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Authors: Tucker, Rebecca C., Rothermel, Betsie B., and Daskin, Joshua H.

Source: Natural Areas Journal, 37(1) : 39-48

Published By: Natural Areas Association

URL: https://doi.org/10.3375/043.037.0107

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RESEARCH ARTICLE

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Preparing Florida Pasture for Grassland Restoration: Seedling Establishment after Herbiciding and Tilling

Rebecca C. Tucker[1,2]

[1]Restoration Ecology and Herpetology Program
Archbold Biological Station
Venus, FL 33960

Betsie B. Rothermel[1,4]
Joshua H. Daskin[1,3]

[2] Present Address: 3734 Henry St.
Wausau, WI 54403

[3] Present Address: Department of Ecology and Evolutionary Biology
Princeton University
Princeton, NJ 08544

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Natural Areas Journal 37:39–48

ABSTRACT: Nonnative pasture grasses established for agriculture and livestock husbandry have replaced countless acres of natural habitat in the last century of Florida history. Many endemic species, such as cutthroat grass (*Coleataenia abscissa*), are now endangered and may not return to areas until the dominant pasture grass has been removed. To determine the best strategy for native revegetation, we examined the effects of two soil preparations (tilling and no tilling) in pasture plots dominated by bahiagrass (*Paspalum notatum*) on the Archbold Reserve in south-central Florida. In preparation for the experiment, the No-Till and Till plots were first herbicided with glyphosate and burned to remove bahiagrass cover and expose the seed bank, then the Till plots were disked to a depth of 5–10 cm. Reference plots were left undisturbed to provide a pre-restoration baseline. The pre-tilling herbicide application and burn reduced bahiagrass cover in experimental plots to an average of 24% compared to 80% in reference plots. In the first year following tilling, overall plant community composition differed between tillage treatments, with 52 and 38 plant taxa found in the No-Till and Till plots, respectively, compared to only 14 in the reference plots. Importantly, native species richness was significantly higher in the No-Till treatment, and tilling resulted in minimal reduction of cover of bahiagrass and other exotic grasses. Seedling species composition differed among experimental blocks, suggesting soil moisture or other local abiotic conditions may significantly influence seedling establishment and restoration outcomes regardless of mechanical soil preparations.

Index terms: bahiagrass, habitat restoration, pasture, seed bank, tilling

INTRODUCTION

Florida contains an estimated 1.64 million head of cattle, many of which are grazed on pastures that have been improved for forage by installing drainage systems and planting a monoculture of nonnative pasture grass (Florida Department of Agriculture and Consumer Services 2015). Bahiagrass (*Paspalum notatum* Flugge) is the most common of these introduced pasture grasses in the southeastern United States, with over two million acres planted in Florida alone (Newman et al. 2015). On the inland sand ridges of peninsular Florida, communities once dominated by native grasses, such as the state-endemic and endangered cutthroat grass *Coleataenia abscissa* (Swallen) LeBlond, are possible targets for restoration from pasture to semi-native or fully native states (US Fish and Wildlife Service 1999). Transitioning managed agricultural land to native vegetation is increasingly desirable to landowners as they may receive ecological benefits from higher plant and animal diversity as well as monetary compensation through enrollment in state and federal conservation easement programs (Sorice et al. 2013; Waddle et al. 2013; Farmer et al. 2015). Reestablishment of native plant communities on these managed lands will likely require an understanding of how environmental factors and site preparation may facilitate or hinder passive seedling establishment in these restoration areas.

Where no active planting measures are undertaken, the success of passive restoration depends greatly on recruitment from the seed bank or via seed dispersal from surrounding areas. Intensive management, such as frequent tilling or haying, reduces seed bank richness (Auestad et al. 2013) and increased time in management further depletes the seeds available for passive restoration (Waldhardt et al. 2001). Furthermore, significant variation in seed bank diversity may exist within fields (Sanderson et al. 2014), suggesting that seed banks can be highly variable, even on small scales (Auestad et al. 2008). Adjoining land use is another important factor that determines the seedling composition of pastures, with decreased distance from the edge of adjacent pastures increasing the similarity of species composition between those communities (Winsa et al. 2015). Site-level environmental conditions, including soil characteristics and water availability, as well as the species composition of the surrounding area, may also contribute to the species composition of managed habitats (Wetzel et al. 2001; Goslee and Sanderson 2010; Ficken and Menges 2013).

