing site preparation intensity, which is consistent with other studies that have examined longleaf pine responses to vegetation control and soil manipulation (Knapp et al., 2006; Loveless et al., 1989). Study treatments that resulted in the greatest tree size and density included both herbicide and soil manipulation, while treatments that produced intermediate size and density included either herbicide or soil manipulation. The treatments that resulted in the smallest tree size and lowest density – CF and F – included neither herbicide nor soil manipulation.

Chopping was not effective for improving conditions for longleaf pine establishment. This is consistent with other studies, which have found that chopping resulted in reduced longleaf pine seedling growth (Freeman and Jose, 2009; Knapp et al., 2006) and increased woody vegetation (Miller, 1980) compared to other vegetation control treatments. Although chopping causes

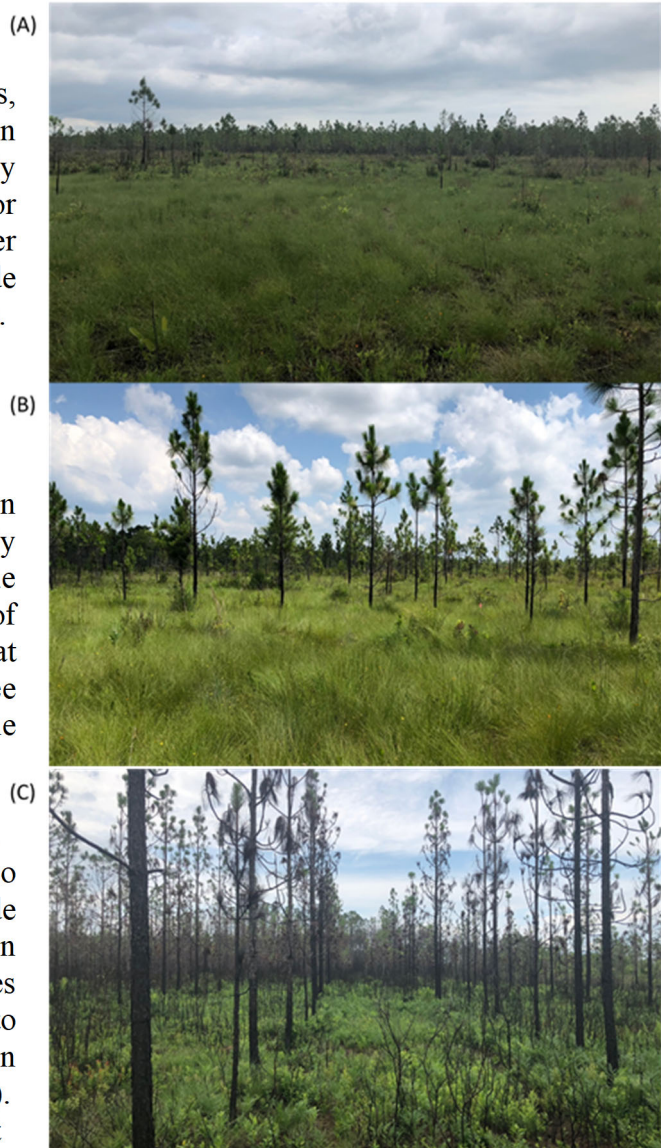


Figure 21. Images to contrast the stand structure and development of A) an untreated control (F); B) chop-bedding treatment (CB); and C) herbicide-bedding treatment (HB).

minimal soil disturbance, has low impact on herbaceous vegetation, and immediately reduces standing woody vegetation, a major drawback is that it promotes sprouting of woody species (Lowery and Gjerstad, 1991). Freeman and Jose (2009) suggested that chopping plus fire may be sufficient for longleaf pine establishment in flatwoods, even though seedling growth may be slow. However, our findings did not support this expectation through 15 years after planting on similar flatwoods and savanna sites. Perhaps this contrasting result is due to interactions between treatments and fire, but an analysis of such interactions is beyond the scope of this study because we lack long-term, replicated data on fire behavior. The contrasting result might also be due to a fire return interval that was too long to control abundant woody competition in our study. The first burn occurred two years after site preparation, but the next two burns occurred at 4-7-year intervals, depending upon the block. This is much less frequent than the study sites' estimated pre-settlement fire return interval of 1-3 years (Frost, 2001). The need for frequent fire, especially during restoration on flatwoods sites, is emphasized because even a slight reduction in frequency could allow shrub encroachment (Glitzenstein et al., 2003).

The herbicide treatment had lasting improvements on growth and survival of planted longleaf pine. Although previous studies have consistently observed increased longleaf pine seedling growth (Addington et al., 2012; Boyer, 1988; Freeman and Jose, 2009; Knapp et al., 2006; Loveless et al., 1989) and earlier grass stage emergence (Boyer, 1988; Freeman and Jose, 2009; Loveless et al., 1989) as a result of herbicide application for site preparation and release, few studies have reported responses beyond six years after planting. Additionally, previous studies have reported that longleaf pine seedling survival is either unaffected (Addington et al., 2012; Loveless et al., 1989) or reduced by herbicide (Boyer, 1988; Freeman and Jose, 2009) within the first few years following planting. Although survival did not differ among treatments through two years after planting in our study (Knapp et al., 2006), herbicide contributed to the highest survival among treatments by year 15. This finding emphasizes the importance of a longer-term perspective when evaluating responses to site preparation and suggests that treatments that encourage early grass emergence may be important for increasing long-term survival.

Soil manipulation treatments resulted in increased longleaf pine growth compared to flat-planting (no treatment) through 15 years after treatment, which is consistent with previous studies of early growth for slash (Haywood, 1987; Outcalt, 1984; Pritchett, 1979) and longleaf pine seedlings (Knapp et al., 2006; Loveless et al., 1989). Survival and density responses to soil manipulation in our study were less clear. Bedding has been shown to increase survival of slash pine seedlings (Pritchett, 1979) but not of longleaf pine seedlings (Knapp et al., 2006; Loveless et al., 1989), while mounding has not been shown to increase survival of slash (Haywood, 1987) or longleaf pine seedlings (Knapp et al., 2006). In our study, treatments with soil manipulation tended to have higher survival in year 15 than the untreated control, yet only those treatments that included herbicide had significantly better survival than the control. Stand density in year 15 on bedded sites was significantly greater than mounded sites, but this was likely due to lower initial planting density on mounds (mean = 886 trees/ha) compared to beds (mean = 1,371 trees/ha) because survival in year 15 was similar among bedded and mounded treatments.

Stem analysis indicated that herbicide and soil manipulation treatments provided both immediate (earlier grass stage emergence) and long-term (increased growth rate after emergence) advantages for longleaf pine establishment. The short-term benefit obtained from vegetation control is

consistent with our expectation that reduced competition results in greater seedling growth and earlier grass stage emergence (Knapp et al., 2006, 2008). However, competition control from herbicide or mechanical treatments should diminish through time following vegetation recovery. For example, Freeman and Jose (2009) found that shrub cover was similar between treated and untreated areas four years after herbicide application on flatwoods sites in Florida. Boyer (1990) suggested that duration of the grass stage has a critical impact on subsequent longleaf pine growth and stand development, with delayed emergence resulting in lower long-term growth rates (Boyer, 1983). Twenty years after a single herbicide release treatment, Michael (1980) reported volume differences that represented an 8-year growth advantage for longleaf pine on treated sites. Although this could be due to more years of growth from earlier grass stage emergence, our results suggest that early competition control results in long-term, sustained increases in growth rate. In contrast to the recovery of vegetation following a single mechanical or chemical application, soil manipulation treatments may have persistent effects for several decades (Brockway et al., 2005). Although mounds and beds are likely to settle over time (Londo and Mroz, 2001), these treatments may have lasting impacts on hydrology. Given that the majority of the root system of longleaf pine is within the upper 30 cm of soil (Boyer, 1990; Heyward, 1933), it is possible that the drier conditions at the soil surface continue to directly benefit tree growth through time.

Our findings suggest that the growth rate after grass stage emergence is more important than the timing of emergence for determining patterns of stand development through year 15. For example, the untreated control (F) did not differ from any treatment other than CF in timing of grass stage emergence, yet resulted in the fewest trees/ha and smallest mean tree size. One explanation for this pattern may be the survivorship bias implicit in our sampling approach. By studying a random sample of trees 15 years after planting, we were limited to studying only those trees that were still alive at sampling, thus basing conclusions on successful trees. On the untreated control, which experienced a high level of mortality during the study period, the stem analysis results suggest that for trees to survive to age 15, they must emerge from the grass stage early. If they fail to do so, competitors will presumably overtake the grass stage seedlings, resulting in suppression and eventual mortality. Because competition and poor drainage were not improved on the control, post-emergence growth remained low for trees that did manage to survive.

Several of our findings suggest that the more intensive site preparation treatments used in this study resulted in a Type A response (Morris and Lowery, 1988; Nilsson and Allen, 2003) through 15 years after treatment. The Type A response indicates that gains in growth rates due to site preparation are maintained through time, such that the magnitude of differences among treated and untreated areas increases through time. For example, survival did not differ among treatments during early seedling establishment, but by year 15, survival ranged from 11.7% on F to 44.7% on HB. The results for growth differences were much more pronounced; differences in relative diameter among treatments were significant in year two and persisted at the same magnitude through year 15. Finally, stem analysis indicated that annual height growth remained consistently higher through time on treated sites compared to F and CF. Differences in annual growth rates observed in our study magnify the cumulative differences in tree size among treatments, suggesting that differences among treatments are likely to persist or increase. Long-term responses of longleaf pine to site preparation have not been well studied, but the Type A response observed in our study is consistent with the growth response of loblolly pine through 18 years after intense site preparation reported by Nilsson and Allen (2003). In contrast, slash pine has generally

exhibited a Type C response after bedding on flatwoods sites (Outcalt, 1984; Zhao et al., 2009). This type of response sees initial benefits of site preparation treatment diminish through time, eventually resulting in the untreated areas outperforming the treated areas. The occurrence of this type of response to site preparation is believed to be due to long-term, detrimental impacts of the site preparation treatments on site quality or nutrient availability, but our results provide no evidence of this situation.

Objective 2 – Ground-Layer Vegetation Response to Site Preparation

Our study was designed to evaluate the effects of a variety of site preparation treatments commonly used to improve seedlings establishment for longleaf pine ecosystem restoration outcomes through time. Although we did not sample a comparative reference community as part of this study, plant community classifications and previous research on longleaf pine ecosystems at Camp Lejeune can be used to describe a reference condition. Frost (2001) described the pre-settlement vegetation community on frequently burned Leon sand at Camp Lejeune as species-rich longleaf pine wet savanna. This community is characterized by an overstory of longleaf pine and an understory dominated by bunchgrasses but also containing a diverse array of grasses, sedges, forbs, and fire-dwarfed shrubs (Frost, 2001). Mitchell et al. (2015) identified three pine-dominated, natural community types on Camp Lejeune - dry pine/oak savanna, wet pine savanna, and high pocosin – which are arranged along a moisture gradient from xeric to hydric, respectively. Many of the indicator species from all three community types (see table 6 in Mitchell et al., 2015) were present in our study sites. This highlights the spatial heterogeneity of topography, soil, and vegetation even at small scales in longleaf pine communities and suggests that a range of restoration outcomes might be suitable for our study sites.

The chop-only (CF) treatment in our study resulted in several characteristics that aligned with the reference conditions for wet pine savanna as described by Frost (2001) and Mitchell et al. (2015). Fifteen years after treatment application, CF resulted in the greatest bunchgrass and wiregrass cover and was among the treatments with the highest diversity. The advantages of low intensity chopping as a form of site preparation include minimal soil disturbance, low impact on herbaceous vegetation, and drier fuels for fire (Johnson and Gjerstad, 2006). Frequent fire promotes dominance of bunchgrasses (Mitchell et al., 2009) and is associated with increased species richness in longleaf pine communities (Walker and Peet, 1984; Glitzenstein et al., 2003). The CF treatment may have provided an advantage to herbaceous vegetation by reducing woody competitors early in the study, with that advantage maintained through time with the periodic prescribed burns. In addition, chopping resulted in significantly more canopy light transmittance than herbicide treatments due to the slower rate of canopy closure. Increased competition for light and resources as the canopy closes is expected to reduce understory plant abundance and diversity in longleaf pine plantations (Harrington et al., 2006).

