Hardwood Reduction in Pinelands
Second Progress Report
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Abstract
We conducted a randomized, controlled trial of four different herbicide formulations for use in woody-encroached longleaf pine sandhills, applied using a semi-directed foliar spray method along with a control: 1) Imazapyr (low rate), 2) Imazapyr (high rate), 3) Fosamine/Triclopyr/Aminopyralid mix, 4) Imazapyr/Triclopyr mix, and 5) Control (no treatment). All four of the herbicide formulations caused large and significant decreases in the measured oak parameters, effectively reducing oak cover and oak stem density in all herbicide treatments. By contrast, herbaceous cover and herbaceous species richness were affected relatively little by the herbicide treatments. Multivariate analysis detected significant post-treatment changes in herbaceous plant community composition that did not occur in the control plots, suggesting that the herbicide treatments caused short-term changes in the relative abundances of native herbaceous species. Additional analysis and longer-term follow-up monitoring will be needed to elucidate the nature of the herbaceous plant community changes.

Introduction

Florida's longleaf pine savannas are fire-maintained ecosystems, which are thought to have experienced a pre-industrial fire regime of low-intensity surface fires recurring every 2-3 years (Frost 2006). The vast majority of remnant longleaf pine savanna fragments have experienced moderate to severe fire suppression relative to the pre-industrial period (Heyward, 1939; Varner & Kush, 2004). When maintained with frequent fire, longleaf pine savannas are characterized by very high levels of biodiversity and endemism (Noss 2015); however, in the absence of fire, woody encroachment and attendant leaf litter accumulation suppress understory herbaceous growth and lead to dramatic local and regional biodiversity loss (Hiers et al. 2007, Freeman et al. 2019).

Mechanical and chemical woody reduction treatments have been widely used to help re-create fine fuel structures that facilitate the application of frequent prescribed fire in longleaf pine systems (Van Lear et al. 2005). Reduction of woody cover in longleaf systems is correlated with increased herbaceous cover and richness (Provencher et al. 2001), due to an increase in light penetration and decrease in suppressive leaf litter (Hiers et al. 2007). When successful, woody reduction treatments can lead to the re-establishment of a herbaceous-dominated understory, especially on sites with remnant groundcover (Provencher et al. 2001, Van Lear et al. 2005,Hiers et al. 2007, Freeman & Jose 2009, Kirkman et al. 2013).

Though widely used, mechanical and chemical treatments are not always successful. One of the most difficult obstacles to the restoration of longleaf pine savannas is the re-sprouting of woody species after removal of above ground biomass by mechanical means or fire (Diaz & Putz 2017). Resprouting ability is a widespread adaptation among woody plants, facilitating persistence in frequently-disturbed ecosystems (Bond and Midgley 2001). Resprouting plants can respond to many types of disturbances that result in topkill by quickly re-growing aboveground biomass from buds located at or below the soil surface (Bond and Midgley 2003).

Intensive mechanical techniques are reported to be effective for preparing sites for longleaf pine establishment (Johnson and Gjerstad, 2006), but are very detrimental to wiregrass (Clewell 1989), and therefore they are not the best options on sites where understory conservation is desired. In addition, mechanically clipped plants typically have more stems post treatment than plants top killed by fire (Williges et al. 2014, Hmielowski et al. 2014). Thus, mowing or roller chopping to reduce woody cover appear to be only short-term remedies (Williges et al. 2006). Felling/girdling is also an effective treatment for shrub cover reduction (Provencher et al. 2001), but the application process is very laborious and time intensive at the landscape level, and the same issues with re-sprouting exist (Diaz & Putz 2017). Herbicides are another fairly commonly applied hardwood control technique for natural areas managers in the southeast, but relatively few studies have been conducted on the use of herbicides in longleaf pine savanna restoration, and it can be difficult for managers to find specific guidance on rates, methods, and natural community impacts for herbicides.

Hexazinone (Velpar) is probably the most common herbicide used in the southeast for control of oaks in natural areas, and several studies have shown that it is effective at reducing oak and other hardwood cover with minimal impacts to herbaceous groundcover vegetation (Brockway et al. 1998, Brockway & Outcalt 2000, Provencher et al. 2001, Freeman & Jose 2009). Triclopyr (Garlon) has been widely used throughout the region since 1980, and basal bark applications are effective in controlling a wide range of woody species (Yeiser 2000). Atwater (2017) describes triclopyr as "the equivalent of a hot summer fire in a bottle" which will attack all broadleaved plants. Triclopyr has the advantage of no residual soil activity. Long term control varies with the species, size, density, timing of application, and the general vigor of the plants prior to, and at the time of application (Atwater 2017). Imazapyr (Arsenal) is described as perhaps the most versatile and flexible herbicide because selectivity can be affected by rate, timing, and tank mix (Atwater 2017). Despite its persistent soil activity, low rates of imazapyr (0.21 kg/ha) have been used effectively as a control of understory shrubs in longleaf pine flatwoods without negatively impacting groundcover (Freeman & Jose 2009). An herbicide mix often used for roadside right-of-way control of hardwoods is a Krenite S (fosamine)/Trycera (triclopyr)/Milestone (aminopyralid) mixture. This tank mix is reported to be effective at promoting grass establishment, with minimum impacts to longleaf and slash pine as it has no residual soil activity (Greg Boozer, Helena Chemical Co., personal communication). This mix has thus far not been widely used or studied in natural areas.

In this study, we compared four different herbicide formulations for use in woody-encroached longleaf pine sandhills, applied using a semi-directed foliar spray method along with a control: 1) Imazapyr (low rate), 2) Imazapyr (high rate), 3) Fosamine/Triclopyr/Aminopyralid mix, 4) Imazapyr/Triclopyr mix, and 5) Control (no treatment). We did not test hexazinone, since it is already well-studied in sandhills with remnant old-growth understory vegetation. We applied these treatments to nine study blocks at three different sandhill preserves, each with pre-treatment conditions characterized by moderate to severe oak encroachment as well as remnant native groundcover. The purpose of the study was to evaluate the effectiveness of the different herbicide treatments for controlling target oak species, as well as to evaluate direct and indirect effects on nontarget native groundlayer species.