Site preparation may further determine the response of pasture to restoration efforts, as the expressed plant community is a combination of what was available in the seed bank and what was stimulated by, or able to thrive, under the current environmental and management conditions, with

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[4] Corresponding author: brothermel@archbold-station.org; 863-465-2571

a number of studies finding discrepancies between seed bank and expressed plant diversity or richness (Tracy and Sanderson 2000; Auestad et al. 2013; Sanderson et al. 2014). Seeds from species that rarely germinate from the seed bank, including natives such as cutthroat grass, have shown poor competitive ability against nonnative pasture grasses, if they are able to regenerate from seed at all (Frances et al. 2010; Hamman and Hawkes 2013; C. Matson, pers. comm.). Thus, removal of dense forage grasses is presumed to be an essential step in passive grassland restoration (Bahm et al. 2015). Single applications of chemical or cultural practices (e.g., herbiciding, controlled burning, mowing) have been shown to remove biomass, aid in weed control, or facilitate tilling, but displayed limited success in terms of preparing existing pastures for native grass restoration (Wilson and Gerry 1995; Washburn et. al. 2000; Ewing 2002; Barnes 2004). Although tilling removes stubble of herbicided plants, the resulting disturbance of the soil surface may facilitate establishment of undesirable weedy or nonnative species, increasing competition with slower colonizing or disturbance-sensitive native species (Bissett 2006; Schutte et al. 2012).

Prior to attempting restoration of a former cutthroat grass community in south-central Florida, we designed an experiment to better understand the potential for passive restoration by removal of exotic pasture grasses. Specifically, we examined species composition and richness of the emergent vegetation in the first year following removal of the dominant bahiagrass, as well as the effects of tillage on seedling establishment.

METHODS

Study Area

The Cutthroat Seep Wetlands Reserve Program (CS-WRP) management unit is an 83-ha pasture located on the Archbold Reserve on the southwestern slope of the Lake Wales Ridge in Highlands County, Florida (27°07.462', −81°22.625'). To make this seepage slope suitable for pasture, previous owners extensively ditched the site between 1974 and 1981. Although we lack specific information on plant community composition prior to ditching, the site's Basinger-St. Johns-Placid soils are typically associated with cutthroat seeps. The CS-WRP site was enrolled in the US Department of Agriculture's Wetlands Reserve Program in 2009, providing an opportunity to restore a hydrologically altered cattle pasture into wet prairie–seepage slope habitat by blocking existing ditches to retain water on site. Scattered patches of cutthroat grass still remain in the pasture, and intact cutthroat seep communities still exist in the adjacent undisturbed flatwoods.

The National Oceanic and Atmospheric Administration operates a Climate Reference Network station (Sebring 23 SSE Florida) three km northeast of CS-WRP, providing accurate climatic data for our study area. Rainfall totals during our study were 32.55 cm in the dry season (January–May 2014, the period between treatment initiation and our first sampling event) and 78.74 cm in the wet season (June–September 2014, between our first and second sampling events) (National Centers for Environmental Information 2016). During the same time periods, mean daily maximum temperatures were 27.12 °C and 32.58 °C and mean daily minimum temperatures were 12.7 °C and 21.53 °C, respectively.

Experimental Design

We used a randomized block design with five blocks (referred to as Blocks A through E), each block containing one 10 × 10–m plot assigned to each treatment (experimental No-Till and Till plots, and an undisturbed Reference plot). The pasture experienced year-round grazing at a low stocking density until cattle were fenced out of all blocks in the fall of 2011. Locations of blocks were chosen to span the hydrological gradient and spatial extent of the entire pasture. All experimental plots (both No-Till and Till) were herbicided in September 2013 (4.4 kg a.i./ha glyphosate with 0.5% non-ionic surfactant), resulting in approximately >90% aboveground mortality; the resulting dried material was completely burned six weeks later in November 2013 to remove bahiagrass prior to the experiment. Plots randomly designated as Till were prepared using a tractor-pulled disk that turned and loosened the top 5–10 cm of burned stubble and soil in December 2013, and finally both Till and No-Till plots were herbicided a second time at the same rate in January 2014, again resulting in nearly complete kill. Each block contained an unmanipulated 10 × 10–m Reference plot that was protected from herbiciding and burning. We also installed wells made of slotted PVC pipes in a corner of each block in March 2012 and sampled groundwater depths monthly using a WaterMark water level meter.

We nondestructively estimated percent cover of each taxon in 12 randomly chosen 1 × 1–m subplots in each experimental plot on 20 May 2014 and again between 30 September and 6 October 2014. In greenhouse studies, a five-month interval has been shown to be sufficient time to capture the diversity presently in the seed bank (Tracy and Sanderson 2000; Auestad et al. 2013; Sanderson et al. 2014). During each sampling event, we estimated percent cover of bare ground, dead vegetation, and live vegetation and recorded presence of all individual plants to species when possible. A limited number of small seedlings could not be identified and were omitted from analyses. In the Reference plots, we estimated the percent cover of 14 abundant focal species as well as the presence of all other taxa in nine subplots per block between 5 and 7 November 2014, for consistency with previous annual sampling.