In contrast to chopping, herbicide had stronger initial effects on the understory plant community, some of which lasted throughout the 15-year study period. Herbicides are commonly used as a form of site preparation because of their ability to target specific plant groups (Walker and Silletti, 2006), and through the first 3 years after treatment, herbicide reduced woody plant and shrub cover without reducing herbaceous plant and graminoid cover. These early results are consistent with other studies that observed understory responses to herbicide for site preparation in longleaf pine plantations (Addington et al., 2012; Freeman and Jose, 2009). However, by year 15 the early

reductions in shrub cover from herbicide were lost, which was not expected in our study. Freeman and Jose (2009) observed recovery of shrubs within 4 years of herbicide application on flatwoods sites; yet in that study, wiregrass cover remained significantly higher on sites treated with imazapyr. In our study, herbicide resulted in significant long-term reductions in cover of bunchgrasses, wiregrass, and forbs as well as lower plant species diversity and evenness compared to chopping. Based on the ordination, herbicide also affected plant community composition, with herbicide treatments separating from non-herbicide treatments in ordination space, a separation that persisted through the duration of the study. The impacts of herbicide were particularly pronounced when combined with soil manipulation treatments. Shrub cover was lowest on the soil manipulation treatments crossed with herbicide through 3 years after treatment. By year 15, though, all treatments had similar shrub cover, and the two treatments that combined herbicide and bedding – HB and CHB – experienced significant reductions in graminoid cover and had significantly lower graminoid cover than CF.

Wiregrass appeared to be particularly sensitive to site preparation, which is concerning because bunchgrasses are important fuels in the longleaf pine ecosystem (Mitchell et al., 2009), and wiregrass, in particular, may have historically dominated wet longleaf pine savannas (Frost, 2001). Wiregrass is easily uprooted by mechanical site preparation, does not reproduce via seed except after growing season fire (Fill et al., 2017), and propagates primarily by clump expansion when burned exclusively in the dormant season, all of which make the species ill-suited for recovery after soil disturbance (Clewett, 1989). Unsurprisingly, bedded and mounded treatments had significantly lower wiregrass cover than flat-planted treatments. Moreover, the abundance of wiregrass was greater within the interbed areas than on the beds within the bedded treatment units. This result highlights the long-lasting effect of the mechanical disturbance caused by bedding on wiregrass but also suggests recovery of wiregrass on beds, as wiregrass cover on beds in 2018 was 4.3% compared to 7.2% in interbed areas.



Figure 22. Bedded site immediately following treatment application, illustrating differences in plant cover on beds and interbed areas.

The herbicide treatment used in our study had resulted in long-term reduction in wiregrass. Imazapyr, which was one of the two herbicides used in our study, is a broad-spectrum herbicide that targets grasses among other species. Previous research has not shown reductions in wiregrass cover after application of imazapyr in flatwoods sites (Freeman and Jose, 2009) or of bunchgrass cover on upland sites (Addington et al., 2012). In contrast, Platt et al. (2015) documented reductions of dominant warm season grasses within the first year after treatment with imazapyr in pine savannas. Our 15-year study suggests that imazapyr may have long-term negative impacts on wiregrass abundance. An important caveat is that imazapyr was applied via broadcast application in our study. Perhaps negative impacts could have been minimized by targeting shrubs with backpack spraying, although this approach is more expensive.

Our results show that stand development is beginning to affect light availability in the understory, with available light (gap light index) reduced in treatments with the greatest tree survival/growth. As tree canopies close within plantations, light levels to the forest floor decline, with concomitant reductions in ground layer vegetation abundance and diversity (Harrington and Edwards, 1999). In addition, light levels were significantly greater on interbeds compared to beds, which would also be expected because trees were planted on the beds. The 15-year-old plantations in our study appear to be at the beginning stages of canopy closure on the site preparation treatments with the best-growing trees. However, available light exceeded 80% of full sunlight across all treatments, and there was little evidence that the reductions in light associated with some of the site preparation treatments were driving response patterns of ground layer vegetation at this time. As these stands continue to develop, canopy closure is expected to reduce light levels at the forest floor and further impact the ground layer plant community.

Management Implications

Site preparation treatments are applied to address site-specific challenges, and the effectiveness of the treatments used in our study would be expected to vary across the wide range of site types on which longleaf pine is managed. On mesic and xeric sites, soil manipulation treatments are likely unnecessary because these site types lack the high water table that inhibits seedling growth and survival on hydric sites (Fox et al., 2007), although bedding has been shown to increase growth, but not survival, of longleaf pine seedlings on well-drained sites (Loveless et al., 1989). In contrast, herbicide is commonly used for site preparation on mesic and xeric sites because of longleaf pine's intolerance to competition (Brockway et al., 2006). Our findings support previous studies and suggest that herbicide site preparation improves longleaf pine seedling growth regardless of site type (Addington et al., 2012; Boyer, 1988; Loveless et al., 1989).

Longleaf pine management may encompass a range of specific objectives and structural or compositional conditions. Site preparation is commonly used in conjunction with timber production of other southern pines, and our results show that intensive site preparation resulted in the greatest growth and survival of longleaf pine. However, management objectives for longleaf pine are often different within the context of ecosystem restoration. Consequently, maintaining or enhancing other attributes, such as ground flora communities, appropriate fire regimes, and soil properties, are also targeted during restoration. The natural community of our study area has been described as longleaf pine hydric flatwoods or wet savanna (Frost, 2001; Mitchell et al., 2015), which has relatively open forest structure and low stand density. Our study shows that planting approximately 1,050 trees/ha with no site preparation results in approximately 125 longleaf pine trees/ha at year 15. Although the trees were small (mean height = 1.2 m), the resulting stand may meet some objectives of savanna restoration if the trees continue to grow and survive to maturity. Although the stand development phase would be prolonged, slow tree growth could be considered beneficial because slower growth is correlated with increased longevity (Black et al., 2008). An alternative restoration scenario could be optimizing longleaf pine stand density and growth rates to minimize time required to satisfy red-cockaded woodpecker (*Leuconotopicus borealis*) habitat guidelines (Shaw and Long, 2007). In this scenario, site preparation may be useful to improve the establishment and long-term growth of planted seedlings on hydric sites.

Although longleaf pine would have naturally regenerated on hydric flatwoods sites under a regime of frequent fire (Frost, 2001; Peet, 2006), loss of overstory longleaf pine and legacies of infrequent

fire have disrupted ecosystem processes and created barriers to restoration success. Feedbacks among longleaf pine dominance, fuel characteristics, and frequent fire contribute to ecosystem function. The disruption of these feedbacks presents a potential ecological threshold for longleaf pine restoration because conditions suitable for regeneration success are limited within current, pre-restoration community states (Martin and Kirkman, 2009; Suding and Hobbs, 2009). Site preparation, as an example of intensive intervention during restoration, may be necessary to promote longleaf pine establishment and overcome the threshold between the current and desired community states. Once longleaf pine is successfully established, the desired community state may be maintained by re-establishing ecosystem function through less intense management (e.g., frequent prescribed fire) (Martin and Kirkman, 2009). Thus, site preparation treatments for longleaf pine restoration must (1) result in adequate longleaf pine regeneration to develop a future stand of desired structure and composition and (2) sustain a frequent fire regime that is continuous in both time and space (Mitchell et al., 2009) to maintain ecosystem function. An evaluation of the study treatments' impacts on the ecology of fire and fuels is beyond the scope of this study; however, our study does demonstrate that site preparation significantly improves establishment and growth of longleaf pine on hydric sites. We also found that survival of planted seedlings is still possible without site preparation, although this approach results in high mortality and slow growth.

Long-term responses to site preparation can be complicated by additional management action post-site preparation as well as indirect effects of site preparation on other management actions. For longleaf pine restoration, frequent fire is critical to maintaining and improving understory conditions through time. There are two likely explanations for increased shrub abundance in our study, both of which are related to post-site preparation fire management. First, the fire return interval on our study sites was too long. The first prescribed fire occurred 2 years after site preparation, but the next two burns occurred at 2-6-year intervals, depending upon the block. The pre-settlement fire return interval for wet pine savannas in these sites may have been 1-3 years (Frost, 2001), so these sites were likely not burned frequently enough between years 3 and 15. Furthermore, hydric sites naturally have a high shrub component, so the need for frequent fire during restoration may be even greater on these sites because a slight reduction in frequency could promote shrub encroachment (Glitzenstein et al., 2003). Second, the site preparation treatments likely affected fire behavior, resulting in indirect effects on the plant community. An analysis of fire behavior during the prescribed fire in 2006 indicated that bedding and mounding interfered with fire behavior (Walker et al., 2009). Bedding rows interrupted the spread of fire because they have minimal vegetation cover and the troughs between the rows often hold standing water. Mounding also produced patches of minimal vegetation with adjacent wet pits, but their discontinuous spatial arrangement allowed for more fire continuity. The long-term impact of these alterations to fire behavior may have been compounded by increased shrub abundance that made subsequent prescribed burns more difficult. These potential explanations underscore the importance of frequent fire in restoring and maintaining longleaf pine-grassland ecosystems.

Conclusions and Implications for Future Research/Implementation

Our results demonstrate the importance of evaluating plantation development over appropriate timescales when assessing the efficacy of site preparation for longleaf pine restoration. Interest in site preparation is conventionally focused on tree responses, with conceptualized response models of tree response fitting into one of three patterns: A) site preparation improves the site and results in increasing differences between treated and untreated sites through time; B) early beneficial effects of site preparation are maintained but differences between treated and untreated sites do not increase after the first few years; and C) early improvements/benefits are transient and untreated sites ‘catch up’ with treated sites through time (Morris and Lowery, 1988). For objectives related to longleaf pine restoration, benefits to tree establishment and growth must balance potential negative impacts on the ground layer plant community.

Our study included several specific hypotheses related to site preparation impacts and plantation development. Regarding planted longleaf pine trees, these included:

- *Hypothesis 1 (O1H1): Site preparation treatments improve total survival of planted trees at age 15.* This hypothesis was supported by our results. We found that the lowest survival was on the untreated control, although the survival was similarly low on the treatment using only chopping (CF).
- *Hypothesis 2 (O1H2): Site preparation results in earlier grass stage emergence, leading to a significant increase in the number of saplings at age 15.* Our results partially supported this hypothesis. We found that the treatment with the latest grass stage emergence was the chop-only treatment (CF). However, the untreated control treatment (F) resulted in average grass stage emergence at ages similar to that of the other site preparation treatments. Despite this, the F treatment resulted in the fewest established trees and the greatest number of seedlings remaining in the grass stage. These results suggest that successful longleaf pine trees in the F treatment had good growth early in order to emerge from the grass stage, but the number of trees that with this rate of growth on F was low (and mortality was high).
- *Hypothesis 3 (O1H3): Site preparation treatments affect mean tree size at year 15 but do not affect annual height growth rates after emergence from the grass stage.* This hypothesis was partially supported by our results. We did find differences in mean tree size at year 15, which we originally expected to be due to differences in the timing of emergence from the grass stage rather than growth rates. However, results indicated that site preparation treatments maintained differences in annual growth rates throughout much of the study period. These results support the Type A pattern of site preparation effect on tree growth from Morris and Lowery (1988).
- *Hypothesis 4 (O1H4): Site preparation treatments that impact planting site (e.g., bedding and mounding) have more persistent effects on tree responses than those that impact competing vegetation (e.g., chopping and herbicide).* This hypothesis was partially supported by our results. In general, the bedding and mounding treatments both increased the rate of plantation development (survival, growth) compared to the flat-planted treatment. On these hydric sites, our results indicate that the improvement to site conditions provided by increasing soil drainage at the root zone is important for tree establishment but also maintained through time. The herbicide treatment also had a persistent effect through time, resulting in more trees established and larger trees than chopping. The first three years

of this study (SI-1303) resulted in similar trends among treatments (Knapp et al., 2006). Consequently, the combination of herbicides with bedding or mounding treatments resulted in the greatest number of established longleaf pine trees and the greatest mean tree sizes. Because the planting density was higher in bedded sites than mounded sites, the basal area of the bedded sites was generally greater than that in the mounded sites.