Methods

Study sites: The study sites were fire-suppressed sandhills located at Chassahowitzka Wildlife Management Area, Perry Oldenburg Mitigation Park (Hernando County), and Watermelon Pond Wildlife and Environmental Area (Alachua County) (Appendix A, Maps of Study Sites). All of the study plots were established in fire-suppressed sandhills with moderate to heavy oak encroachment and low to moderate herbaceous understory cover, with many remnant old growth savanna groundlayer species present in low to moderate cover levels. The Perry Oldenburg study site, which is on Arrendondo fine sands sloping 0-8%, had the highest levels of remnant herbaceous cover prior to treatment. The Watermelon pond study site is on Candler fine sand with a slope of 0-5%. Laurel oak and sand live oak were the most prominent oak species found at Watermelon Pond, and remnant herbaceous cover ranged from absent to patchy. The Chassahowitzka study site is on Candler fine sands and was predominantly encroached by myrtle oak and sand live oak, with moderate, patchy remnant native groundcover consisting of many old-growth savanna specialists.

Treatments: Three study blocks were established at each of the three sites, within which each of 5 treatment plots received one of the following treatments: 1) **Imazapyr (low rate)**: Arsenal or Polaris AC (12 oz/ac imazapyr) + 32 oz/ac Methylated Seed Oil surfactant referred to as LRIMAZ throughout the report; 2) **Imazapyr (high rate)**: Arsenal or Polaris AC (16 oz/ac imazapyr) + 1 gal/ac Methylated Seed Oil surfactant (HRIMAZ); 3) **Fosamine/ Triclopyr/ Aminopyralid mix**: Krenite S (64 oz/ac) + Trycera (triclopyr, 32 oz/ac) + Milestone (7 oz/ac aminopyralid) + Methylated Seed Oil surfactant (24 oz/ac) (KTM); 4) **Imazapyr/Triclopyr mix:** Arsenal or Polaris AC (16 oz/ac) + Trycera (32 oz/ac) + Methylated Seed Oil surfactant (24 oz/ac) (TRYIMAZ); and 5) **Control** (no treatment).

Semi-directed foliar spray was applied using a truck-mounted tank and hose. The hose allowed for a large volume of herbicide to be directed at the oak layer with more control than an aerial broadcast application, but less control than a targeted application (such as basal bark or hack and squirt). The target species were sand live oak, myrtle oak, laurel oak, and turkey oak with stems < 8 cm DBH. Turkey oak was avoided at Perry Oldenburg per request of the WEA biologists. Herbicide treatments took place in 2018 at: Perry Oldenburg on June 5th- June 11th, Chassahowitzka on June 11th - June 18th and Watermelon Pond June 18th– June 27th (Appendix 1 & 2).

Sampling: Within each 1-acre treatment plot boundary, 10 random sample points were established at least 5 m apart, with an internal 10m buffer along the plot perimeter to minimize any potential edge effects. The sample points were permanently marked on the ground within the plot using 18" metal tag stake and fitted with a consecutively numbered metal tag. A 2-m radius circular quadrat was centered on each sample point (**Figure 1**). All herbaceous plants rooted

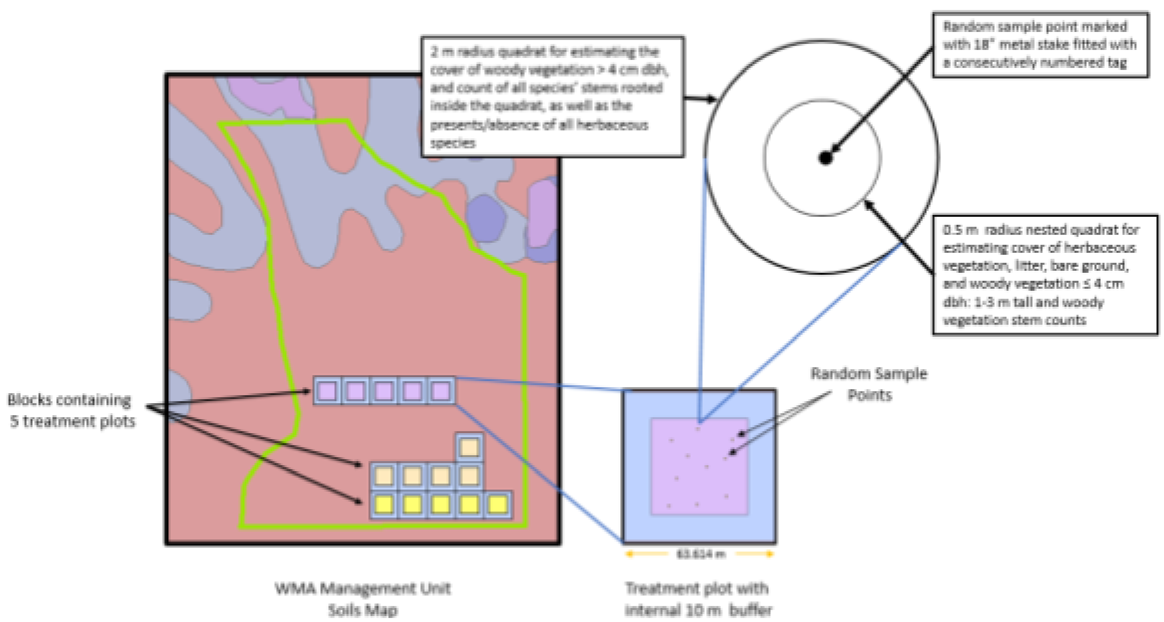

Figure 1. Schematic diagram showing the block, plot, and sampling point design.

within the 2m quadrat were identified to species. Woody vegetation cover, occurring but not required to be rooted within, was visually estimated in the 2-m radius quadrat with no height restrictions on observations. All visual cover estimates in this study were taken using the Daubenmire cover class system which minimizes inter-observer variability. Stems of woody species rooted within the 2-m radius quadrat with a DBH ≥ 4-cm were also recorded for each species. Within the central 0.5m-radius circle, we visually estimated the percent cover of ground strata including herbaceous vegetation, woody vegetation <3m, litter, and bare ground and counted the stems of woody plants with a DBH < 4-cm. Pre-treatment baseline vegetation data was collected in April and May of 2018, and post-treatment response data was collected in April and May of 2019.