Data Analyses

We used univariate analyses of variance (ANOVAs; IBM SPSS Statistics, Version 22) to examine the effects of block and field preparation treatment in the experimental plots on bahiagrass cover, native and exotic plant cover, bare ground cover, and species richness (i.e., number of distinct taxa, whether recorded to genus or species level), with treatment treated as a fixed effect, and block as random. For bahiagrass cover, we analyzed the data from fall 2014 only (i.e., regrowth after nine months), using the same statistical model to examine block and treatment effects. The remaining cover estimates and richness values were derived by averaging or pooling across the spring and fall sampling periods, respectively. We generated rank-abundance curves from data on species relative abundances and ranked abundances averaged across blocks (McCune et al. 2002; Magurran 2004). Because our sampling of Reference plots differed in timing and intensity of sampling (e.g., fewer subplots, less detailed composition data) relative to experimental plots, we only used data from Reference plots for qualitative comparisons.

We performed nonmetric multidimensional scaling (NMS) ordinations with Sorensen distance measures to examine block and treatment differences in vegetation composition in the 10 experimental plots as represented by percent cover of 56 taxa, based on 100 iterations with real data and 500 iterations with randomized data. To examine differences in community composition between the Till and No-Till treatments, we ran blocked multi-response permutation procedures (MRPP) using Euclidean similarity distance and groups defined by treatments (group $n = 2$ treatments, within group $n = 5$ blocks). Indicator species analyses were performed with the Monte Carlo test to see if species were associated with particular experimental treatments or blocks (4999 permutations; PC-Ord Version 6.08; Peck 2010; McCune and Mefford 2011).

RESULTS

We found a total of 59 distinct taxa in the experimental and reference plots combined, of which 38 were identified to species and 21 to genus. Most identifiable taxa were perennials, with 24 perennial taxa found in the No-Till treatment compared to 16 in the Till treatment, and only 8 in the Reference plots (Appendix). Bahiagrass cover in the fall sampling period was considerably lower in the experimental plots (No-Till 33.92 ± 20.13 SD, Till 37.20 ± 12.78) compared to the Reference plots (80.00 ± 26.6 SD) as a result of the pre-experiment pasture grass removal. However, bahiagrass cover in experimental plots did not differ significantly among blocks or between treatments (Table 1). Cumulatively, there were fewer unique taxa found in the Reference plots ($n = 14$) compared to the No-Till ($n = 52$) and Till plots ($n = 38$) (Appendix). Within the experimental plots, mean total species richness was significantly higher in the No-Till plots (22.4 ± 7.5 SD) compared to the Till plots (17.6 ± 6.7 SD) (Table 1).

Table 1. Summary of univariate ANOVAs of effects of block and treatment in the experimental plots. *P* values <0.05 were considered significant and are in bold.

	Block		Treatment	
	$F_{1,4}$	P	$F_{1,4}$	P
Total species richness	**23.659**	**0.005**	**14.049**	**0.02**
Native species richness	**19.889**	**0.007**	**9.796**	**0.035**
Exotic species richness	0.875	0.55	0.063	0.815
Bahiagrass % cover	1.041	0.485	0.097	0.771
Bare ground % cover	2.31	0.219	2.413	0.195
Native species % cover	6.256	0.052	2.755	0.172
Exotic species % cover	3.674	0.118	0.382	0.57

Because mean richness of exotic species did not differ between experimental treatments (Table 1), the differences in total richness were largely a function of significantly greater richness of native species in the No-Till treatment compared to the Till treatment (16.6 ± 7.9 SD and 12.0 ± 7.1 SD, respectively; Table 1). On average, No-Till plots had higher richness of grasses, forbs, and shrubs (Figure 1). No taxa were determined to be indicator species when the groups were assigned by treatment, and only *Sporobolus indicus* (L.) R. Br. had a significant observed indicator value associated with Block B when the groups were assigned by block (IV = 62.2, $P = 0.0378$). The average relative abundance plotted against species rank on a log scale approximated a log normal distribution in both experimental treatments (Magurran 2004), with bahiagrass being found most frequently and with the highest percent cover in both the Till and No-Till treatments (Figure 2). Total species richness also differed significantly among blocks (Table 1). Twenty-two of the total 56 taxa were found only in one block, and 18 of those taxa were found only in the No-Till treatment. No other richness or percent cover metrics differed significantly among blocks or between treatments (Table 1).