The hypotheses related to the ground layer vegetation included:

- *Hypothesis 1 (O2H1): Early effects of herbicides on woody species richness and abundance diminish through time and are no longer significant 15 years following establishment, resulting in long-term reductions in herbaceous vegetation abundance.* This hypothesis was partially supported by our results. The herbicides used in this study were selected to target woody vegetation and were effective immediately following application. This hypothesis was correct in that woody vegetation recovered on herbicide treatments through 15 years after application. Results on herbaceous vegetation were more variable. Across all herbaceous species, herbicide treatments generally increased abundance during early years, with similar levels of herbaceous cover in year 15 as compared to year 3. However, cover of graminoids, and specifically bunchgrasses including wiregrass, were reduced by herbicides across the entire study period. As a consequence, the relative abundance of herbaceous plants (compared to woody plants) decreased on the treatments with herbicide through year 15.
- *Hypothesis 2 (O2H2): Greater tree size and density from improved plantation establishment correlate to locally reduced ground layer vegetation abundance but do not affect species richness at the stand-level.* This hypothesis was not supported. Although we found significantly greater light levels within the untreated control (F) and the chop only treatment (CF) than in the more intensive site preparation treatments, there was little evidence that the reductions in light availability affected the vegetation community. Because light availability exceeded 80% full sunlight across all treatments, it is likely that the plantations have not yet developed to a point of crown closure to reduce light levels. Given the effects of the site preparation treatments on the rate of stand development, however, it is likely that crown closure and subsequent light interception will vary greatly among the treatments in the future.
- *Hypothesis 3 (O2H3): Bedding treatments increase within-stand variation of ground layer abundance due to differences between treated (on-bed) and untreated (between-bed) locations.* Our results partially support this hypothesis. We found important differences in vegetation cover between the bedded and interbed areas. In particular, the bedded portions of the stands had greater cover of woody vegetation than the interbed areas and lower cover of graminoids, including bunchgrasses and specifically wiregrass, when compared to the interbed areas. Light levels were slightly greater in the interbed areas than in the bedded areas, likely due to the location of planted trees on the beds, but measures of diversity did not differ between bedded and interbed areas.

Taken together, our results provide several key findings:

- Longleaf pine tree establishment improves with increased intensity of site preparation treatments, as measured by the number of trees established, mean tree size, and stand basal area. Therefore, intensive site preparation treatments (including soil manipulation and herbicide) would maximize rate of stand development.
- Plantation establishment with no site preparation (other than burning, which was applied uniformly across all study units), resulted in the lowest rate of plantation establishment, although there were still around 100 trees per hectare on the untreated control units. Approximately 30 of these trees remained in the grass stage 15 years following planting, and it is likely that these trees will eventually die. However, the low density stand conditions associated with the untreated control site or the chop-only treatment may satisfy objectives related to longleaf pine savanna establishment through time.
- To improve longleaf pine tree establishment, competition control through herbicides and soil manipulation both contributed to successful outcomes. This is evident from the herbicide-only treatment (HF) resulting in smaller mean tree size than the HM, HB, or CHB treatments, and from the mounding or bedding treatments with chopping instead of herbicide resulting in lower basal areas at year 15.
- Early benefits of site preparation to planted longleaf pine tree growth are maintained through stand development.
- Both timing of emergence from the grass stage and rate of growth after emergence from the grass stage are important for long-term stand development.
- Broadcast herbicide had long-lasting impact on the ground layer vegetation: 1) initial reductions in woody vegetation in the short-term following herbicide were lost 15 years after treatment; 2) reductions in wiregrass persisted 15 years after treatment; 3) shifts in community composition persisted 15 years after treatment.
- Species richness of the ground layer did not differ among treatments 15 years after treatment, but diversity and evenness were highest on the chop-only treatment (CF). The untreated control (F) had among the lowest species richness, diversity, and evenness, indicating that site preparation did not negatively affect these characteristics.
- The mechanical site preparation treatments (mounding and bedding) both reduced abundance of wiregrass compared to flat planting.
- Bedding resulted in local variation in the ground layer vegetation, with greater wiregrass in the untreated interbed areas than on the beds.

Future research needs: The role of fire in maintaining and promoting these sites cannot be understated. These sites have been burned repeatedly since the study began, with intervals ranging from 3 to 5 years. In recent years, the fire interval has been closer to 5 years, which has likely affected the development of abundant woody vegetation. It is not clear how the need for and effects of site preparation would interact with burning at different frequencies or intensities. For example, the early reductions in woody plant cover may have been maintained with more aggressive burning. It is also unclear how the site preparation treatments affect fire behavior. Finally, these sites had histories of previous pine plantations and do not represent reference longleaf pine communities. We anticipate that effects of site preparation on ground layer vegetation would differ with different starting conditions.

Response to IPR Action Items

Following the April 2020 In Progress Review, the following Action Item was assigned:

“In your final report, detail the history and background of the site and address the efficacy of the treatment in relation to the cost”

The study locations were selected to represent common conditions for longleaf pine restoration on hydric soils of Marine Corps Base Camp Lejeune and other similar sites within the Atlantic Coastal Plain. As common with many areas in the region, these sites included a history of pine production by the timber industry. The study plots on the western portion of MCBCL, in the Greater Sandy Run area (Blocks 1, 2, 5, and 7), had been under ownership of International Paper Company prior to MCBCL acquisition in 1992. These areas had been planted in either loblolly pine or slash pine for timber production and remained as plantations of these species prior to study initiation in 2003. According to Frost (2001), many of these areas experienced fire suppression through the late 1990s, after which MCBCL began using fire on a frequent (1-3 year) return interval. The study site east of New River (Block 3) was a bit different, remaining as a natural mixed pine stand prior to the study initiation in 2003 (personal communication with MCBCL forestry staff). This stand was regenerated in the 1930s. Aerial imagery from 1938 indicated that this area had not been cleared for agriculture at that time.

The fire histories varied slightly among the blocks. All study areas were burned prior to the planting longleaf pine seedlings for the study, with records from MCBCL indicated that fires occurred between July and September 2003. All study blocks were burned again in March 2006, which occurred prior to the Year 3 data collection. It is likely that effects of this burn are observed in the 2006 (Year 3) vegetation data. In particular, there was a general decrease of woody plant cover in 2006, representing the recovery of woody plants to the fire treatment. Two of the blocks were burned in 2009, with Block 3 burned in January 2009 and Block 5 burned in July 2009. These two areas are more accessible than Blocks 1, 2, and 7 due to range activities, and it is likely that burns in Blocks 1, 2, and 7 were not feasible in 2009 due to scheduling constraints. The next prescribed burns occurred in January-February 2012 for Blocks 1, 2, 3, and 7. Block 5 had been burned by a wildfire in March 2011. The final burns were conducted in December 2016-February 2017 in Blocks 3, 5, and 7 or December 2017 in Blocks 1 and 2.

The variation in the burn schedule among blocks is likely to have some impact on responses, particularly related to the ground layer vegetation, but these impacts are not likely to obfuscate site preparation treatment effects. Recommended burn frequencies for these sites are 1-3 year intervals (Frost, 2001). Among the study blocks, Block 3 was closest to this burn frequency, with prescribed fires in 2003, 2006, 2009, 2012, and 2016. Each other block had at least one period of longer fire frequency, with Blocks 1, 2, and 7 each having 6-year return intervals between 2006 and 2012 and then 5-year return intervals between 2012 and 2017. The longer fire return intervals likely provided opportunity for greater development of woody vegetation in place of herbaceous vegetation. Despite this potential source of variation, our experimental design includes a block factor to account for variation at the block level, and any variation associated with variable fire histories did not overcome the observed treatment effects. However, our study is not able to determine if outcomes would differ with greater fire frequency applied across all study areas.

Costs for the site preparation treatments were compiled from communication with the forestry staff at Marine Corps Base Camp Lejeune and from available literature (Maggard and Barlow, 2018). For longleaf pine plantation establishment, tree planting, seedlings, and prescribed burning are required costs and were not tested as treatments in our study. Tree planting/seedling costs are for estimates of 1285 trees per hectare (520 trees per acre). The study sites were planted at slightly lower densities of 930 trees per hectare (around 380 trees per acre). The low survival rates in CF and F treatments (< 25% survival) demonstrate low cost efficiency as well but would require such planting densities to result in tree establishment.

Table 6. Summary of estimated costs for site preparation treatments used in the study.

Action	Cost per acre	
	MCBCL foresters	Maggard and Barlow (2018)
Tree planting	\$135	\$72
Seedlings	\$125	\$66
Prescribed burning	NA	\$32
Broadcast herbicide	\$150-225	\$84
Mechanical chopping (one pass)	\$100-150	\$130
Mechanical bedding (one pass)	\$185	NA

Herbicide application is typically lower cost than mechanical treatments, although this depends on the type of chemical and the type of application. In this study, the broadcast herbicide was applied with a tractor, thus requiring costs associated with fuel in addition to the chemical itself. The treatment also included a mixture of two chemicals. These factors contribute to the higher cost of the treatment as reported by MCBCL forestry compared to that in Maggard and Barlow (2018). In our study, the combination of the broadcast herbicide and the mechanical chopping treatments (which were also combined with bedding) represented the most intensive treatment. This treatment generally resulted in the largest longleaf pine trees and the greatest stand basal area. However, there was essentially no additional benefit of combining the chopping and herbicide treatments compared to the treatment of herbicide and bedding. The added financial cost of combining these two treatments is not realized in any ecological or biological effect.

The combination of bedding with either chopping or herbicide showed additive benefit to longleaf pine tree establishment relative to either chopping or herbicide alone. Given that the costs of chopping and herbicide treatments, as provided by MCBCL, were similar, the combination of bedding with one of these treatments offers benefit for tree establishment with similar cost. Decisions on which treatment to use may be dictated by the management objective: with greater interest in increasing tree survival or growth, bedding and herbicide would be the superior choice; with greater interest in reducing impact to the ground layer plant community (specifically reducing negative impact on wiregrass and reducing woody vegetation dominance), bedding and chopping would be the superior choice.

Literature Cited

- Addington, R.N., Greene, T.A., Elmore, M.L., Prior, C.E., Harrison, W.C. 2012. Influence of herbicide site preparation on longleaf pine ecosystem development and fire management. *Southern Journal of Applied Forestry* 36: 173–180.
- Addington, R.N., Knapp, B.O., Sorrell, G.G., Elmore, M.L., Wang, G.G., and Walker, J.L. 2015. Factors affecting broadleaf woody vegetation in upland pine forests managed for longleaf pine restoration. *Forest Ecology and Management* 354: 130-138.
- Aschenbach, T.A., Foster, B.L., and Imm, D.W. 2010. The initial phase of a longleaf pine-wiregrass savanna restoration: species establishment and community response. *Restoration Ecology* 18: 762-771.
- Barnhill, W.L. 1992. Soil Survey of Onslow County, North Carolina. U.S. Department of Agriculture, Soil Conservation Service, Washington, DC.
- Bates, D., Maechler, M., Bolker, B., Walker, S. 2015. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* 67: 1–48.
- Battaglia, M.A., Mitchell, R.J., Mou, P.P., Pecot, S.D. 2003. Light transmittance estimates in a longleaf pine woodland. *Forest Science* 49: 752–762.
- Black, B.A., Colbert, J.J., Pederson, N. 2008. Relationships between radial growth rates and lifespan within North American tree species. *Ecoscience* 15: 349–357.
- Boyer, W.D. 1983. Variations in height-over-age curves for young longleaf pine plantations. *Forest Science* 29: 15-27.
- Boyer, W.D. 1988. Effects of site preparation and release on the survival and growth of planted bare-root and container-grown longleaf pine (Georgia Forest Research Paper No. 76). Research Division, Georgia Forestry Commission.
- Boyer, W.D. 1990. *Pinus palustris* Mill. longleaf pine, in: Burns, R.M., Honkala, B.H. (Eds.), *Silvics of North America*, Vol. 1, Conifers. USDA Handbook 654. pp. 405–412.
- Brockway, D.G. and Lewis, C.E. 1997. Long-term effects of dormant-season prescribed fire on plant community diversity, structure and productivity in a longleaf pine wiregrass ecosystem. *Forest Ecology and Management* 96: 167-183.
- Brockway, D.G., Outcalt, K.W., and Wilkins, R.N. 1998. Restoring longleaf pine wiregrass ecosystems: plant cover, diversity and biomass following low-rate hexazinone application on Florida sandhills. *Forest Ecology and Management* 103: 159-175.