Analysis: For this progress report, we conducted univariate analysis on pre- and post-treatment point-level means of 6 variables (total oak cover (2m circle), oak cover <3m (1m circle), oak stem density >4cm dbh (2m circle), oak stem density <4cm dbh (1m circle), herbaceous species cover (1m circle), and herbaceous species richness (1m circle)). For clarity of interpretation and reporting, we assessed whether any variables could be dropped due to redundancy or sparseness, and determined that oak cover <3m was redundant with total oak cover, and the oak stem density >4cm dbh parameter was too sparse to be analyzed statistically (typically only 0 to 2 large oaks were present in the circle, with mostly zeroes). The oak cover results reported here are from that 2m-radius circle parameter that included all heights, and the oak stem density results are for oaks <4cm dbh taken in the smaller circle. We used a mixed-model, repeated-measures ANOVA to analyze these variables using linear models in the R package lme4, with *Treatment* and *Time* as a fixed effects and *Block* and *Site* as nested random effects. For non-normally distributed variables (of the retained variables, this included only oak stem density), we used a generalized linear mixed model with a Poisson distribution, also in lme4. Within treatments, we compared pre- and post-treatment means using Tukey's HSD test.

In order to explore multivariate patterns of change in vegetation structure, we conducted Principal Components Analysis (PCA) using the R stats (v.3.6.2) and ggbiplot packages, on a matrix containing oak cover (all heights), oak stem density (<4cm dbh), and herbaceous cover (all species). We obtained single plot-level values for the ordination by summing the point-level data for each variable within each plot. The data were scaled and square-root transformed to meet the assumptions of PCA. We also conducted Non-Metric Multidimensional Scaling (NMDS) to explore differences between groups in herbaceous plant species composition using a Bray-Curtis distance measure. NMDS is a type of unconstrained ordination that plots points according to their similarity/dissimilarity to each other and produces a visualization plot similar to PCA, in which points more similar to each other are plotted closer together. NMDS is more suitable than PCA for data containing a large number of zeroes (as is the case with most species-level plant community composition data) because it can be used with a variety of distance measures appropriate for such data, whereas PCA can only be used with a Euclidean distance measure. When group differences are suspected based on inspection of the NMDS plot, PerMANOVA (nonparametric multivariate ANOVA) can be conducted in order to determine whether observed differences between groups are significant. Species with fewer than 3 occurrences were dropped from the data prior to conducting NMDS and PerMANOVA, because rare species can exert an undue influence and create noise in multivariate results. We conducted NMDS and PerMANOVA using the vegan package in R (Oksanen et al. 2013).

Results

Table 1. Mixed-model ANOVA comparison of mean percent oak cover in the 2m quadrat and mean oak stem count in the 1m quadrat among treatments at Time 1 and Time 2.

		Oak cover (2m)		Oak stems (1m)	
Factor	**Df**	**F**	**p**	**F**	**p**
Treatment	4	5.22	0.0003	122.7	<0.0001
Time	1	312.9	<0.0001	524.1	<0.0001
Treatment*Time	4	21.1	<0.0001	128.0	<0.0001
Site variance	2	0.0		0.1	
Block variance	8	0.7		0.1	

A mixed-model, repeated-measures ANOVA with *Treatment* and *Time* as fixed effects and *Block* and *Site* as nested random effects showed that overall there were significant differences in mean percent oak cover in the 2m-radius circle at Time 1 (pre-treatment) and Time 2 (post-treatment), as well as significant variation between treatments (**Table 1**). There was also a significant *Treatment* x *Time* interaction, indicating differences among treatments in the amount of change over time (**Table 1**). The strength of the treatment effect was primarily due to the lack of change in the Control treatment (**Figure 2**), which differed strongly from all the other treatments, showing no significant pre- and post-treatment change in oak cover, whereas all of the herbicide treatments had very significant pre- and post-treatment changes in oak cover (Tukey's HSD, $p < 0.0001$ for pre- and post- treatment change within all herbicide treatments).

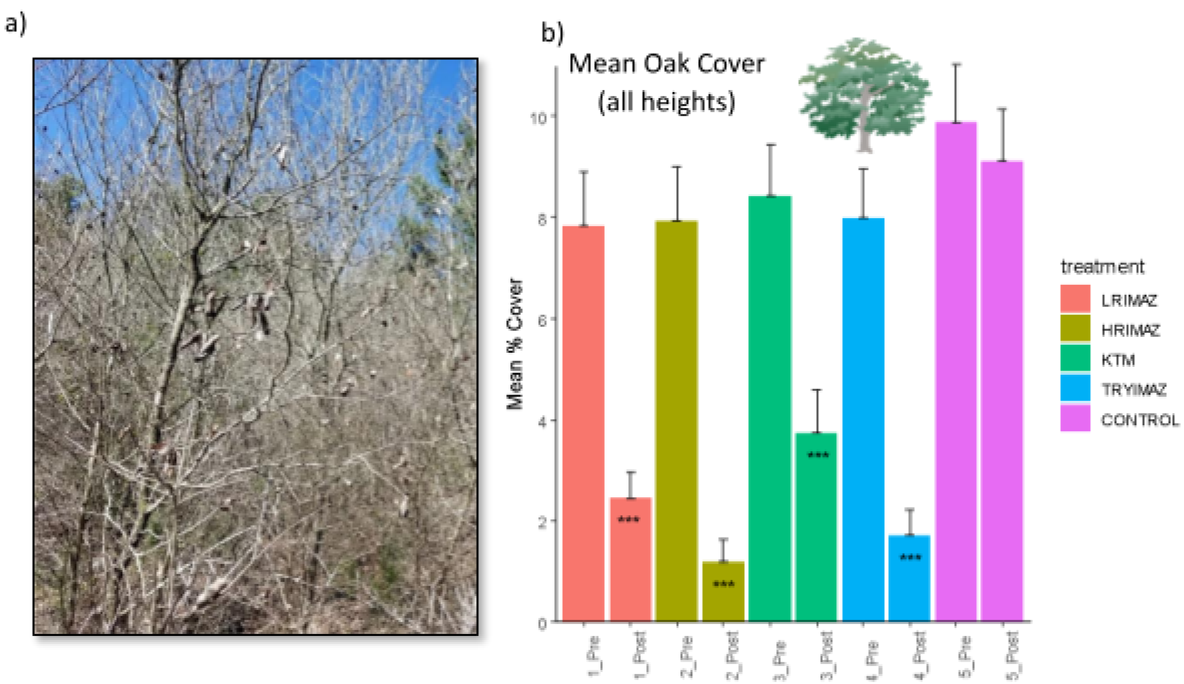

Figure 2. a) Post-treatment oak stems killed by herbicide. b) Pre- and post-treatment change in oak cover (all heights) at the 2m quad level in all treatments. (***) indicates $p < 0.0001$, based on Tukey's HSD test.