The NMS ordination found a three-dimensional solution with low stress (0.00393) that represented 91.12% of the variation ($r^2 = 0.253$, $r^2 = 0.433$, and $r^2 = 0.226$ for axes 1, 2, and 3, respectively). Blocks A and C were distinct from the remaining three blocks in NMS ordination space (Figures 3A and 3B). The Till and No-Till Treatments differed much more in Blocks A and E than in other blocks; the Till treatment in Block E clustered with Blocks D and B, separating from the corresponding No-Till treatment in Block E along axes 2 and 3 (Figures 3A and 3B). Block C showed no separation between treatments along any axes, but was separated from the majority of the other blocks along axis 3 as well as axis 2, and showed strong associations with *Hemarthria altissima* (Poir.) Stapf & C. E. Hubbard, *Mimosa quadrivalvis* L., *Dichanthelium* sp., and *Indigofera hirsuta* L. (Figure 3B). Several taxa were found only or preferentially in Block E (Figures 3A and 3B). The magnitude and direction of variation of the depth to ground water vectors were strongly represented by axes 1 and 3, corresponding with the separation of Block A from the remaining blocks (Figure 3A). Three taxa were found only in the No-Till plot of Block A (*Asimina* sp., *Crotalaria* sp., and *Galactia elliottii* Nutt.; Figures 3A and 3B), however MRPP analysis did not show a significant probability of higher heterogeneity of species within treatments than expected by chance ($A = -0.0167$, $P = 0.587$).

DISCUSSION

Experimental plots contained three times the number of taxa on average compared

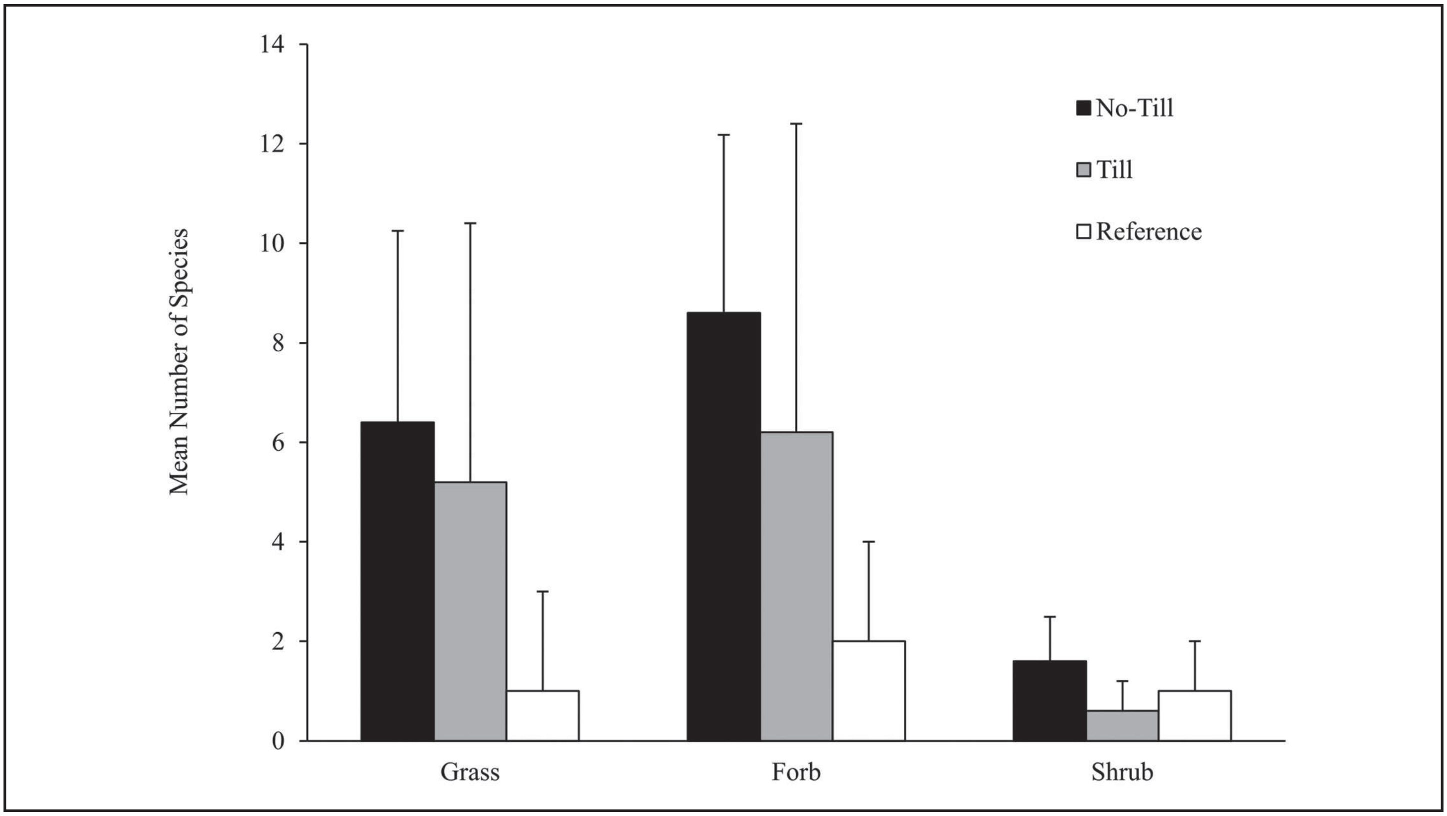

Figure 1. Species richness of native grasses, forbs, and shrubs in the No-Till and Till treatments as well as Reference plots. For each type of plot, the number of species was pooled within each block then averaged across blocks. Error bars are one standard deviation.