Brockway, D.G., Outcalt, K.W., Tomczack, D.J., and Johnson, E.E. 2005. Restoration of longleaf pine ecosystems. Gen. Tech. Rep. SRS-83. USDA Forest Service, Southern Research Station. Asheville, NC. 34 p.

Brockway, D.G., Outcalt, K.W., Boyer, W.D. 2006. Longleaf pine regeneration ecology and methods, in: Jose, S., Jokela, E.J., Miller, D.L. (Eds.), *The Longleaf Pine Ecosystem: Ecology, Silviculture, and Restoration*. Springer, New York, pp. 95–133.

Brockway, D.G., Outcalt, K.W., Estes, B.L., and Rummer, R.B. 2009. Vegetation response to midstory mulching and prescribed burning for wildfire hazard reduction and longleaf pine (*Pinus palustris* Mill.) ecosystem restoration. *Forestry*. 82, 299-314.

Brooks, J.R. and Jack, S.B. 2006. A whole stand growth and yield system for young longleaf pine plantations in southwest Georgia. In: Connor K.F. (ed.), *Proceedings of the 13th Biennial Southern Silvicultural Research Conference*. Gen. Tech. Rep. SRS-92. USDA Forest Service, Southern Research Station, Asheville, NC. 317-318.

Brooks, J.R. and Jack, S.B. 2014. An updated whole stand growth and yield system for young longleaf pine plantations in southwest Georgia. In: Schweitzer, C.J., Clatterbuck, W.K., and Oswalt, C.M. (eds.), *Proceedings of the 18th Biennial Southern Silvicultural Research Conference*. Gen. Tech. Rep. SRS-212. USDA Forest Service, Southern Research Station, Asheville, NC. 258-263.

Canham, C.D. 1988. An index for understory light levels in and around canopy gaps. *Ecology* 69, 1634–1638.

Carmean, W.H. 1972. Site index curves for upland oaks in the Central States. *Forest Science* 18: 109–120.

Carter, M.C. and Foster, C.D. 2004. Prescribed burning and productivity in southern pine forests: a review. *Forest Ecology and Management* 191: 93-109.

Chapman, H.H. 1932. Is the longleaf type a climax? *Ecology* 13: 328-334.

Clewell, A.F. 1989. Natural history of wiregrass (*Aristida stricta* Michx., Gramineae). *Natural Areas Journal* 9: 223-233.

Conde, L.F., Swindel, B.F., and Smith, J.E. 1983a. Plant species cover, frequency, and biomass: early responses to clearcutting, chopping, and bedding in *Pinus elliottii* flatwoods. *Forest Ecology and Management* 6: 307-317.

Conde, L.F., Swindel, B.F., and Smith, J.E. 1983b. Plant species cover, frequency, and biomass: early responses to clearcutting, burning, windrowing, discing, and bedding in *Pinus elliottii* flatwoods. *Forest Ecology and Management* 6: 319-331.

De Rosario-Martinez, H. 2015. phia: Post-Hoc Interaction Analysis.

- Dean, T.J. and Baldwin, V.C. Jr. 1993. Using a density-management diagram to develop thinning schedules for loblolly pine plantations. Res. Pap. SO-275. USDA Forest Service, Southern Forest Experiment Station. New Orleans, LA. 7 p.
- Drewa, P.B., Platt, W.J., and E.B. Moser. 2002. Fire effects on resprouting of shrubs in headwaters of southeastern longleaf pine savannas. *Ecology* 83: 755-767.
- Dyer, M.E., Bailey, R.L. 1987. A test of six methods for estimating true heights from stem analysis data. *Forest Science* 33, 3–13.
- Farrar, R.M., Jr. 1985. Volume and growth predictions for thinned even-aged natural longleaf pine stands in the east gulf area. Res. Pap. SO-220. USDA Forest Service, Southern Forest Experiment Station. New Orleans, LA. 12 p.
- Fill, J.M., Glitzenstein, J.S., Streng, D.R., Stowe, J. and Mousseau, T.A. 2017. Wiregrass (*Aristida beyrichiana*) may limit woody plant encroachment in longleaf pine (*Pinus palustris*) ecosystems. *American Midland Naturalist* 177: 153-161.
- Fort Benning, 2014. Integrated Natural Resources Management Plan. U.S. Department of the Army. 1033 p.
- Fort Bragg, 2011. Integrated Natural Resources Management Plan. U.S. Department of the Army. 370 p.
- Fox, T.R., Jokela, E.J., and Allen, H.L. 2007. The development of pine plantation silviculture in the southern United States. *Journal of Forestry* 105: 337-347.
- Freeman, J.E. and Jose, S. 2009. The role of herbicide in savanna restoration: Effects of shrub reduction treatments on the understory and overstory of a longleaf pine flatwoods. *Forest Ecology and Management* 257: 978-986.
- Frost, C. 2001. Presettlement Vegetation and Natural Fire Regimes of Camp Lejeune. North Carolina Department of Agriculture, Plant Conservation Program, Raleigh, NC.
- Frost, C. 2006. History and future of the longleaf pine ecosystem. In: Jose, S., Jokela, E.J., Miller, D.L. (eds.), *The longleaf pine ecosystem: ecology, silviculture, and restoration*. Springer, New York, NY, pp. 9-42.
- Glitzenstein, J.S., Streng, D.R., Wade, D.D., and Brubaker, J. 2001. Starting new populations of longleaf pine ground-layer plants in the outer coastal plain of South Carolina, USA. *Natural Areas Journal* 21, 89-110.
- Glitzenstein, J.S., Streng, D.R., and Wade, D.D. 2003. Fire frequency effects on longleaf pine (*Pinus palustris* P. Miller) vegetation in South Carolina and northeast Florida, USA. *Natural Areas Journal* 23: 22-37.

Graves, S., Piepho, H.-P., Selzer, L. 2015. multcompView: Visualizations of Paired Comparisons.

Gonzalez-Benecke, C.A., Gezan, S.A., Martin, T.A., Cropper, W.P. Jr., Samuelson, L.J., and Leduc, D.J. 2014. Individual tree diameter, height, and volume functions for longleaf pine. *Forest Science* 60: 43-56.

Hanula, J.L. and Engstrom, T. 2000. Comparison of red-cockaded woodpecker (*Picoides borealis*) nestling diet in old-growth and old-field longleaf pine (*Pinus palustris*) habitats. *The American Midland Naturalist* 144: 370-376.

Harrington, T.B. 2006. Plant competition, facilitation, and other overstory-understory interactions in longleaf pine ecosystems. in: Jose, S., Jokela, E.J., Miller, D.L. (Eds.), *The Longleaf Pine Ecosystem: Ecology, Silviculture, and Restoration*. Springer, New York, pp. 135–156.

Harrington, T.B. 2011. Overstory and understory relationships in longleaf pine plantations 14 years after thinning and woody control. *Canadian Journal of Forest Research* 41: 2301-2314.

Harrington, T.B. and Edwards, M.B. 1999. Understory vegetation, resource availability, and litterfall responses to pine thinning and woody vegetation control in longleaf pine plantations. *Canadian Journal of Forest Research* 29: 1055-1064.

Haywood, J.D. 1987. Response of slash pine planted on mounds in central and southwestern Louisiana. *New Forests* 1, 291–300.

Haywood, J.D. 2005. Effects of herbaceous and woody plant control on *Pinus palustris* growth and foliar nutrients through six growing seasons. *Forest Ecology and Management* 214: 384-397.

Haywood, J.D., Harris, F.L., Grelen, H.E., and Pearson, H.A. 2001. Vegetative response to 37 years of seasonal burning on a Louisiana longleaf pine site. *Southern Journal of Applied Forestry* 25: 122-130.

Hedman, C.W., Grace, S.L., and King, S.E. 2000. Vegetation composition and structure of southern coastal plain pine forests: an ecological comparison. *Forest Ecology and Management* 134: 233-247.

Hervé, M. 2020. RVAideMemoire: Testing and Plotting Procedures for Biostatistics. R package version 0.9-78.

Heyward, F. 1933. The root system of longleaf pine on the deep sands of western Florida. *Ecology* 14: 136–148.

Hu, H., Wang, G.G., Walker, J.L., and Knapp, B.O. 2012. Silvicultural treatments for converting loblolly pine to longleaf pine dominance: effects on planted longleaf pine seedlings. *Forest Ecology and Management* 276: 209-216.

James, F.C., Hess, C.A., Kicklighter, B.C., and Thum, R.A. 2001. Ecosystem management and the nice gestalt of the red-cockaded woodpecker in longleaf pine forests. *Ecological Applications* 11: 854-870.

Johnson, R. and Gjerstad, D. 2006. Restoring the overstory of longleaf pine ecosystems. In: Jose, S., Jokela, E.J., Miller, D.L. (eds.), *The longleaf pine ecosystem: ecology, silviculture, and restoration*. Springer, New York, NY, pp. 271-295.

Jose, S., Ranasinghe, S., and Ramsey, C.L. 2010. Longleaf pine (*Pinus palustris* P. Mill.) restoration using herbicides: overstory and understory vegetation responses on a Coastal Plain flatwoods site in Florida, U.S.A. *Restoration Ecology* 18: 244-251.

Jost, L. 2006. Entropy and diversity. *Oikos* 113: 363–375.

Kaesler, M.J. and Kirkman, L.K. 2010. The effects of pre- and post-emergent herbicides on non-target native plant species of the longleaf pine ecosystem. *Journal of the Torrey Botanical Society* 137: 420-430.

Kassambara, A., and Kosinski, M. 2018. survminer: Drawing Survival Curves using ggplot2.

Kirkman, L.K., Mitchell, R.J., Kaesler, M.J., Pecot, S.D., and Coffey, K.L. 2007. The perpetual forest: using undesirable species to bridge restoration. *Journal of Applied Ecology* 44: 604-614.

Knapp, B.O., Wang, G.G., Walker, J.L., Cohen, S. 2006. Effects of site preparation treatments on early growth and survival of planted longleaf pine (*Pinus palustris* Mill.) seedlings in North Carolina. *Forest Ecology and Management* 226, 122–128.

Knapp, B.O., Wang, G.G., Walker, J.L. 2008. Relating the survival and growth of planted longleaf pine seedlings to microsite conditions altered by site preparation treatments. *Forest Ecology and Management* 255, 3768–3777.

Knapp, B.O. and Walker, J.L. 2013. Using existing growth models to predict RCW habitat development following site preparation: pitfalls of the process and potential growth response. In: Guldin, J.M. (ed.), *Proceedings of the 15th Biennial Southern Silvicultural Research Conference*. Gen. Tech. Rep. SRS-175. USDA Forest Service, Southern Research Station, Asheville, NC. 499-506.

Knapp, B.O., Wang, G.G., and Walker, J.L. 2013. Effects of canopy structure and cultural treatments on the survival and growth of *Pinus palustris* Mill. seedlings underplanted in *Pinus taeda* L. stands. *Ecological Engineering* 57: 46-56.

Lavoie, M., Kobziar, L., Long, A., and Hains, M. 2011. Problems and needs for restorationists of longleaf pine ecosystems: a survey. *Natural Areas Journal* 31: 294-299.

Lenth, R., 2019. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lewis, C.E., Swindel, B.F., and Tanner, G.W. 1988. Species diversity and diversity profiles: concept, measurement, and application to timber and range management. *Journal of Range Management* 41: 466-469.

Litt, A.R., Herring, B.J., and Provencher, L. 2001. Herbicide effects on ground-layer vegetation in southern pinelands, USA: a review. *Natural Areas Journal* 21: 177-188.

Lohrey, R.E. and Bailey R.L. 1977. Yield tables and stand structure for unthinned longleaf pine plantations in Louisiana and Texas. Res. Pap. SO-133. USDA Forest Service, Southern Forest Experiment Station. New Orleans, LA. 53 p.

Londo, A.J., Mroz, G.D. 2001. Bucket mounding as a mechanical site preparation technique in wetlands. *Northern Journal of Applied Forestry* 18: 7–13.