Table 2. Mixed-model ANOVA comparison of mean herb cover and mean herb richness at the 1m-quadrat level among treatments at Time 1 and Time 2

		Herb cover		**Herb richness**	
Factor	**Df**	**F**	**p**	**F**	**p**
Treatment	4	1.32	0.2619	1.65	0.16
Time	1	31.24	<0.0001	27.07	<0.0001
Treatment*Time	4	0.52	.072	0.35	0.85
Site variance	2	4.5		0.65	
Block variance	8	0.7		0.09	

There were no significant treatment differences overall in herbaceous cover or herbaceous species richness based on a mixed-model, repeated-measures ANOVA model with *Treatment* and *Time* as fixed effects and *Block* and *Site* as random effects (**Table 2**), though there was a significant effect of *Time* for both herbaceous parameters. All of the treatments, including the control, decreased both herbaceous cover and herbaceous species richness slightly between Time 1 and Time 2 (**Figure 3**), but this change was only significant for herb cover in low-rate imazapyr treatment, and herb richness in the high-rate imazapyr treatment. The random *Site* variable contributed a substantial amount to the variance in quadrat-level herb cover, most likely due to much higher herbaceous cover at Perry Oldenburg (both pre- and post-treatment) than any of the other sites.

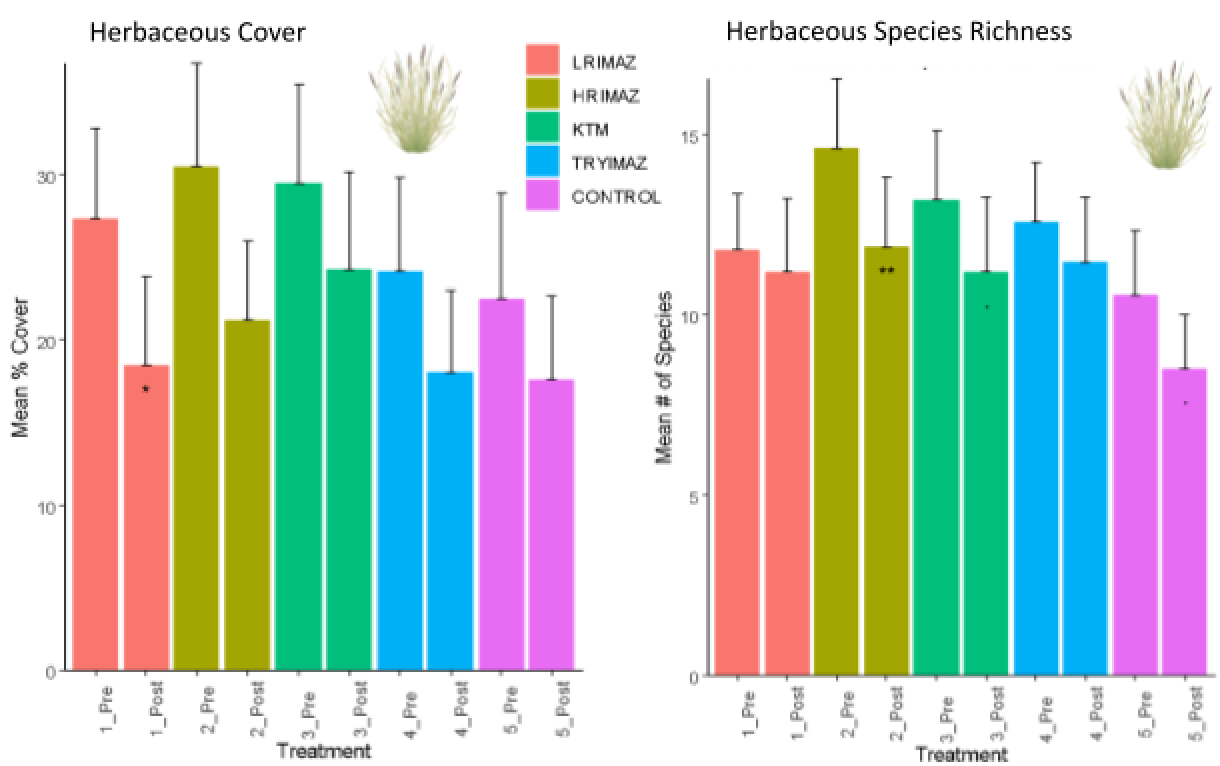

Figure 3. Pre- and post-treatment herbaceous plant cover (left) and species richness (right) at the 1m quad level in all treatments. (*) indicates a significant post-treatment change in understory within a treatment at the p < 0.05 - 0.001 level, and (**) indicates a significant change at the p < 0.001 - 0.0001 (Tukey's HSD test, α = 0.05).

Principal Components Analysis (PCA) of a matrix containing mean oak cover, herbaceous cover, and oak stem density summed for each 1-acre treatment plot at Time 1 and Time 2 showed very similar directional trajectories among all herbicide-treated plots, though the amount of change (indicated by the length of the arrows between the pre- and post-treatment point locations) varied (**Figure 4**). The two PCA axes shown in Figure 2 explain a total of 92.5% of the variation between plots, indicating that this two-dimensional plot is a very good representation of the differences between plots in these three structural variables. The majority of herbicide-treated plots showed little change along the herbaceous cover gradient, but experienced dramatic change along the oak cover and oak stem density gradients. In contrast, the control plots changed very little and did not move in consistent directions. Plot trajectories pointing slightly down and to the left on the PCA plot, parallel to the oak cover and oak stem density gradients, indicate decreases in the oak cover/oak stems with relatively little change in herbaceous cover. Steeper downward angles, which are in the minority, indicate more substantial changes in herbaceous cover.