to the untreated pasture plots following removal of most bahiagrass cover. The predominance of perennial species in our experimental plots is consistent with this pasture having been a mostly undisturbed monoculture of nonnative pasture grass for nearly 50 years. Bahiagrass is very competitive with weedy annuals (Sanderson et al. 2014; Newman et al. 2015), which may have limited the accumulation of seeds from annual plants over time. Our intensive field preparations designed to remove bahiagrass may also have reduced any weedy annuals prior to initiation of the experimental treatments. Most of the taxa that were only found in the experimental plots were native, perennial, and early successional species. Additionally, the disturbed patches we created may not have been large enough or timed appropriately to collect potentially colonizing species from outside the plots in the short time they were bare (Renne et al. 2006).

There were few other significant differences between the tilled and untilled experimental treatments. We found more total species in untilled plots, and the subsequent tillage did not confer any further reduction in bahiagrass, nor did total percent cover of native species differ significantly between treatments. Many minor species were found that contributed to the overall richness but did not contribute much in terms of percent cover in the plot. However, higher species richness in the No-Till treatment may have implications for future succession depending on site environmental conditions (Goslee and Sanderson 2010). The number of nonnative species was similar in both field preparation treatments, suggesting that the extra disturbance of tillage did not increase the likelihood of invasion by undesirable exotics. Tilling did, however, appear to have a negative effect on shrub establishment, including potentially weedy natives such as *Baccharis halimifolia* L. (see Appendix), which may be an important consideration in the recovery of mesic grasslands (Van Deelen 1991; Lett and Knapp 2003).

Local site conditions (Barnes 2004; Sanderson 2014) and disturbance may determine plant seed bank and community composition of grazed pastures (Goslee and Sanderson 2010). We found large differences in taxa among blocks, with a number of species limited to only one block. Blocks A and C had very distinct communities, including a number of unique taxa, such as *Asimina* sp. and *Galactia elliottii*. The Till plots in Block E also showed separation from the other plots, again with a large number of unique taxa. Variation in groundwater depth, and presumably soil moisture, across the site may have contributed to the localized variation seen within the pasture. The greatest between-treatment differences in total and native species richness occurred in Blocks A and E, suggesting that the effects of tilling were accentuated only in the driest and wettest blocks, respectively.

Compared to cultivation or haying, grazing is a low-impact management regime that may limit the seed bank to fewer, but persistent, perennial species (Auestad et al. 2013). Individual species may have growth habits that allow them to persist or possibly thrive in the diverse hydrological and disturbance conditions prevailing in

