

# Landscape Pattern–Marl Prairie/Slough Gradient; patterns and trends in Shark Slough marshes and associated marl prairies

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Cover photo: Marl prairie on western portion of Transect M4, Date: 03/29/2017 (Photo - Jay Sah)

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## **General Background**

The Monitoring and Assessment Plan (MAP), the primary tool of the REstoration COordination and VERification (RECOVER) program to assess the performance of the Comprehensive Everglades Restoration Plan (CERP) provides the data and analytical support necessary to implement adaptive management. In the Everglades, marsh vegetation in both marl prairie and ridge and slough landscapes is sensitive to large-scale restoration activities associated with the CERP authorized by the Water Resources Development Act (WRDA) of 2000. More specifically, changes in hydrologic regimes at both local and landscape scales are likely to affect vegetation composition in the transition zone between these two landscapes, resulting in a shift in boundary between plant communities. In order to track these dynamics, Florida International University (Dr Michael Ross-PI and Dr. Jay Sah-Co-PI) has under taken a study of vegetation structure and composition in relation to physical and biological processes along the marl prairie-slough gradient since 2005. Later, since the Fall of 2014, the study is led by Dr. Jay Sah, while Dr. Michael Ross is also actively involved as the Co-PI in the study.

Vegetation monitoring transects in the Shark Slough basin, funded by US Army Corps of Engineers (USACOE) under RECOVER-MAP, capture the full range of marl prairie and slough plant communities, and address Performance Measure (PM):GE-15(Landscape Pattern–Marl Prairie/Slough gradient),by "...detecting spatio-temporal change in vegetation structure and composition in response to natural and restoration-induced hydrologic changes...". Monitoring of vegetation along the marl prairie/slough gradients addresses a working hypothesis that 'Spatial patterning and topographic relief of ridges and sloughs are directly related to the volume, timing and distribution of sheet flow and related water depth patterns', identified in the hypothesis cluster "Landscape Patterns of Ridge and Slough Peatlands and Adjacent Marl Prairies in Relation to Sheet Flow, Water Depth Patterns and Eutrophication" (RECOVER 2009). The study also addresses the hypothesis that resumption of historical flow and related patterns of hydroperiod, water depth, and fire with the implementation of CERP will cause a noticeable change in plant community composition and structure along the gradient and in the transition zone between marl prairie and peat-dominated ridge and sloughs.

Initiated in 2005 as an expansion on Shark Slough study transects that had been established and sampled in 1998-2000 with funding from DOI's Critical Ecosystems Study Initiative (CESI), the ongoing study is in the fifth sampling cycle beginning from Fall 2014. The results from the Year-1 (2014/2015) and Year-2 (2015/2016) study of this cycle were reported in Sah et al. (2017a, b). This document summarizes results for the Year-3 (2016/2017) (2014-2019), and it updates the results, including those from previous two years (Year-1 and Year-2) of the current cycle. The report now includes the summary of the vegetation dynamics along Transect M4, which was initially sampled in 2007/2008, and then sampled every three years (2010/2011, 2013/2014, and 2016/2017).

## 1. Introduction

In the Everglades, plant communities arranged along environmental gradients are expressions of ecosystem functional processes associated with underlying physico-chemical drivers that vary in space and time. Hence, determining the responses to spatio-temporal changes in key environmental drivers of plant assemblages along gradients, and the boundaries between them, is important for conservation and ecosystem restoration. The landscape in both Shark River and Taylor Slough basins of the Everglades includes long hydroperiod sloughs, flanked by short hydroperiod marl prairies. Particularly in the Shark River Slough (SRS) basin, vegetation structure and composition change gradually along an elevation and water depth gradient, from short-hydroperiod marl prairies to ridge and slough, which are characteristic features of the landscape of central SRS (Olmsted and Loope 1984; Olmsted and Armentano1997; Ross et al. 2003). Hydrology is one of the major drivers of species differences between marl prairie and ridge-and-slough landscapes. Hence, alterations in hydrologic conditions usually cause a shift in vegetation structure and composition within each landscape; extreme changes can lead to even dominance of hydric vegetation in marl prairie or various levels of degradation of landforms in the ridge and slough (R&S) landscape. In the past century, changes in the amount and flow patterns of water, resulting from the construction and operation of a series of canals, levees and water structures (Light and Dineen1994, McVoy et al. 2011), have altered the proportions of prairie and slough vegetation in the region. Furthermore, changes in water management associated with the ongoing Comprehensive Everglades Restoration Plan (CERP 2000) are likely to affect vegetation composition in the transition zone between these ecosystems, resulting in a shift in the boundary between marl prairie and slough communities. It is therefore important to understand how restoration impacts the dynamics of prairie and slough landscapes and the boundaries between the two.