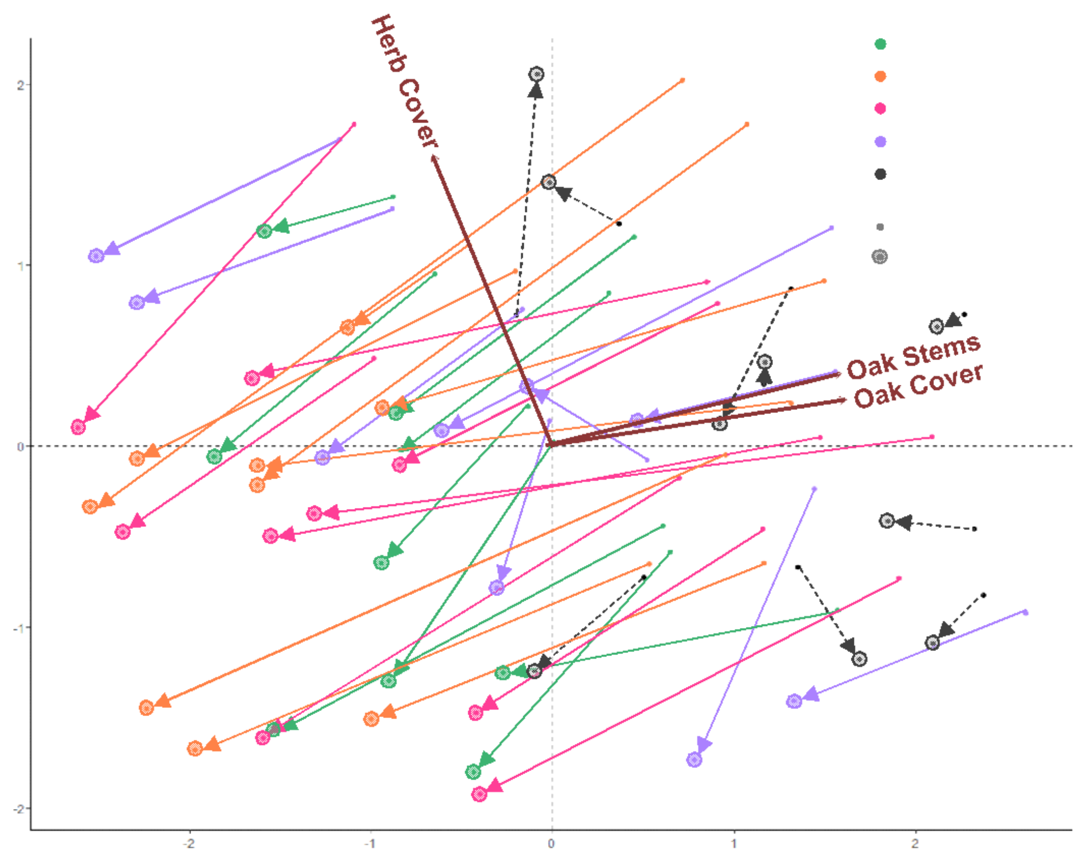

Figure 4. Principal Components Analysis of Year 1 and Year 2 mean plot-level herbaceous cover, oak cover, and oak stem density. Arrows between points represent the magnitude and direction of change in the same plots, with the base of the arrow (small closed circle) at the pre-treatment position of the and the end of the arrow (large open circle) at the post-treatment position of the plot. The bold biplot lines show the strength and direction of the relationships between the variables and the PCA axes.

Non-metric multidimensional scaling (NMDS) ordination of a matrix containing plant species percent cover values, summed for each 1-acre treatment plot (**Appendix 2,** example species data matrix), showed a clear distinction between the herbaceous plant communities on the three study sites, as well as a separation between years for the treated plots on all sites (circles vs. triangles) (**Figure 5**). PerMANOVA based on the individual 1m-circle plant species % cover data points showed that the pre-treatment herbaceous plant community composition differed significantly from the post-treatment composition in all of the herbicide treatments: KTM ($p = 0.001$), LRIMAZ ($p = 0.001$), TRYIMAZ ($p = 0.001$), HRIMAZ ($p = 0.001$), but not the control treatment ($p = 0.373$). If desired, further analysis can be done to identify the changes in presence/absence or abundance of individual plant species that are resulting in the overall compositional change detected by NMDS and PerMANOVA.