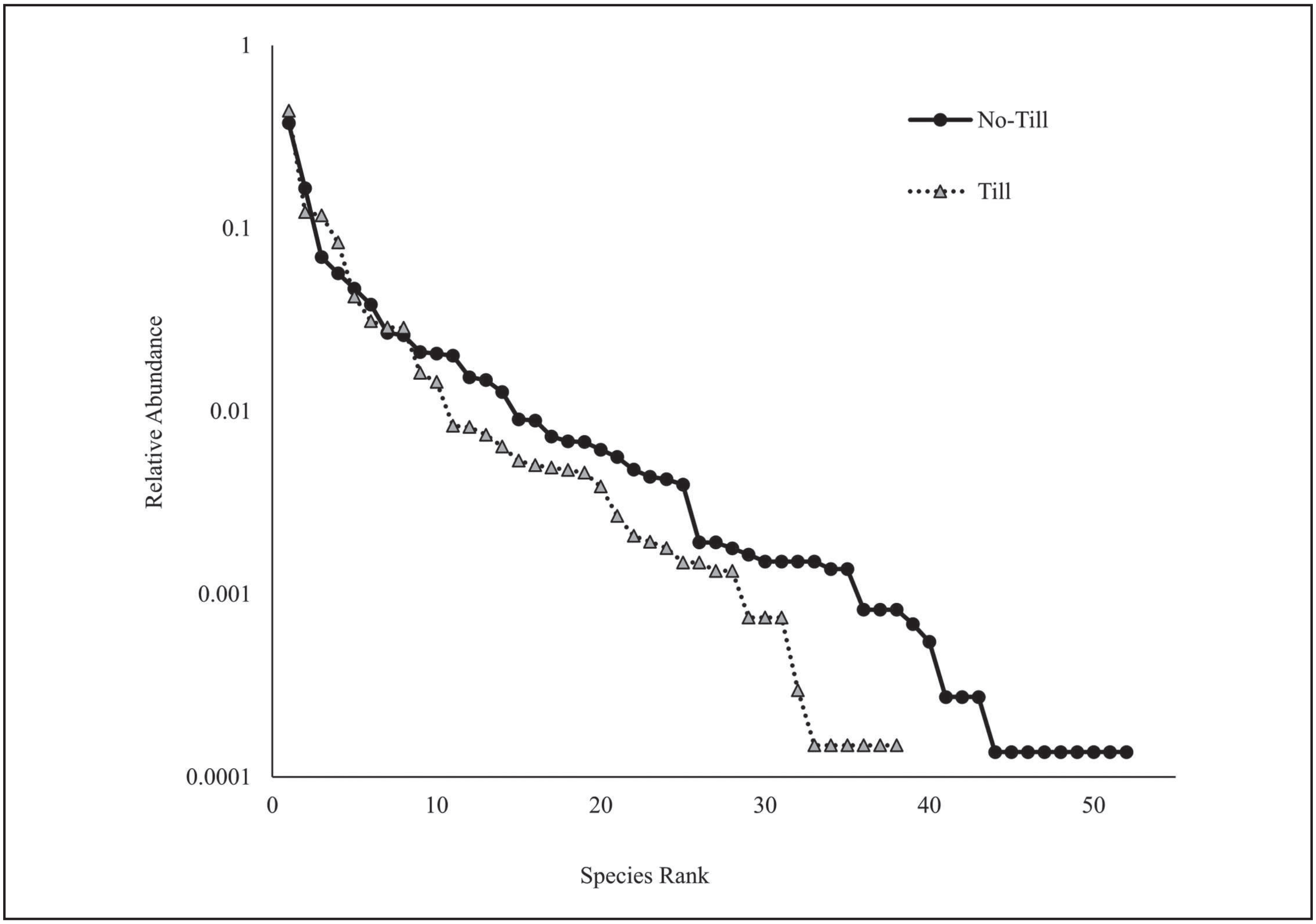

Figure 2. Relative abundance of species (average percent cover) versus ordered rank of species abundance on a log scale in experimental Till and No-Till plots.

this experiment. For example, *Eupatorium capillifolium* (Lam.) Small and *Rhynchospora miliacea* (Lam.) A. Gray were the most dominant species after bahiagrass. There were, however, more species that were found only in one block than there were species found only in one treatment. Overall, our results imply that the hydrology of the site may be more important to initial seedling establishment than additional mechanical disturbance. Although passive field conversion approaches that rely solely on hydrological restoration may achieve increased plant diversity (Ficken and Menges 2013), they may be influenced by local environmental conditions, which could differ within and between sites, making for a less predictable restoration result (Goslee and Sanderson 2010). Passive restorations may also contain fewer desirable native plants, especially species that are dispersal-limited or do not readily germinate from seed, such as cutthroat grass (C. Matson, pers. obs.). Depending on restoration goals, active planting may be needed to attain the desired outcomes (Wetzel et. al. 2001; Zedler 2005; Standish et. al. 2007).

CONCLUSIONS

The goal of this experiment was to understand the response of a cattle pasture to the removal of the dominant pasture grass and to the additional disturbance of shallow cultivation. Initial removal of the dominant bahiagrass proved crucial for any restoration efforts, and further research may be needed to determine the most efficient and effective technique for this first step. However, undertaking further mechanical field preparations beyond the removal of the dominant pasture grass may not accrue widespread benefits for native grassland restoration, except in the removal of woody or shrubby plants.

Within the first year following removal of the dominant monoculture, we observed establishment of a large number of predominantly perennial species. We presume most were contained in the previously suppressed seed bank, although some may also have established via seed dispersal from adjacent native areas. Even at this relatively small spatial scale, hydrological or other environmental variation influenced the species that responded to the removal of the dominant pasture grass by herbicide and tillage. Addressing this within-site variation may be critical to the success of native plant restoration in similar landscapes.