Loveless, R.W., Pait III, J.A., McElwain, T. 1989. Response of longleaf pine to varying intensity of silvicultural treatments, in: Miller, J.H. (Ed.), *Proceedings of the Fifth Biennial Southern Silvicultural Research Conference*. Gen. Tech. Rep. SO-74. U.S. Department of Agriculture, Forest Service, Southern Research Station, New Orleans, LA, pp. 159–164.

Lowery, R.F., Gjerstad, D.H. 1991. Chemical and mechanical site preparation, in: Duryea, M.L., Dougherty, P.M. (Eds.), *Forest Regeneration Manual*, Forestry Sciences. Springer, Dordrecht, pp. 251–261.

Maggard, A., Barlow, R. 2018. Costs and trends of southern forestry practices. FOR-2073. Alabama Cooperative Extension Service.

Marine Corps Base Camp Lejeune. 2015. 2015-2020 Integrated Natural Resources Management Plan. U.S. Department of the Navy. 844 p.

Martin, K.L. and Kirkman, L.K. 2009. Management of ecological thresholds to re-establish disturbance-maintained herbaceous wetlands of the south-eastern USA. *Journal of Applied Ecology* 46: 906-914.

Miller, J.H. 1980. Competition after windrowing or single-roller chopping for site preparation in the southern piedmont. *Proceedings of the Southern Weed Science Society* 33: 139–245.

Miyata, E.S., Steinbilb, H.M., Sajdak, R.L., and Coffman, M. 1982. Roller chopping for site preparation in Wisconsin: a case study. Res. Pap. NC-223. USDA Forest Service, North Central Forest Experiment Station. St. Paul, MN 8 p.

- McKee, W.H. and Wilhite, L.P. 1986. Loblolly pine response to bedding and fertilization varies by drainage class on lower Atlantic Coastal Plain sites. *Southern Journal of Applied Forestry* 10: 16-21.
- McNab, W.H., Cleland, D.T., Freeouf, J.A., Keys, Jr., J.E., Nowacki, G.J., Carpenter, C.A. 2007. Description of ecological subregions: sections of the conterminous United States (General Technical Report No. WO-76B). United States Department of Agriculture Forest Service, Washington, DC.
- Michael, J.L. 1980. Long-term impact of aerial application of 2,4,5-T to longleaf pine (*Pinus palustris*). *Weed Science* 28, 255–257.
- Mitchell, R.J., Hiers, J.K., O'Brien, J., and Starr, G. 2009. Ecological forestry in the southeast: understanding the ecology of fuels. *Journal of Forestry* 107: 391-397.
- Mitchell, S., Palmquist, K., Cohen, S. and Christensen N.L. 2015. Patterns of vegetation composition and diversity in pine-dominated ecosystems of the Outer Coastal Plain of North Carolina: Implications for ecosystem restoration. *Forest Ecology and Management* 356: 64–73.
- Moore, W.H., Swindel, B.F., and Terry, W.S. 1982. Vegetation response to clearcutting and chopping in a north Florida flatwood forest. *Journal of Range Management* 35: 214-218.
- Morris, L.A. and Lowery, R.F. 1988. Influence of site preparation on soil conditions affecting stand establishment and tree growth. *Southern Journal of Applied Forestry* 12: 170-178.
- Nilsson, U. and Allen, H.L. 2003. Short- and long-term effects of site preparation, fertilization, and vegetation control on growth and stand development of planted loblolly pine. *Forest Ecology and Management* 175: 367-377.
- NRCS, 2014. Official Soil Description-Leon Series. Natural Resources Conservation Service, USDA.
- O'Brien, J.J., Hiers, J.K., Callaham, M.A. Jr., Mitchell, R.J. and Jack, S.B. 2008. Interactions among overstory structure, seedling life-history traits, and fire in frequently burned neotropical pine forests. *Ambio* 37, 542-547.
- Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre P., McGlinn D., et al. 2020. *vegan: Community Ecology Package*. R Package version 2.5-7.
- Oswalt, C.M., Cooper, J.A., Brockway, D.G., Brooks, H.W., Walker, J.L., Connor, K.F., Oswalt, S.N., and Conner, R.C. 2012. History and current condition of longleaf pine in the southern United States. Gen. Tech. Rep. SRS-166. USDA Forest Service, Southern Research Station. Asheville, NC. 51 p.
- Outcalt, K.W. 1984. Influence of bedding height on the growth of slash and loblolly pine on a Leon fine sand in northeast Florida. *Southern Journal of Applied Forestry* 8: 29-31.

Outcalt, K.W. and Sheffield, R.M. 1996. The longleaf pine forest: trends and current conditions. Res. Bull. SRS-9. USDA Forest Service, Southern Research Station. Asheville, NC, 23 p.

Outcalt, K.W., Williams, M.E., and Onokpise, O. 1999. Restoring *Aristida stricta* to *Pinus palustris* ecosystems on the Atlantic Coastal Plain, U.S.A. Restoration Ecology 7: 262-270.

Peet, R.K. 2006. Ecological classification of longleaf pine woodlands, in: Jose, S., Jokela, E.J., Miller, D.L. (Eds.), The Longleaf Pine Ecosystem: Ecology, Silviculture, and Restoration. Springer, New York, pp. 51–93.

Peet, R.K., Lee, M., Boyle, F. Wentworth, T., Schafale, M., and Weakly, A. 2012. Vegetation-plot database of the Carolina Vegetation Survey. Biodiversity and Ecology 4: 243-253.

Pessin, L.J. 1944. Stimulating early height growth of longleaf pine seedlings. Journal of Forestry 42: 95-98.

Pinherio, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models.

Platt, W.J., Entrup, A.K., Babl, E.K., Coryell-Turpin C., Dao, V., Hebert, J.A., LaBarbera, C.D., Noto, J.F.L., Ogundare, S.O. Stamper, L.K., and Timilsina, N. 2015. Short-term effects of herbicides and a prescribed fire on restoration of shrub-encroached pine savanna. Restoration Ecology 23: 909–917.

Pritchett, W.L. 1979. Site preparation and fertilization of slash pine on a wet savanna soil. Southern Journal of Applied Forestry 3: 86-90.

Ramsey, C.L., Jose, S., Brecke, B.J., and Merritt, S. 2003. Growth response of longleaf pine (*Pinus palustris* Mill.) seedlings to fertilization and herbaceous weed control in an old field in southern USA. Forest Ecology and Management 172: 281-289.

Schultz, R.P. and Wilhite, L.P. 1974. Changes in a flatwoods site following intensive preparation. Forest Service 20: 230-237.

Shaw, J.D. and Long, J.N. 2007. A density management diagram for longleaf pine stands with application to red-cockaded woodpecker habitat. Southern Journal of Applied Forestry 31: 28-38.

Sorrie, B.A. and Weakley, A.S. 1999. Coastal plain vascular plant endemics: phytogeographic patterns. Castanea 66: 50-82.

Suding, K.N., Hobbs, R.J. 2009. Threshold models in restoration and conservation: a developing framework. Trends in Ecology and Evolution 24: 271–279.

Sutton, R.F. 1993. Mounding site preparation: a review of European and North American experience. New Forests 7: 151-192.

Swindel, B.F., Conde, L.F., and Smith, J.E. 1983. Plant cover and biomass response to clear-cutting, site preparation, and planting in *Pinus elliottii* flatwoods. *Science*. 219, 1421-1422.

USFWS. 2013. Recovery plan for the red-cockaded woodpecker (*Picoides borealis*). U.S. Fish and Wildlife Service, Atlanta, Georgia, USA. 296 p.

Van Lear, D.H., Carroll, W.D., Kapeluck, P.R., Johnson, R. 2005. History and restoration of the longleaf pine-grassland ecosystem: Implications for species at risk. *Forest Ecology and Management* 211, 150-165.

Walker, J. 1993. Rare vascular plant taxa associated with the longleaf pine ecosystems: patterns in taxonomy and ecology. In: Hermann, S.M. (ed.), *Proceedings of the Tall Timbers fire ecology conference*, No. 18. The longleaf pine ecosystem: ecology, restoration, and management. Tall Timbers Research Station, Tallahassee, FL, pp. 105-127.

Walker, J. and Peet, R.K. 1984. Composition and species diversity of pine-wiregrass savannas of the Green Swamp, North Carolina. *Vegatatio* 55, 163-179.

Walker, J.L. and Silletti, A.M. 2006. Restoring the ground layer of longleaf pine ecosystems. in: Jose, S., Jokela, E.J., Miller, D.L. (Eds.), *The Longleaf Pine Ecosystem: Ecology, Silviculture, and Restoration*. Springer, New York, pp. 297–325.

Walker, J.L., Cohen, S. and Knapp, B.O. 2009 Regenerating Longleaf Pine on Hydric Soils: Short- and Long-Term Effects on Native Ground-Layer Vegetation. Strategic Environmental Research and Development Program Project No. SI-1303.

Wang, G.G., Pile, L.S., Knapp, B.O., Hu, H. 2016. Longleaf pine adaptation to fire: Is early height growth pattern critical to fire survival?, in: Schweitzer, C.J., Clatterbuck, W.K., Oswalt, C.M. (Eds.), *Proceedings of the 18th Biennial Southern Silvicultural Research Conference*. Gen. Tech. Rep. SRS-212. U.S. Department of Agriculture, Forest Service, Southern Research Station, Asheville, NC, pp. 214–218.

Welles, J.M., Cohen, S. 1996. Canopy structure measurement by gap fraction analysis using commercial instrumentation. *Journal of Experimental Botany* 47: 1335–1342.

Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag New York.

Wickham, H., Francois, R., Henry, L., Muller, K. 2019. *dplyr: A Grammar of Data Manipulation*.

Zhao, D., Kane, M., Borders, B., and Harrison, M. 2009. Long-term effects of site preparation treatments, complete competition control, and repeated fertilization on growth of slash pine plantations in the flatwoods of the southeastern United States. *Forest Science* 55: 403-410.

Zutter, B.R. and Miller, J.H. 1998. Eleventh-year response of loblolly pine and competing vegetation to woody and herbaceous plant control on a Georgia flatwoods site. *Southern Journal of Applied Forestry* 22: 88-95.

Appendices

Appendix 1. Complete species list of ground flora, including functional group designations and period of species identification.

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Anacardiaceae	Rhus	copallinum	woody	shrub	tallshrub	both
Anacardiaceae	Toxicodendron	radicans	woody	vine		year 15
Apiaceae	Centella	asiatica	herb	forb	perforb	year 15
Aquifoliaceae	Ilex	coriacea	woody	shrub	tallshrub	both
Aquifoliaceae	Ilex	glabra	woody	shrub	tallshrub	both
Aquifoliaceae	Ilex	myrtifolia	woody	shrub	tallshrub	both
Aquifoliaceae	Ilex	opaca	woody	tree		both
Asclepiadaceae	Asclepias	pedicellata	herb	forb	perforb	year 15
Asteraceae	Acer	rubrum	woody	tree		both
Asteraceae	Ambrosia	artemisiifolia	herb	forb	anforb	years 1-3
Asteraceae	Aster	spp.	herb	forb	perforb	years 1-3
Asteraceae	Baccharis	halimifolia	woody	shrub	tallshrub	both
Asteraceae	Carphephorus	tomentosus	herb	forb	perforb	both
Asteraceae	Coreopsis	falcata	herb	forb	perforb	year 15
Asteraceae	Coreopsis	linifolia	herb	forb	perforb	year 15
Asteraceae	Erechtites	hieraciifolius	herb	forb	anforb	year 15
Asteraceae	Erigeron	vernus	herb	forb	anforb	years 1-3
Asteraceae	Eupatorium	capilliofolium	herb	forb	perforb	years 1-3
Asteraceae	Eupatorium	leucolepis	herb	forb	perforb	both
Asteraceae	Eupatorium	mohrii	herb	forb	perforb	both
Asteraceae	Eupatorium	pilosum	herb	forb	perforb	both
Asteraceae	Eupatorium	rotundifolium	herb	forb	perforb	both
Asteraceae	Eurybia	paludosa	herb	forb	perforb	both
Asteraceae	Euthamia	caroliniana	herb	forb	perforb	both
Asteraceae	Euthamia	galetorum	herb	forb	perforb	years 1-3