Along the marl prairie-slough gradient, vegetation in the marl prairie portion of the gradient is likely to respond to hydrologic changes more rapidly than vegetation in the slough portion. Armentano et al. (2006) also argued that the transition from one vegetation type to another (e.g., prairie to marsh) in response to hydrology may take place in as little as 3 to 4 years. However, the transition from marsh to prairie may take longer. In the southern Everglades, recent water management efforts have been directed towards ameliorating the adverse effects caused of previous water management activities. In this respect, a series of water detention ponds have been brought into operation along the eastern boundary of the park to mitigate the wet-season water reversals that were prevalent in this region due to the loss of water from the rocky glades to the canal (Van Lent et al. 1999). In contrast, strategic regulation of water deliveries through the S12 structures along US 41 has been in place since 2002 to reverse the damage that were caused by the extended wet conditions that resulted from both high water deliveries and rains in the mid-to-late -1990s. These modifications in water management activities, along with those planned under Central Everglades Planning Project (CEPP), including construction and operation of Tamiami Bridges, have affected, and are likely to influence water conditions within the Park, resulting in changes in vegetation communities and ecological processes.

In 2005, we initiated a long-term study of vegetation dynamics in relation to changes in under lying environmental drivers, especially hydrology, along the MP-S gradient. The broader goal of

the study is to assess the impact of Everglades restoration activities on plant communities along the gradient, and to detect any shift in position and attributes of boundaries between those communities. The study is conducted on five transects that extend across SRS into adjacent marl prairies. Shark Slough portions of the transects over lap transects that were established and sampled under different sponsorship in 1998-2000, providing the prospect of assessing long-term temporal change in vegetation in those areas.

In this study, our specific objectives were, i) to characterize recent vegetation composition along the marl prairie-slough gradient, and ii) to assess changes in vegetation in both the Shark Slough and marl prairie portions of the transects over a seventeen-year period (1999-2016). We hypothesized that variation in vegetation composition along the MP-S gradient is mainly driven by hydrology, i.e. duration and depth of flooding. We also hypothesized that Shark River Slough vegetation follows the temporal trend in hydrologic regimes, and over the last fifteen years has changed in species composition toward assemblages more indicative of relatively dry conditions. In addition, in compliance with the differential water management goals on both sides of SRS, we hypothesized that marl prairie vegetation follows the spatially differentiated temporal trend in hydrologic regimes, and over the ten years (2006-2016) vegetation in eastern portion of marl will change toward a wetter character while vegetation in the western marl prairies would shift toward a drier type.

# 2. Methods

# 2.1 Study Sites

Sites sampled in 2016-2017 were part of the ongoing long-term vegetation monitoring along marlprairie slough gradient in the southern Everglades. The study design includes 5 Transects (MAP Transect M1 to M5), varying in length from 9.0 km to 35.8 km. These transects were established in 2005, when the systematic sampling began along the gradient. Three transects, M1, M3 and M4 extend across the Shark Slough to adjacent short-hydroperiod marl prairie habitat (**Figure 1**). M1, located in Northeastern Shark Slough (NESRS), extends to the marl prairie only to the east of the slough. M2 originally covered an area restricted to Shark River Slough (SRS), extending on both sides of L-67S canal. But in 2015, this transect was extended towards east for the other 5 km which covers prairie vegetation along eastern boundary of the ENP and transitional zone between marl prairie in NESRS and ridge & slough landscape in SRS. Transect M5 covers an area between fresh to brackish water ecosystems in the southeastern corner of SRS, extending to the east into fresh water marl prairies located on both sides of the main Park road.

In Transects M3 and M4, sampled in 2015/16 and 2016/17, respectively, the vegetation sampling plots were established at 200 to 500 m intervals. In the marl prairie section of the transects, the plots were established at 300 m intervals, and in the SRS portion of the transects, the plot density varied between 2 to 4 plots per km (200-500 meter intervals). **Table 1** summarizes the years and numbers of sites sampled on Transects M1-M4. The slough portion of these transects was sampled in the wet season (July to November), accessing the sites by airboat or helicopter. Marl prairie portions of the transect were sampled in the dry season (Jan to May) and were accessed by helicopter for drop off and pickup, and on foot for sampling. In 2016, however, the dry season sampling on Transect M3 continued through June 13<sup>th</sup>, primarily due to unusual high water level

in early dry season, and partly due unavailability of helicopter caused by high demand during squeezed period of sampling in late spring by various research groups. In the same year, sampling in slough portion of Transect 4 began late and continued through December, primarily due to unforeseen turn-over of the research staff responsible for operating the airboat for field sampling. Likewise, sampling days on prairie portion of the Transect M4 were spread over more than three months, primarily due to limited availability of helicopter caused by active fire season.

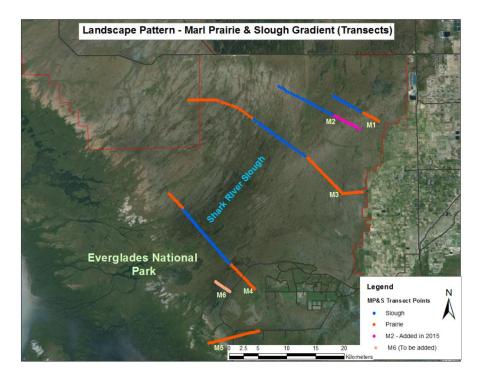


Figure 1: Location map of Marl prairie-Slough Gradient Study plots on Transects M1-M5.