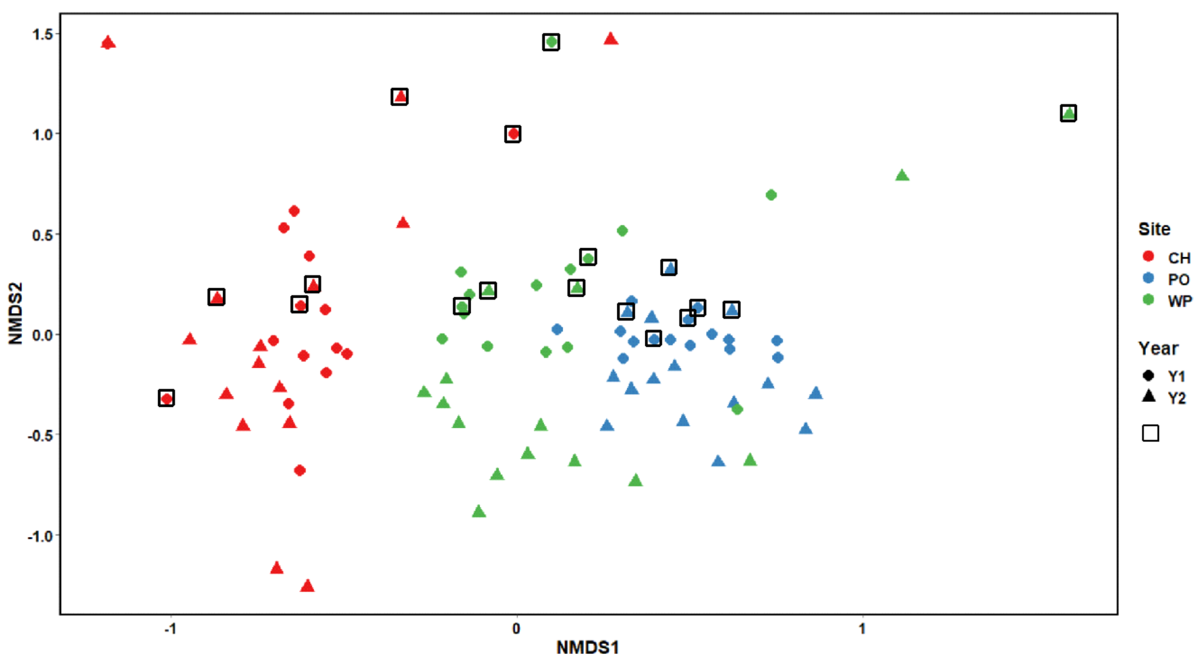

Figure 5. NMDS plot of plant community composition by site and year, based on Bray-Curtis distances between plot-level % cover sums for all species. Sites are indicated by color (red = Chassahowitzka, blue = Perry Oldenburg, and green – Watermelon Pond. Circles denote Year 1 values, and triangles denote Year 2 values. Control treatment plots are indicated by an open square.

Discussion

All four of the herbicide formulations caused large and significant decreases in the measured oak parameters, effectively reducing oak cover and oak stem density in all herbicide treatments. By contrast, herbaceous cover and herbaceous species richness were affected relatively little by the herbicide treatments. We measured small but significant decreases in herbaceous cover and herbaceous richness in the low-rate imazapyr and high-rate imazapyr treatments, respectively; however, decreases of similar magnitude were also evident in the control plots. The slight decreases in herbaceous species richness and cover in the control plots could be the result of natural successional processes: without fire, the accumulation of oak litter and oak growth between years can be expected to suppress herbaceous cover and diversity (Hiers et al. 2007). Similarly, the observed decreases in herbaceous cover and species richness in the herbicide plots could be due in part to litterfall from dead oaks as well as direct foliar impacts from the herbicide spray, which was directed at the oak layer. Litter cover was measured in this study and will be analyzed in the final report, which will include post-fire results following prescribed fires that are currently being conducted on all of the study plots.

While the aggregate herbaceous parameters (percent cover and species richness) suggested only small herbaceous changes in the herbicide treatment plots relative to the controls, multivariate plant community analysis using NMDS and PerMANOVA suggests that significant species compositional changes occurred in the herbicide plots that did not occur in the control plots. Since the univariate analysis did not reveal large changes in herbaceous species richness pre- and post-treatment relative to the controls, it is likely that the multivariate compositional analysis reflects changes in relative abundances of individual species rather than changes in overall species richness. It may be that some herbaceous species were more susceptible than others to direct herbicide impacts or indirect litterfall impacts.

If the herbaceous community changes reflect any negative impacts to native species, they may be short-term and ultimately lead to a more sustainable herbaceous-dominated community, as concluded by other herbicide studies in longleaf pine savannas (Freeman & Jose 2009, Kirkman et al. 2013). We recommend that further multivariate analysis of this plant community data be conducted to elucidate the specific changes in relative abundance of individual species most responsible for driving the multivariate patterns. Additional data collection events following prescribed fires will help determine the longer-term trajectory of herbaceous community assembly following treatment.

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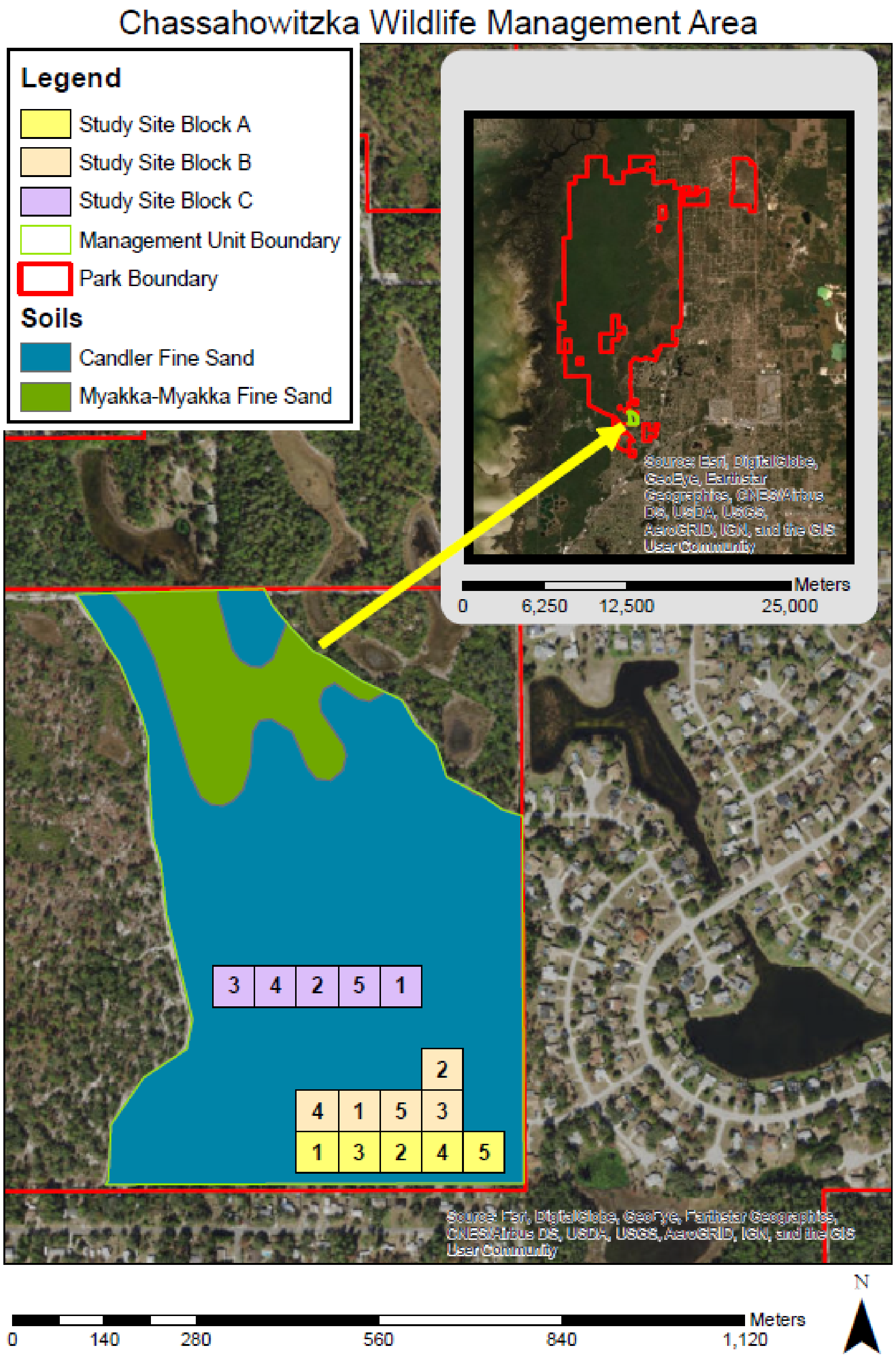
Appendi