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Asteraceae	Hieracium	gronovii	herb	forb	perforb	year 15
Asteraceae	Ionactis	linariifolia	herb	forb	perforb	both
Asteraceae	Liatris	pilosa	herb	forb	perforb	both
Asteraceae	Liatris	spicata var. resinosa	herb	forb	perforb	year 15
Asteraceae	Marshallia	graminifolia	herb	forb	perforb	year 15
Asteraceae	Pityopsis	graminifolia	herb	forb	perforb	both
Asteraceae	Pterocaulon	pycnostachyum	herb	forb	perforb	year 15
Asteraceae	Sericocarpus	tortifolius	herb	forb	perforb	year 15
Asteraceae	Solidago	fistulosa	herb	forb	perforb	both
Asteraceae	Solidago	spp.	herb	forb	perforb	years 1-3
Asteraceae	Solidago	verna	herb	forb	perforb	both
Asteraceae	Solidago	odora	herb	forb	perforb	both
Asteraceae	Solidago	pulchra	herb	forb	perforb	both
Asteraceae	Symphyotrichum	dumosum	herb	forb	perforb	year 15
Asteraceae	Symphyotrichum	walteri	herb	forb	perforb	year 15
Asteraceae	Taraxacum	spp.	herb	forb	perforb	years 1-3
Asteraceae	Trilisa	odoratissima	herb	forb	perforb	both
Asteraceae	Trilisa	paniculata	herb	forb	perforb	both
Blechnaceae	Anchistea	virginica	herb	fern		both
Blechnaceae	Lorinseria	areolata	herb	fern		both
Campanulaceae	Lobelia	nuttallii	herb	forb	perforb	both
Caprifoliaceae	Viburnum	nudum	woody	shrub	tallshrub	year 15
Cistaceae	Lechea	pulchella var. ramosissima	herb	forb	perforb	year 15
Clethraceae	Clethra	alnifolia	woody	shrub	tallshrub	both
Clusiaceae	Hypericum	cistifolium	woody	shrub	shortshrub	both
Clusiaceae	Hypericum	crux-andreae	woody	shrub	shortshrub	both
Clusiaceae	Hypericum	hypericoides	woody	shrub	shortshrub	both
Clusiaceae	Hypericum	tenuifolium	woody	shrub	shortshrub	both

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Cornaceae	Nyssa	biflora	woody	tree		year 15
Cuscutaceae	Cuscuta	compacta	herb	forb	perforb	both
Cyperaceae	Carex	striata	herb	graminoid	sedgerush	year
Cyperaceae	Fimbristylis	puberula	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	cephalantha	herb	graminoid	sedgerush	year 15
Cyperaceae	Rhynchospora	chalarocephala	herb	graminoid	sedgerush	year 15
Cyperaceae	Rhynchospora	chapmanii	herb	graminoid	sedgerush	year 15
Cyperaceae	Rhynchospora	ciliaris	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	fascicularis	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	galeana	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	inexpansa	herb	graminoid	sedgerush	year 15
Cyperaceae	Rhynchospora	pallida	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	plumosa	herb	graminoid	sedgerush	both
Cyperaceae	Rhynchospora	rariflora	herb	graminoid	sedgerush	year 15
Cyperaceae	Rhynchospora	spp.	herb	graminoid	sedgerush	years 1-3
Cyperaceae	Scleria	ciliata var. glabra	herb	graminoid	sedgerush	year 15
Cyperaceae	Scleria	minor	herb	graminoid	sedgerush	year 15
Cyperaceae	Scleria	muehlenbergii	herb	graminoid	sedgerush	year 15
Cyperaceae	Scleria	pauciflora var. caroliniana	herb	graminoid	sedgerush	year 15
Cyrillaceae	Cyrilla	racemiflora	woody	shrub	tallshrub	both
Dennstaedtiaceae	Pteridium	latiusculum	herb	fern		both
Diaspensiaceae	Pyxidanthera	barbulata	herb	shrub	shortshrub	year 15
Droseraceae	Dionaea	muscipula	herb	forb	perforb	both
Droseraceae	Drosera	brevifolia	herb	forb	perforb	years 1-3
Droseraceae	Drosera	capillaris	herb	forb	perforb	both
Droseraceae	Drosera	intermedia	herb	forb	perforb	both
Ebenaceae	Diospyros	virginiana	woody	tree		both
Ericaceae	Chamaedaphne	calyculata	woody	shrub	shortshrub	both

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Ericaceae	Eubotrys	racemosus	woody	shrub	tallshrub	year 15
Ericaceae	Gaylussacia	dumosa	woody	shrub	shortshrub	both
Ericaceae	Gaylussacia	frondosa	woody	shrub	shortshrub	both
Ericaceae	Kalmia	carolina	woody	shrub	tallshrub	both
Ericaceae	Lyonia	ligustrina	woody	shrub	shortshrub	both
Ericaceae	Lyonia	lucida	woody	shrub	tallshrub	both
Ericaceae	Lyonia	mariana	woody	shrub	shortshrub	both
Ericaceae	Rhododendron	atlanticum	woody	shrub	shortshrub	both
Ericaceae	Vaccinium	crassifolium	woody	shrub	shortshrub	both
Ericaceae	Vaccinium	formosum	woody	shrub	tallshrub	both
Ericaceae	Vaccinium	fuscatum	woody	shrub	tallshrub	both
Ericaceae	Vaccinium	stamineum	woody	shrub	shortshrub	year 15
Ericaceae	Vaccinium	tenellum	woody	shrub	shortshrub	both
Ericaceae	Zenobia	pulverulenta	woody	shrub	tallshrub	both
Eriocaulaceae	Eriocaulon	decangulare	herb	forb	perforb	both
Eriocaulaceae	Lachnocaulon	anceps	herb	forb	perforb	both
Fabaceae	Lespedeza	cuneata	herb	forb	perforb	years 1-3
Fagaceae	Quercus	falcata	woody	tree		year 15
Fagaceae	Quercus	nigra	woody	tree		both
Fagaceae	Quercus	virginiana	woody	tree		year 15
Gentianaceae	Bartonia	virginica	herb	forb	anforb	year 15
Gentianaceae	Gentiana	autumnalis	herb	forb	perforb	year 15
Gentianaceae	Sabatia	difformis/quadrangula	herb	forb	perforb	both
Grossulariaceae	Itea	virginica	woody	shrub	tallshrub	year 15
Haemodoraceae	Lachnanthes	caroliniana	herb	forb	perforb	both
Hamamelidaceae	Liquidambar	styraciflua	woody	tree		both
Iridaceae	Iris	verna	herb	forb	perforb	both
Juncaceae	Juncus	biflorus	herb	graminoid	sedgerush	year 15

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Juncaceae	Juncus	pelocarpus	herb	graminoid	sedgerush	year 15
Juncaceae	Juncus	spp.	herb	graminoid	sedgerush	years 1-3
Juncaceae	Juncus	scirpoides	herb	graminoid	sedgerush	year 15
Lauraceae	Persea	borbonia	woody	shrub	tallshrub	years 1-3
Lauraceae	Persea	palustris	woody	shrub	tallshrub	year 15
Lentibulariaceae	Pinguicula	pumila	herb	forb	perforb	year 15
Lentibulariaceae	Utricularia	junceae/subulata	herb	forb	perforb	both
Liliaceae	Aletris	spp.	herb	forb	perforb	both
Liliaceae	Hypoxis	wrightii	herb	forb	perforb	year 15
Liliaceae	Lilium	catesbaei	herb	forb	perforb	year 15
Liliaceae	Pleea	tenuifolia	herb	forb	perforb	both
Liliaceae	Stenanthium	densum	herb	forb	perforb	both
Liliaceae	Triantha	racemosa	herb	forb	perforb	year 15
Liliaceae	Zigadenus	glaberrimus	herb	forb	perforb	both
Loganiaceae	Gelsemium	sempervirens	woody	vine		both
Lycopodiaceae	Lycopodiella	alopecuroides	herb	fern		year 15
Magnoliaceae	Magnolia	virginiana	woody	tree		both
Melastomataceae	Rhexia	alifanus	herb	forb	perforb	both
Melastomataceae	Rhexia	lutea	herb	forb	perforb	years 1-3
Melastomataceae	Rhexia	mariana	herb	forb	perforb	years 1-3
Melastomataceae	Rhexia	nashii	herb	forb	perforb	both
Melastomataceae	Rhexia	petiolata	herb	forb	perforb	both
Myricaceae	Morella	caroliniensis	woody	shrub	tallshrub	both
Myricaceae	Morella	cerifera	woody	shrub	tallshrub	both
Onagraceae	Ludwigia	alternifolia	herb	forb	perforb	years 1-3
Onagraceae	Ludwigia	maritima	herb	forb	perforb	year 15
Orchidaceae	Calopogon	pallidus	herb	forb	perforb	year 15
Orchidaceae	Cleistes/Pogonia	spp.	herb	forb	perforb	both

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Orchidaceae	Platanthera	blephariglottis	herb	forb	perforb	both
Orchidaceae	Spiranthes	laciniata	herb	forb	perforb	both
Osmundaceae	Osmundastrum	cinnamomeum	herb	fern		both
Pinaceae	Pinus	elliottii	woody	tree		both
Pinaceae	Pinus	palustris	woody	tree		both
Pinaceae	Pinus	serotina	woody	tree		both
Pinaceae	Pinus	taeda	woody	tree		both
Poaceae	Amphicarpum	amphicarpon	herb	graminoid	othergram	both
Poaceae	Andropogon	gyrans	herb	graminoid	bunch	year 15
Poaceae	Andropogon	spp.	herb	graminoid	bunch	years 1-3
Poaceae	Andropogon	capillipes	herb	graminoid	bunch	both
Poaceae	Andropogon	glaucopsis	herb	graminoid	bunch	both
Poaceae	Andropogon	virginicus	herb	graminoid	bunch	both
Poaceae	Aristida	palustris	herb	graminoid	bunch	year 15
Poaceae	Aristida	stricta	herb	graminoid	bunch	both
Poaceae	Aristida	virgata	herb	graminoid	bunch	year 15
Poaceae	Arundinaria	tecta	herb	shrub	tallshrub	both
Poaceae	Coleataenia	rigidula	herb	graminoid	othergram	year 15
Poaceae	Ctenium	aromaticum	herb	graminoid	bunch	year 15
Poaceae	Dichanthelium	chamaelonche	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	consanguineum	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	dichotomum	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	ensifolium	herb	graminoid	othergram	both
Poaceae	Dichanthelium	erectifolium	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	mattamuskeetense	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	scabriusculum	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	scoparium	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	spp.	herb	graminoid	othergram	years 1-3

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Poaceae	Dichanthelium	strigosum var. leucoblepharis	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	strigosum var. strigosum	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	webberianum	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	leucothrix	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	portoricense	herb	graminoid	othergram	year 15
Poaceae	Dichanthelium	tenue	herb	graminoid	othergram	year 15
Poaceae	Eragrostis	refracta	herb	graminoid	bunch	year 15
Poaceae	Muhlenbergia	expansa	herb	graminoid	bunch	years 1-3
Poaceae	Panicum	spp.	herb	graminoid	othergram	years 1-3
Poaceae	Panicum	verrucosum	herb	graminoid	othergram	year 15
Poaceae	Paspalum	setaceum	herb	graminoid	othergram	year 15
Poaceae	Poa	spp.	herb	graminoid	bunch	years 1-3
Poaceae	Schizachyrium	scoparium	herb	graminoid	bunch	both
Poaceae	Sporobolus	brevipilis	herb	graminoid	bunch	year 15
Poaceae	Sporobolus	pinetorum	herb	graminoid	bunch	both
Polygalaceae	Polygala	brevifolia	herb	forb	anforb	year 15
Polygalaceae	Polygala	lutea	herb	forb		both
Polygalaceae	Polygala	ramosa	herb	forb	anforb	year 15
Polygalaceae	Polygala	cruciata	herb	forb	anforb	both
Rosaceae	Aronia	arbutifolia	woody	shrub	tallshrub	both
Rosaceae	Rubus	cuneifolius	woody	shrub	tallshrub	year 15
Rosaceae	Rubus	pensilvanicus	woody	shrub	tallshrub	year 15
Rosaceae	Rubus	spp.	woody	shrub	tallshrub	years 1-3
Rubiaceae	Mitchella	repens	herb	forb	perforb	year 15
Sarraceniaceae	Sarracenia	flava	herb	forb	perforb	both
Sarraceniaceae	Sarracenia	purpurea	herb	forb	perforb	both
Scrophulariaceae	Agalinis	purpurea	herb	forb	anforb	year 15
Scrophulariaceae	Gratiola	pilosa	herb	forb	perforb	year 15

Family	Genus	Species	Woody/Herb	Main Functional Groups	Sub Groups	ID Period
Scrophulariaceae	Seymeria	cassioides	herb	forb	anforb	years 1-3
Smilacaceae	Smilax	bona-nox	woody	vine		years 1-3
Smilacaceae	Smilax	glauca	woody	vine		both
Smilacaceae	Smilax	laurifolia	woody	vine		both
Smilacaceae	Smilax	rotundifolia	woody	vine		years 1-3
Smilacaceae	Smilax	spp.	woody	vine		years 1-3
Symplocaceae	Symplocos	tinctoria	woody	shrub	tallshrub	both
Theaceae	Gordonia	lasianthus	woody	tree		both
Violaceae	Viola	primulifolia	herb	forb	perforb	both
Vitaceae	Muscadinia	rotundifolia	woody	vine		both
Xyridaceae	Xyris	caroliniana	herb	forb	perforb	both
Xyridaceae	Xyris	platylepis	herb	forb	perforb	both
Xyridaceae	Xyris	ambigua	herb	forb	perforb	both
Xyridaceae	Xyris	baldwiniana	herb	forb	perforb	both
Xyridaceae	Xyris	brevifolia	herb	forb	perforb	both

Appendix 2. Taxonomic reconciliation of species lists from the early and later sampling periods.