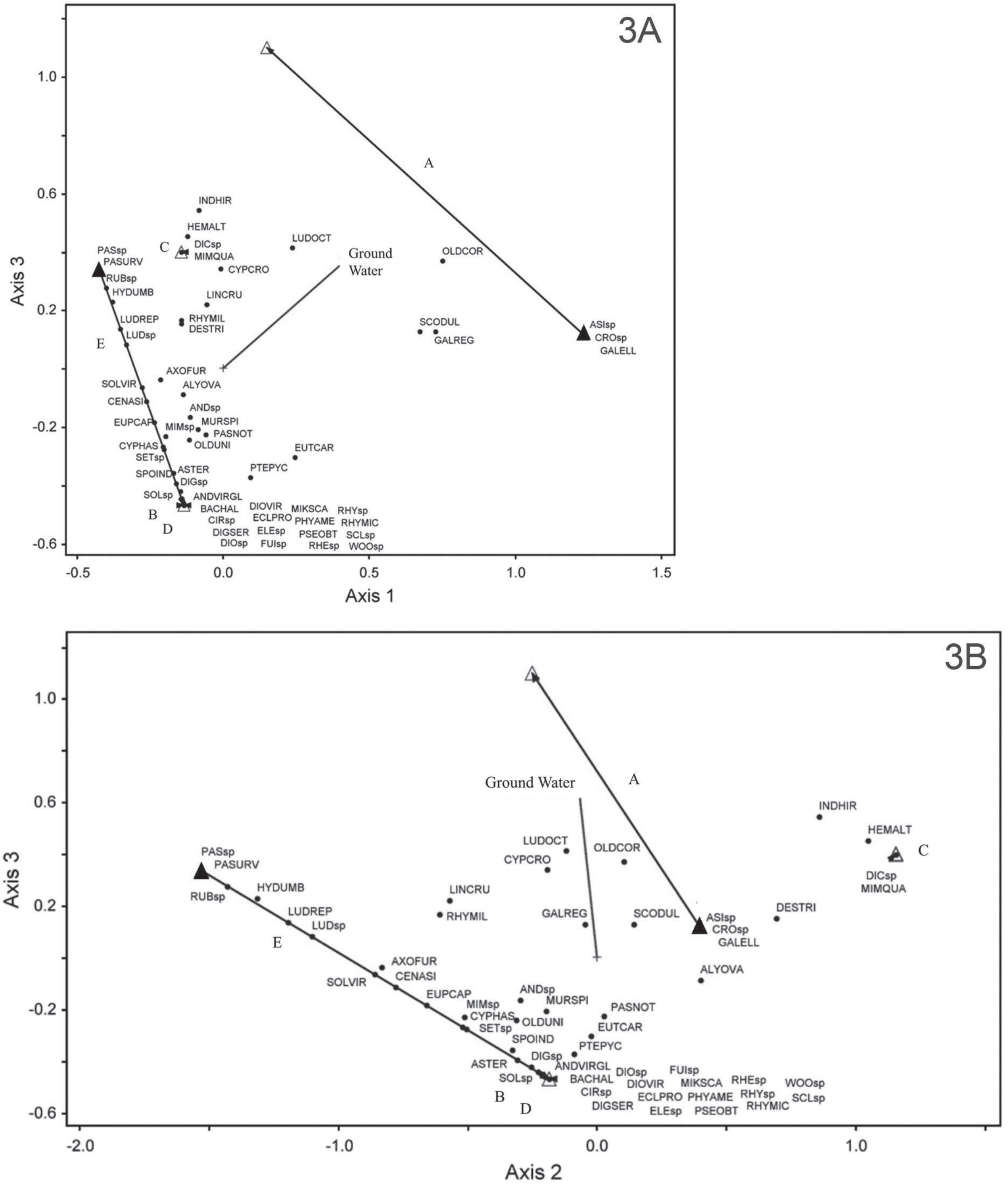

Figure 3. NMS ordination of percent cover of native species within experimental plots. Blocks are labeled with letters, the No-Till treatment is represented by black triangles, and the Till treatment by open triangles (*P* = 0.2455, 0.2295, and 0.0020 for axis 1, 2, and 3 respectively). Treatments within the same block are connected by a line, ground water depth is plotted as a vector, and labeled species are plotted as dots in ordination space. Reference plots were not included in the ordination.

ACKNOWLEDGMENTS

We would like to thank the staff of Archbold Biological Station for their invaluable help with implementing this study, in particular: K. Main, B. Crawford, and the ABS maintenance crew for burning and tilling the experimental plots; E. Boughton as well as the ABS Plant Ecology Program for numerous plant identifications and analytical guidance; and H. Goldspiel, Z. Forsburg, and S. Dillon for helping with field data collection. We also thank the Florida State Office of the Natural Resources Conservation Service for assisting with WRP implementation, and Lightsey Cattle Co. for establishment of the exclosures.

Rebecca C. Tucker, M.S., was the Research Assistant in the Restoration Ecology and Land Management programs at Archbold Biological Station from 2013 to 2016. Her work focused on implementing and assessing outcomes of NRCS Wetlands Reserve Program restorations on a working cattle ranch.