Chassahowitzka Wildlife Management Area
Legend
Study Site Block A
Study Site Block B
Study Site Block C
Management Unit Boundary
Park Boundary
Soils
Candler Fine Sand
Myakka-Myakka Fine Sand
Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
Meters
0
6,250
12,500
25,000
3
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Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community
0
140
280
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840
1,120
Meters
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Figure A1. This map identifies the study site, soil types and locations of herbicide treatments at Chassahowitzka Wildlife Management Area in Hernando county Florida. This site is part of the Florida Fish and Wildlife Conservation Commission's study to control encroaching oak species in fire suppressed sandhill plant communities. 1-5 represent the randomized experimental treatments for a given test plot.

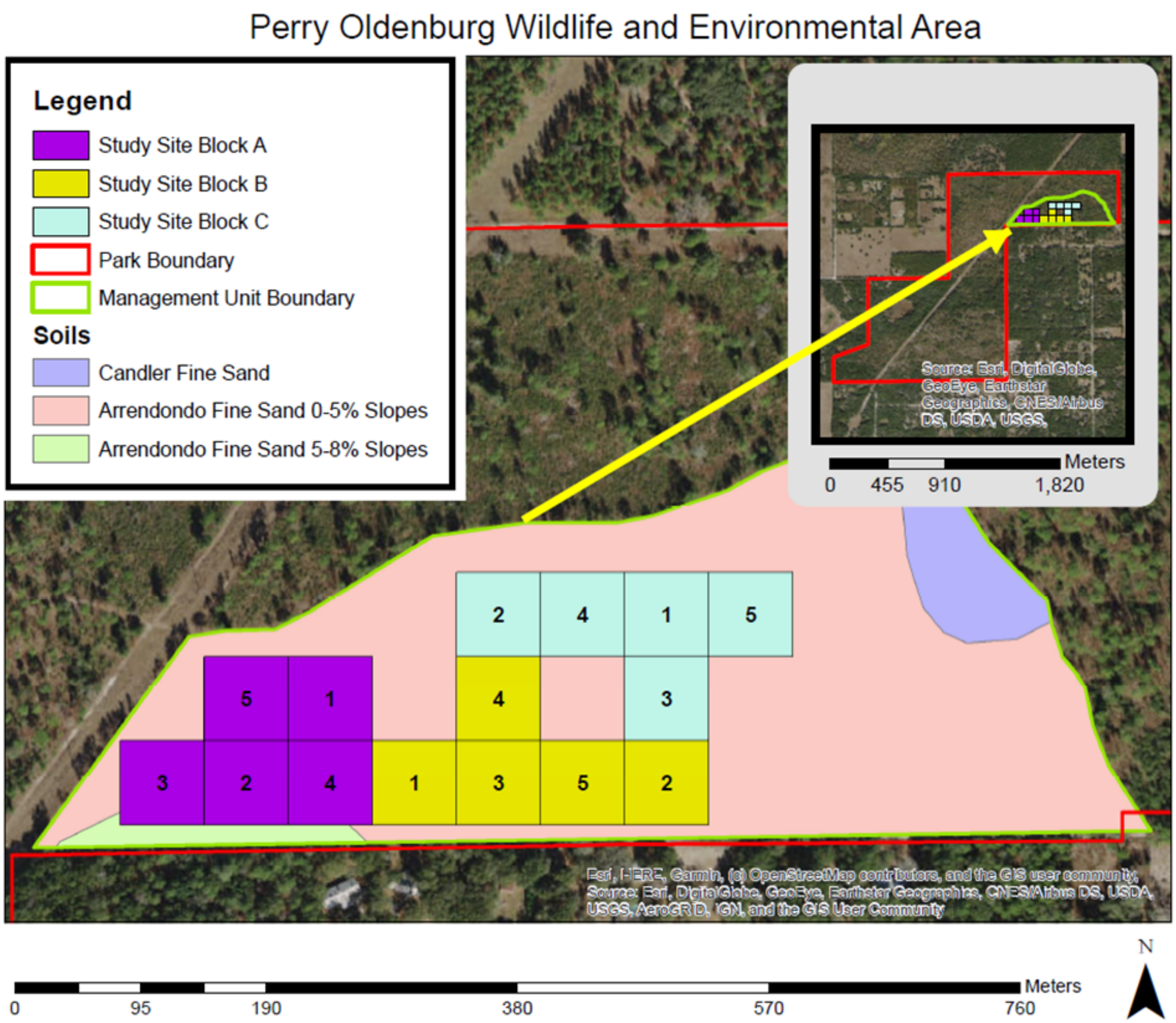

Figure A2. This map identifies the study site, soil types and locations of herbicide treatments at Perry Oldenburg Wildlife and Environmental Area in Hernando county Florida. This site is part of the Florida Fish and Wildlife Conservation Commission's study to control encroaching oak species in fire suppressed sandhill plant communities. This is one of 3 study locations. Numbers 1-5 represent the randomized experimental treatments for a given test plot.