Combined analysis	Years 1-3 (2004-2006)	Year 15 (2018)
<i>Acer rubrum</i>	<i>Acer rubrum</i>	<i>Acer rubrum</i>
<i>Agalinis purpurea</i>		<i>Agalinis purpurea</i>
<i>Aletris</i> sp.	<i>Aletris farinosa</i>	<i>Aletris aurea</i>
<i>Ambrosia artemisiifolia</i>	<i>Ambrosia artemisiifolia</i>	
<i>Amphicarpum amphicarpon</i>	<i>Amphicarpum amphicarpon</i>	<i>Amphicarpum amphicarpon</i>
<i>Anchistea virginica</i>	<i>Woodwardia virginica</i>	<i>Anchistea virginica</i>
<i>Andropogon glaucopsis</i>	<i>Andropogon capillipes</i>	<i>Andropogon capillipes</i>
<i>Andropogon glaucopsis</i>	<i>Andropogon capillipes</i>	<i>Andropogon glaucopsis</i>
<i>Andropogon</i> sp.		<i>Andropogon glomeratus</i>
<i>Andropogon</i> sp.		<i>Andropogon gyrans</i>
<i>Andropogon</i> sp.	<i>Andropogon</i> sp.	
<i>Andropogon virginicus</i>	<i>Andropogon virginicus</i>	<i>Andropogon virginicus</i>
<i>Aristida palustris</i>		<i>Aristida palustris</i>
<i>Aristida stricta</i>	<i>Aristida stricta</i>	<i>Aristida stricta</i>
<i>Aristida virgata</i>		<i>Aristida virgata</i>
<i>Aronia arbutifolia</i>	<i>Aronia arbutifolia</i>	<i>Aronia arbutifolia</i>
<i>Arundinaria tecta</i>	<i>Arundinaria tecta</i>	<i>Arundinaria tecta</i>
<i>Asclepias pedicellata</i>		<i>Asclepias pedicellata</i>
<i>Aster</i> sp.	<i>Aster</i> sp.	<i>Sericocarpus tortifolius</i>
<i>Aster</i> sp.	<i>Aster</i> sp.	<i>Symphyotrichum dumosum</i>
<i>Aster</i> sp.	<i>Aster</i> sp.	<i>Symphyotrichum walteri</i>
<i>Baccharis halimifolia</i>	<i>Baccharis halimifolia</i>	<i>Baccharis halimifolia</i>
<i>Bartonia virginica</i>		<i>Bartonia virginica</i>
<i>Calopogon pallidus</i>		<i>Calopogon pallidus</i>
<i>Carex striata</i>		<i>Carex striata</i>
<i>Carphephorus tomentosus</i>	<i>Carphephorus tomentosus</i>	<i>Carphephorus tomentosus</i>
<i>Centella asiatica</i>		<i>Centella asiatica</i>
<i>Chamaedaphne calyculata</i>	<i>Chamaedaphne calyculata</i>	<i>Cassandra calyculata</i>
<i>Clethra alnifolia</i>	<i>Clethra alnifolia</i>	<i>Clethra alnifolia</i>
<i>Cleistes/Pogonia</i>	<i>Cleistes/Pogonia</i>	<i>Cleistes</i> sp.
<i>Coreopsis falcata</i>		<i>Coreopsis falcata</i>
<i>Coreopsis linifolia</i>		<i>Coreopsis linifolia</i>
<i>Ctenium aromaticum</i>		<i>Ctenium aromaticum</i>
<i>Cuscuta compacta</i>	<i>Cuscuta</i> sp.	<i>Cuscuta compacta</i>
<i>Cyrilla racemiflora</i>	<i>Cyrilla racemiflora</i>	<i>Cyrilla racemiflora</i>
<i>Dichanthelium</i> sp.	<i>Dichanthelium</i> sp.	<i>Dichanthelium chamaelonche</i>
<i>Dichanthelium</i> sp.	<i>Dichanthelium</i> sp.	<i>Dichanthelium consanguineum</i>
<i>Dichanthelium</i> sp.	<i>Dichanthelium</i> sp.	<i>Dichanthelium dichotomum</i>

Combined analysis

Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dionaea muscipula
Diospyros virginiana
Drosera brevifolia
Drosera capillaris
Drosera intermedia
Eragrostis refracta
Erechtites hieraciifolius
Eriocaulon decangulare
Eriocaulon decangulare
Erigeron vernus
Eupatorium capillifolium
Eupatorium leucolepis
Eupatorium mohrii
Eupatorium pilosum
Eupatorium rotundifolium
Euthamia caroliniana
Fimbristylis puberula
Gaylussacia dumosa
Gaylussacia frondosa
Gelsemium sempervirens
Gentiana autumnalis
Gordonia lasianthus
Hieracium gronovii
Hypericum cistifolium
Hypericum crux-andreae
Hypericum hypericoides
Hypericum tenuifolium
Hypericum tenuifolium
Hypoxis wrightii
Ilex coriacea

Years 1-3 (2004-2006)

Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dichanthelium sp.
Dionaea muscipula
Diospyros virginiana
Drosera brevifolia
Drosera capillaris
Drosera sp.

Eriocaulon compressum
Eriocaulon sp.
Erigeron sp.
Eupatorium capillifolium
Eupatorium sp.
Eupatorium album
Eupatorium pilosum
Eupatorium rotundifolium
Euthamia tenuifolia
Cyperus sp.
Gaylussacia dumosa
Gaylussacia frondosa
Gelsemium sempervirens

Gordonia lasianthus

Hypericum sp.
Hypericum stans
Hypericum hypericoides
Hypericum reductum
Hypericum densiflorum

Ilex coriacea

Year 15 (2018)

Dichanthelium ensifolium
Dichanthelium erectifolium
Dichanthelium leucothrix
Dichanthelium mattamuskeetense
Dichanthelium portoricense
Dichanthelium scabriusculum
Dichanthelium scoparium
Dichanthelium strigosum
Dichanthelium tenue
Dichanthelium webberianum
Dionaea muscipula
Diospyros virginiana

Drosera capillaris
Drosera intermedia
Eragrostis refracta
Erechtites hieraciifolius
Eriocaulon decangulare
Eriocaulon decangulare

Eupatorium leucolepis
Eupatorium mohrii
Eupatorium pilosum
Eupatorium rotundifolium
Euthamia caroliniana
Fimbristylis puberula
Gaylussacia dumosa
Gaylussacia frondosa
Gelsemium sempervirens
Gentiana autumnalis
Gordonia lasianthus
Hieracium gronovii
Hypericum cistifolium
Hypericum crux-andreae
Hypericum hypericoides
Hypericum tenuifolium
Hypericum tenuifolium
Hypoxis wrightii
Ilex coriacea

Combined analysis

Ilex glabra
Ilex myrtifolia
Ilex opaca
Iris verna
Itea virginica
Juncus sp.
Juncus sp.
Juncus sp.
Kalmia carolina
Lachnocaulon anceps
Lachnocaulon anceps
Lachnanthes caroliniana
Lechea pulchella
Lespedeza cuneata
Eubotrys racemosus
Liatris pilosa
Liatris spicata
Lilium catesbaei
Liquidambar styraciflua
Lobelia nuttallii
Lorinseria areolata
Ludwigia alternifolia
Ludwigia maritima
Lycopodiella alopecuroides
Lyonia ligustrina
Lyonia lucida
Lyonia mariana
Magnolia virginiana
Marshallia graminifolia
Mitchella repens
Morella caroliniensis
Morella cerifera
Muhlenbergia expansa
Muscadinia rotundifolia
Nyssa biflora
Osmundastrum cinnamomeum
Coleataenia rigidula
Panicum sp.
Panicum sp.
Paspalum setaceum

Years 1-3 (2004-2006)

Ilex glabra
Ilex myrtifolia
Ilex opaca
Iris sp.

Juncus sp.
Juncus sp.
Juncus sp.
Kalmia carolina
Lachnocaulon anceps
Lachnocaulon sp.
Lachnanthes caroliniana

Lespedeza cuneata

Liatris graminifolia

Liquidambar styraciflua
Lobelia nuttallii
Woodwardia areolata
Ludwigia alternifolia

Lyonia ligustrina
Lyonia lucida
Lyonia mariana
Magnolia virginiana

Myrica heterophylla
Myrica cerifera
Muhlenbergia expansa
Vitis sp.

Osmunda cinnamomea

Panicum sp.
Panicum sp.

Year 15 (2018)

Ilex glabra
Ilex myrtifolia
Ilex opaca
Iris verna
Itea virginica
Juncus biflorus
Juncus pelocarpus
Juncus scirpoides
Kalmia carolina
Lachnocaulon anceps
Lachnocaulon anceps
Lachnanthes caroliniana
Lechea pulchella

Eubotrys racemosus
Liatris pilosa
Liatris spicata
Lilium catesbaei
Liquidambar styraciflua
Lobelia nuttallii
Lorinseria areolata

Ludwigia maritima
Lycopodiella alopecuroides
Lyonia ligustrina
Lyonia lucida
Lyonia mariana
Magnolia virginiana
Marshallia graminifolia
Mitchella repens
Morella caroliniensis
Morella cerifera
Muhlenbergia expansa
Muscadinia rotundifolia
Nyssa biflora
Osmundastrum cinnamomeum
Coleataenia rigidula
Panicum verrucosum
Coleataenia rigidula
Paspalum setaceum

Combined analysis

Persea palustris
Pinus elliottii
Pinus elliottii
Pinus palustris
Pinguicula pumila
Pinus serotina
Pinus taeda
Pityopsis graminifolia
Platanthera blephariglottis
Pleea tenuifolia
Polygala brevifolia
Polygala cruciata
Polygala lutea
Polygala lutea
Polygala ramosa
Pseudognaphalium obtusifolium
Pteridium latiusculum
Pterocaulon pycnostachyum
Pyxidanthra barbulate
Quercus falcata
Quercus nigra
Quercus virginiana
Rhexia alifanus
Rhexia lutea
Rhexia mariana
Rhexia mariana
Rhexia nashii
Rhexia petiolata
Rhododendron atlanticum
Rhus copallinum
Rhynchospora ciliaris
Rhynchospora fascicularis
Rhynchospora galeana
Rhynchospora pallida
Rhynchospora plumosa
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.