Betsie B. Rothermel, Ph.D., is a research biologist with more than 14 years of experience conducting applied ecological research in the southeastern United States, with a particular focus on the ecology and conservation of amphibians and reptiles in Coastal Plain and Blue Ridge ecosystems. As a Research Program Director at Archbold Biological Station (since 2008), she coordinates wetland restoration projects and conducts research in agriculturally impacted wetlands on the southern Lake Wales Ridge in Florida.

Joshua H. Daskin, M.Sc. and M.A., is a former Research Assistant in the Restoration Ecology program at Archbold Biological Station. He is currently a Ph.D. candidate at Princeton University studying war-driven mammal declines in Mozambique, as well as furthering his studies of amphibian response to fire in seasonal ponds in the Florida scrub.

Footnote: Matt Bahm, Associate Editor, served as Editor for this manuscript due to a potential conflict of interest with the current Editor, Eric Menges.

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APPENDIX: Identifiable seedlings present in the Till, No-Till, and Reference plots in both the first and second sampling period; * indicates an annual species, ** a perennial species, and * a biennial species according to the USDA Plants Database (US Department of Agriculture 2015).**

	Till	No- Till	Ref
NATIVE GRAMINOIDS			
Andropogon sp.	x	x	x
Andropogon virginicus var. *glaucus* Hack.**		x	
Axonopus furcatus (Flüggé) Hitch.**	x	x	
Cyperus croceus Vahl**	x	x	
Cyperus haspan L.**	x	x	
Dichanthelium sp.		x	
Digitaria serotina (Walter) Michx.*	x	x	
Digitaria sp.	x	x	
Eleocharis sp.	x	x	x
Fuirena sp.		x	
Paspalum sp.		x	
Rhynchospora microcarpa Baldwin ex A. Gray**		x	
Rhynchospora miliacea (Lam.) A. Gray**	x	x	
Rhynchospora sp.	x	x	
Scleria sp.		x	
Setaria parviflora (Poir.) Kerguélen**	x	x	
NATIVE FORBS			
Aster sp.	x	x	
Buchnera americana L.*, **, or ***			x
Centella sp.	x	x	
Cirsium sp.	x	x	
Diodia sp.	x		
Diodia virginiana L.* or **		x	
Eclipta prostrata (L.) L.* or **	x		
Euthamia caroliniana (L.) Greene ex Porter & Britton**	x	x	x
Galactia elliottii Nutt.**		x	
Galactia regularis (L.) Britton, Sterns & Poggenb.**	x	x	
Hydrocotyle umbellata L.**	x	x	x
Ludwigia octovalvis (Jacq.) P.H. Raven**	x	x	
Ludwigia repens J.R. Forst.**	x	x	
Ludwigia sp.	x	x	
Mikania scandens (L.) Willd.**		x	
Mimosa quadrivalvis L.**		x	
Mimosa sp.	x	x	
Oldenlandia uniflora L.*	x	x	x
Phytolacca americana L.*		x	
Pseudognaphalium obtusifolium (L.) Hillard & B.L. Burtt* or ***	x	x	
Pterocaulon pycnostachyum (Michx.) Elliott**		x	
Rhexia sp.	x		x
Scoparia dulcis L.* or **	x	x	
Solidago sp.	x	x	
Woodwardia sp.		x	

Continued

APPENDIX (*Continued*)

NATIVE SHRUBS			
Asimina sp.		x	
Baccharis halimifolia L.**		x	
Crotalaria sp.		x	
Eupatorium capillifolium (Lam.) Small**	x	x	x
Hypericum sp.			x
Rubus sp.		x	
NON-NATIVE GRAMINOIDS			
Hemarthria altissima (Poir.) Stapf & C.E. Hubbard**	x	x	
Paspalum notatum Flueggé**	x	x	x
Paspalum urvillei Steud.**		x	x
Sporobolus indicus (L.) R. Br.**	x	x	x
NON-NATIVE FORBS			
Alysicarpus ovalifolius (Schumach.) J. Léonard* $^{\text{or}}$ **	x	x	
Desmodium triflorum (L.) DC.**	x	x	x
Indigofera hirsuta L.*	x	x	
Lindernia crustacea (L.) F. Muell.* $^{\text{or}}$ **	x	x	
Ludwigia peruviana (L.) H. Hara**			x
Murdannia spirata (L.) Bruckner* $^{\text{or}}$ **	x		
Oldenlandia corymbosa L.* $^{\text{or}}$ **	x	x	
NON-NATIVE SHRUBS			
*Solanum viarum***	x	x	

	Till	No-Till	Ref
Native graminoids	10	16	2
Native forbs	17	21	5
Native shrubs	1	5	2
Non-native graminoids	3	4	3
Non-native forbs	6	5	2
Non-native shrubs	1	1	0
Total:	38	52	14