Watermelon Pond Wildlife and Environmental Area

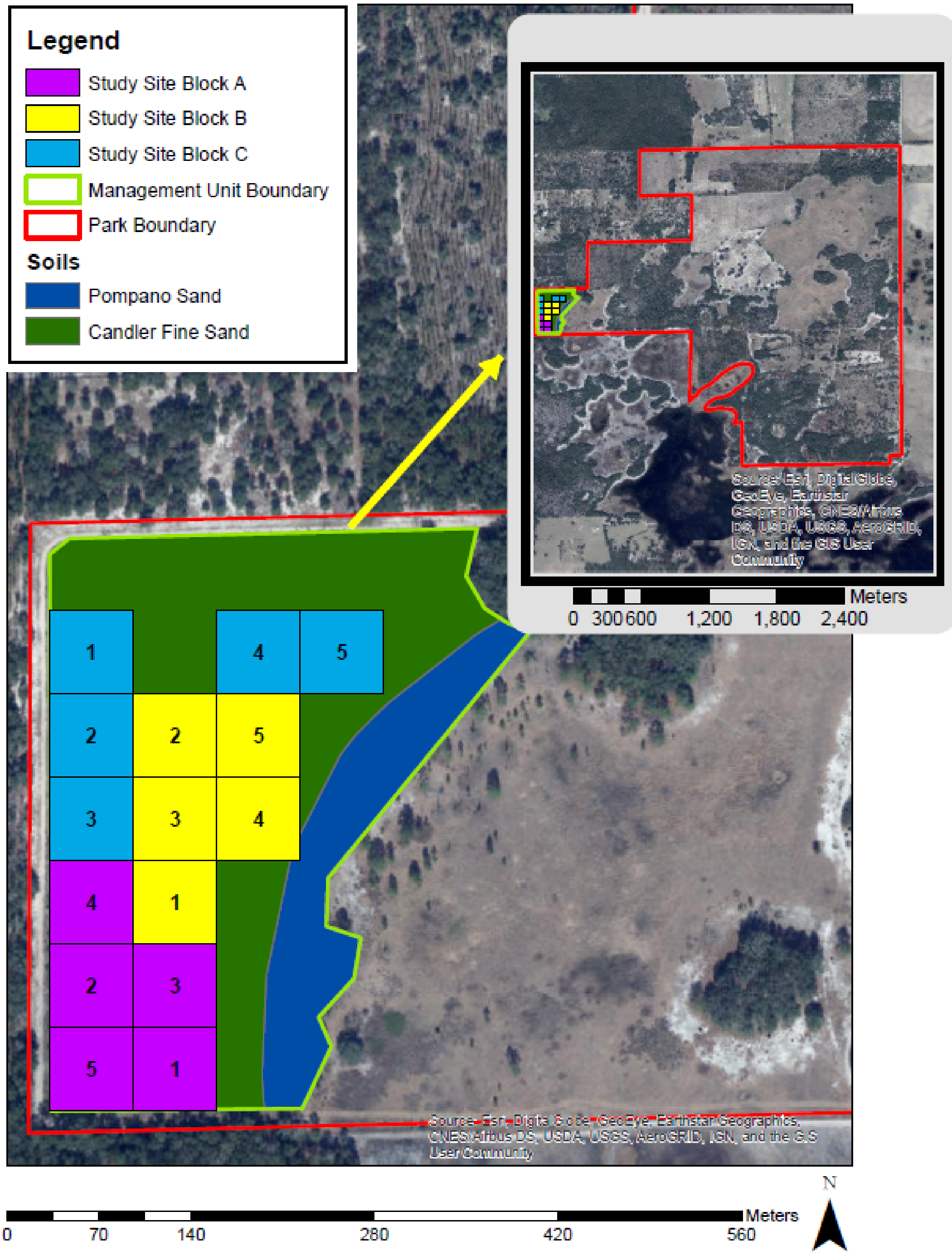

Figure A3. This map identifies the study site, soil types and locations of herbicide treatments at Watermelon Pond Wildlife and Environmental Area in Alachua county Florida. This site is part of the Florida Fish and Wildlife Conservation Commission's study to control encroaching oak species in fire suppressed sandhill plant communities. Numbers 1-5 represent the randomized experimental treatments for a given test plot.

Appendix B. Snapshot of a plot-by-species data matrix containing percent cover values for every species in every plot, illustrating the type of data structure used for multivariate plant community analysis.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		AGEJUC	AMOHER	ANDGYR	ANDTER	ANDVIR	ANDVIRGL	ARIPUR	ARISER	ARISTR	BALANG	BULCIL	CARCOR	CHANIC	CHRGOS	CHRSCA	CLIMAR
2	CA1_Y1	0	0	0	5	0	0	20	0	51.5	0	5	5	0	0	0	
3	CA1_Y2	0	0	0	5	5	0	5	0	15.5	0	5	5	0	0	0	
4	CA2_Y1	0	0	0	0	0	0	0	0	10	0	5	0	0	0	0	
5	CA2_Y2	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	
6	CA3_Y1	0	0	0	5	0	0	0	0.5	30	0	0	0	0	0	0	1
7	CA3_Y2	0	0	0	0	0	0	0	0	1.5	0	0	0	0	0	0	
8	CA4_Y1	0	0	0	5	0.5	0	0	0	5	0	0	0	0.5	0	0	
9	CA4_Y2	0	0	0	0	0	0	0	0	0	0	0	0	0.5	0	0	
10	CA5_Y1	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	
11	CA5_Y2	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0	
12	CB1_Y1	0	0	0	5	0	0	0.5	0	21	0	0	20	0	0	0	
13	CB1_Y2	0	0	0	0	0	0	0	0	1.5	0	0	1.5	0	0	0	
14	CB2_Y1	0	0	0	10	0	0	5	0	20	0	0.5	10	0	0	0	
15	CB2_Y2	0	0	0	5	0	0	0	0	20	0	0.5	0.5	0	0	0	
16	CB3_Y1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
17	CB3_Y2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
18	CB4_Y1	0	0	0	5	0	0	0	0	66	0	0.5	0	0	0	0	
19	CB4_Y2	0	0	0	0	0	0	0	0	26.5	0	0	0	0	0	0	
20	CB5_Y1	0	0	0	0	0	0	5.5	0	5.5	0	0.5	0.5	0	0	0	
21	CB5_Y2	0	0	0	0	0	0	10	0	5.5	0	0	20	0	0	0	
22	CC1_Y1	0	0	0	5	0	0	15	0	70.5	0	10.5	45	0	5	0	
23	CC1_Y2	0	0	0	5	0	0	5.5	0	21	0	0	5.5	0.5	0	0	

Final

Ready Display Settings