Years 1-3 (2004-2006)

Persea palustris
Pinus elliottii
Pinus sp.
Pinus palustris

Pinus serotina
Pinus taeda
Pityopsis graminifolia
Platanthera sp.
Pleea tenuifolia

Polygala cruciata
Polygala lutea
Polygala nana
Polygala ramosa
Gnaphalium obtusifolium
Pteridium aquilinum
Pterocaulon pycnostachyum

Quercus sp.

Rhexia alifanus
Rhexia lutea
Rhexia mariana
Rhexia sp.
Rhexia nashii
Rhexia petiolata
Rhododendron atlanticum
Rhus copallinum
Rhynchospora ciliaris
Rhynchospora fascicularis
Rhynchospora breviseta
Dichromena latifolia
Rhynchospora plumosa
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.
Rhynchospora sp.

Year 15 (2018)

Persea palustris
Pinus elliottii
Pinus elliottii
Pinus palustris
Pinguicula pumila
Pinus serotina
Pinus taeda
Pityopsis graminifolia
Platanthera blephariglottis
Pleea tenuifolia
Polygala brevifolia
Polygala cruciata
Polygala lutea

Polygala ramosa

Pteridium latiusculum
Pterocaulon pycnostachyum
Pyxidanthra barbulate
Quercus falcata
Quercus nigra
Quercus virginiana
Rhexia alifanus

Rhexia nashii
Rhexia petiolata
Rhododendron atlanticum
Rhus copallinum
Rhynchospora ciliaris
Rhynchospora fascicularis
Rhynchospora galeana
Rhynchospora pallida
Rhynchospora plumosa
Rhynchospora cephalantha
Rhynchospora chalarocephala
Rhynchospora chapmanii
Rhynchospora inexpansa
Rhynchospora rariflora

Combined analysis

Rubus sp.
Rubus sp.
Sabatia sp.
Sarracenia flava
Sarracenia purpurea
Schizachyrium scoparium
Scleria ciliata var. glabra
Scleria minor
Scleria muehlenbergii
Scleria pauciflora var. caroliniana
Seymeria cassioides
Smilax bona-nox
Smilax glauca
Smilax laurifolia
Smilax rotundifolia
Smilax sp.
Solidago fistulosa
Solidago odora
Solidago pulchra
Solidago verna
Sophronanthe pilosa
Spiranthes laciniata
Sporobolus brevipilis
Sporobolus pinetorum
Stenanthium densum
Symplocus tinctoria
Taraxacum sp.
Toxicodendron radicans
Trilisa odoratissima
Trilisa paniculata
Triantha racemosa
Utricularia juncea
Vaccinium crassifolium
Vaccinium formosum
Vaccinium fuscatum
Vaccinium stamineum
Vaccinium tenellum
Viburnum nudum
Viola primulifolia
Xyris ambigua

Years 1-3 (2004-2006)

Rubus sp.
Rubus sp.
Sabatia sp.
Sarracenia flava
Sarracenia purpurea
Schizachyrium scoparium

Seymeria cassioides
Smilax bona-nox
Smilax glauca
Smilax laurifolia
Smilax rotundifolia
Smilax sp.
Solidago fistulosa
Solidago odora
Solidago pulchra
Solidago verna

Spiranthes sp.

Sporobolus pinetorum
Zigadenus densus
Symplocus tinctoria
Taraxacum sp.

Carphephorus odoratissimus
Carphephorus sp.

Utricularia juncea
Vaccinium crassifolium
Vaccinium corymbosum
Vaccinium atrococcum

Vaccinium tenellum

Viola sp.
Xyris ambigua

Year 15 (2018)

Rubus cuneifolius
Rubus pensilvanicus
Sabatia difformis/quadrangula
Sarracenia flava
Sarracenia purpurea
Schizachyrium scoparium
Scleria ciliata var. glabra
Scleria minor
Scleria muehlenbergii
Scleria pauciflora var. caroliniana

Smilax glauca
Smilax laurifolia

Solidago fistulosa
Solidago odora
Solidago pulchra
Solidago nemoralis
Sophronanthe pilosa
Spiranthes laciniata
Sporobolus brevipilis
Sporobolus pinetorum
Stenanthium densum
Symplocus tinctoria

Toxicodendron radicans
Trilisa odoratissima
Trilisa paniculata
Triantha racemosa
Utricularia subulata
Vaccinium crassifolium
Vaccinium formosum
Vaccinium fuscatum
Vaccinium stamineum
Vaccinium tenellum
Viburnum nudum
Viola primulifolia
Xyris ambigua

Combined analysis

Xyris brevifolia
Xyris caroliniana
Xyris sp.
Xyris sp.
Zenobia pulverulenta
Zigadenus glaberrimus

Years 1-3 (2004-2006)

Xyris brevifolia
Xyris caroliniana
Xyris sp.
Xyris sp.
Zenobia pulverulenta
Amianthium muscaetoxicum

Year 15 (2018)

Xyris brevifolia
Xyris caroliniana
Xyris baldwiniana
Xyris platylepis
Zenobia pulverulenta
Zigadenus glaberrimus

Appendix 3. Species scores for each axis from non-metric multidimensional scaling ordination.

Species	NMDS1	NMDS2	NMDS3
acerub	-0.3599574	0.26053898	0.11542378
agapur	-0.6146666	-0.6375383	0.2321104
alespp	0.08546253	0.74078808	1.08675386
ambart	0.57785483	0.74238964	0.40521702
ampamp	0.04359936	0.53358843	0.82015856
ancvir	-0.1813786	0.56419225	0.36289733
andgla	0.23314688	0.1439894	-0.164054
andspp	0.20322317	-0.0749566	0.058609
andvir	0.12112877	-0.3538233	0.0427755
aristr	-0.026454	-0.4344104	-0.0779744
arivir	-0.5255728	0.51554556	1.28634914
aroarb	-0.2368213	0.05922678	-0.1586401
arutec	-0.2193275	-0.0346814	-0.2641749
astspp	0.37737047	0.05699348	0.16088606
barvir	-0.5423904	-0.4796135	0.4451007
carstr	-0.6136756	0.19474567	0.90402431
cartom	0.27902074	-0.2951929	-0.1788869
clealn	0.18886976	-0.1128282	0.55990251
clespp	0.01206202	-0.17225	-0.4126901
cyrrac	-0.0371384	0.01266541	0.57950766
dicspp	0.37879067	0.07699913	-0.2325551
diomus	-0.2445774	-0.5869638	0.14218584
drobre	0.60196416	0.53936491	0.11424705
drocap	0.62077889	0.25553206	0.28556547
droint	-0.2181665	0.63272473	1.16013347
eridec	0.28898	0.40634624	-0.2691032
eubrac	-0.228302	-1.3093443	0.65880972
eupcap	1.03538079	0.62963784	-0.0409924
eupleu	0.61318344	0.37052959	-0.0746927
eupmoh	0.18318538	0.20100706	0.21507358
euppil	0.35949923	0.12077349	0.30761233
euprot	0.23681986	-0.3675843	-1.2090691
eurpal	0.72823234	-0.81892	-0.1889709
eutcar	0.25458438	-0.50342	0.14592391
fimpub	0.1718336	0.26628182	0.33147742
gaydum	-0.4520843	-0.2015209	-0.0769168
gayfro	-0.3713391	0.00209397	-0.2626114
gelsem	-0.4272047	0.11056649	-0.3018472

Species	NMDS1	NMDS2	NMDS3
genaut	-0.2907204	-1.2742383	0.20829736
gorlas	-0.7758395	0.12132676	-0.2859671
hypcis	0.56914006	0.69618371	1.17043724
hypcru	0.70842409	0.78578541	0.36969566
hypten	0.50709363	-0.3519666	0.43677952
ilecor	-0.4921893	0.18974054	-0.1844473
ilegla	-0.5312371	0.14948636	-0.0902843
ilemyr	0.14195384	0.59074598	0.55923952
ileopa	-0.5176429	0.43502448	-0.6273298
ionlin	-0.0043462	-0.2429319	0.06613477
iriver	0.16571723	-0.1184455	-0.0120289
junspp	-0.2275236	-0.262315	0.44121038
kalcar	-0.3498585	-0.0758712	-0.1310391
lacanc	0.10114882	0.15711905	-0.2634341
laccar	0.16139764	0.56937646	0.15349691
liapil	0.68084351	-0.4833502	0.11783028
lobnut	0.51700587	-0.0611041	0.97230543
lorare	0.19774721	0.94888362	0.63555989
ludalt	0.52756014	0.9058782	-0.7406094
lyolig	-0.3340608	-0.243153	0.59140875
lyoluc	-0.8889383	0.28356107	-0.0860938
lyomar	0.15590816	-0.5844609	-0.0601075
magvir	-0.4318798	0.29652983	-0.1807944
morcar	-0.1127729	0.26785528	0.40995788
morcer	0.03171091	-0.277881	-0.1120967
muhexp	-0.2187786	0.00837223	-0.2578809
nysbif	-0.6400059	-0.1182328	0.92592293
osmcin	-0.1694527	0.45422425	0.32271785
panspp	0.30648327	0.73626418	0.85754344
perpal	-0.2699379	-0.0210679	-0.0600619
pinell	0.2543124	-0.3796956	0.33113064
pinpal	0.0795391	0.04045575	-0.0951843
pinser	-0.6875728	-0.5369698	0.56128552
pintae	0.48824597	0.02480979	-0.7233447
pitgra	0.44820899	-0.699225	0.18218045
plable	-0.3347105	-0.1190772	0.36392911
pleten	-0.4570327	-0.1484027	0.09765651
poasp	0.62823443	-0.0034576	0.21140911
polcru	-0.3257922	0.18970716	0.66647071
pollut	0.39725087	0.05663902	-0.2243465

Species	NMDS1	NMDS2	NMDS3
ptelat	0.1189345	0.14941227	-0.2983957
pyxbar	-0.5657055	-0.4253715	0.16023607
rheali	0.05766334	0.02407063	-0.1519877
rhenas	0.50641133	0.45175259	0.04899937
rhepet	0.15038973	0.01542599	0.32512322
rhoatl	0.26173176	-0.7774815	0.08316708
rhucop	-0.0820705	-0.941416	-0.2582786
rhycil	-0.5352756	-0.0255695	-0.0725122
rhyfas	-0.6101538	-0.489779	0.26849037
rhypal	-0.8626971	-0.4764883	0.87847728
rhylu	-0.1326538	-0.1835815	0.08358749
rhyspp	0.40017795	0.38639455	-0.0563742
rubsp	0.96579104	0.0135171	-0.1012201
sabsp	-0.5677789	0.29648872	1.35220491
sarfla	-0.4172077	0.06303555	0.37590844
sarpur	-0.3327878	-0.0930905	0.11598875
schsch	0.16134018	-0.2587099	-0.0717616
sclcil	-0.5340272	-0.5693497	-0.1002661
smigla	-0.1490389	0.31091276	-0.1828724
smilau	-0.3755222	0.39202872	0.15004153
smirot	0.21540236	0.25461891	-0.1672634
smispp	-0.60792	0.88451748	-0.4552236
sofis	0.56597399	0.41378422	0.41013989
solpul	-0.1869196	-0.5204908	0.18929533
solspp	0.90659669	0.72707759	0.11185719
spilac	0.74557865	-0.3863953	-0.2884957
spopin	-0.5842426	-0.6932924	0.07629227
steden	-0.0740498	-0.1271887	0.35704186
syntin	0.24730904	0.78191799	1.10514416
tarspp	0.54649287	0.61193026	0.34034333
triodo	0.16787624	-0.5179735	-0.0945309
tripan	-0.3803363	-0.4373025	0.20264069
utrjun	0.32599453	-0.1059047	-0.2779989
vaccra	-0.0199015	-0.1719483	-0.1368845
vacfor	-0.2663802	-0.1095816	0.18632326
vacfus	-0.5544024	-0.0433382	0.40563412
vacten	-0.1317606	-0.2134751	-0.205869
viopri	0.38360096	0.03033396	0.29862036
xyramb	-0.4696716	0.04125065	0.59643645
xyrbre	-0.2969756	-0.8434801	-0.3526069

Species	NMDS1	NMDS2	NMDS3
xycar	-0.1201163	-0.6503922	0.26675661
xyspp	0.29964943	0.11627488	0.08889612
zenpul	0.01301989	-0.6438104	0.44644767
ziggla	-0.5981353	-0.5200376	0.59915979