	Sampling Event	Sites Sampled				
Transect		Prairie sites		Slou	gh sites	
		Year	No. of Sites	Year	Number of Sites	
	E1	2006	11	2005	20	
M1	E2	2009	11	2008	20	
	E3	2012	11	2011	20	
	E4	2015	11	2014	20	
	E1			2005	25	
M2	E2			2008	26	
	E3			2011	25	
	E4	2015	15	2014	25	
	E1	2007	72	2006	37	
М3	E2	2010	72	2009	37	
IVIS	E3	2013	72	2012	37	
	E4	2016	71	2015	37	
	E1	2008	32	2007	55	
M4	E2	2011	32	2010	55	
	E3	2014	32	2013	55	
	E4	2017	30	2016	50	

 Table 1: Sites sampled on MAP transects M1-M4 between 2006 and 2017.

# 2.2 Vegetation sampling

Vegetation was sampled in a nested-plot design that allowed for efficient sampling of the range of plant growth forms (herbs, shrubs and trees) present along the transects (Ross et al. 2005; Sah et al. 2013). Plots were sampled at every 200-500m intervals. Higher intensity sampling occurred in areas accessible by airboat, and was based on the contention that increased sampling intensity would enable us to make a more meaningful comparison of current vegetation with that present on the same transects in 1999 (Ross et al. 2001; Ross et al. 2003).

At each sampling site, a PVC tube marked the SE corner of a 10x10m tree plot. Nested within each tree plot, a 5x5m herb/shrub plot was laid out, leaving a 1-m buffer strip along the southern and eastern border of the tree plot. In the 10x10m tree plots, we measured the DBH and crown length and width of any woody individual  $\geq$  5cm DBH, and then calculated species cover assuming horizontally-flattened elliptical crown form. Within each 5x5m herb/shrub plot, we estimated the cover class of each species of shrub (woody stems>1m height and <5cm DBH) and woody vines, using the following categories: <1%, 1-4%, 4-16%, 16-33%, 33-66%, and >66%. We estimated the cover % of herb layer species (all herbs, and woody plants <1m height) in five 1-m<sup>2</sup> subplots located at the four corners (NE, NW, SE and SW) and the center (CN) of the 5x5m plot. Species present in the 5x5m plot but not found in any of the 1m<sup>2</sup> subplots was assigned a mean cover of 0.01%. In addition, a suite of structural parameters was recorded in a 0.25m<sup>2</sup> quadrat in the SW corner of each of the 5 subplots. Structural measurements included the following attributes: 1) The height and species of the tallest plant in the plot; 2) Canopy height, i.e., the tallest vegetation present within a cylinder of ~5cm width, measured at 4 points in each 0.25m<sup>2</sup> quadrat; 3) Total vegetative cover, in %, and 4) Live vegetation percent cover, expressed as a % of total cover.

# 2.3 Water depth measurements

Field water depths in combination with EDEN (Everglades Depth Estimation Network, <u>http://sofia.usgs.gov/eden</u>) water surface elevation data serve the basis for calculation of ground elevation and estimation of hydrologic conditions at each site. Water depth was measured at each site of the transect, whether marl prairie or slough. We measured water depths at the PVC, the marker of the plot, and in the center of five vegetation sub-plots in a 5x5m plot. At the marl prairie sites of M1 and M3, water depths once measured in the Fall of 2008, when there was standing water, were used to estimate hydrologic conditions.

# 2.4 Soil and Plant analysis

In 2015-2016, soil and plant samples were collected from 15 sites on Transect 3. Five sites were in eastern portion of marl prairie, five in transition zone and other five sites were within ridge & slough portion of the transect. At each site, three of five  $1x1m^2$  sub-plots used for vegetation sampling were randomly selected for soil sampling. In each of three selected subplots, one soil core of 10 cm length was collected using 5.7 cm diameter core tube. At one site, M3-11400, soil samples were collected only in two sub-plots, as other plots are very rocky and there was not enough soil to be collected. Soil samples were placed in Ziploc bags, labelled, and brought to the lab at FIU, where they were placed in refrigerator until further analysis. Each core's compaction and length were measured in the field and recorded.

The net weight of wet soil samples was obtained. Samples were oven-dried at 80°C for 48 hours or until a constant weight was achieved. For each sample, dry weight and volume were obtained, and the sample bulk density was calculated following Blake and Hartge (1986). We removed extraneous macro materials, including roots and rocks. Samples were then ground to pass a 2-mm sieve. Later, 2-3 mg dry samples were obtained and enclosed in tin (Sn) capsules, and then delivered to the SERC Nutrient Analysis Lab at FIU for Total Nitrogen (TN) and Total Carbon (TC) Analysis. Another 2-3 g of dry sample were obtained and transferred to small screw cap glass vials (7 mL), and then delivered to FIU Freshwater Biochemistry Lab for Total Phosphorus (TP) and other analysis. Soils were also analyzed for Phosphatase and Glucosidase enzyme activity. Sub-samples of soils for such analysis were also delivered to the FIU Freshwater Biogeochemistry Lab.

# Soil nutrient analysis

Measured soil parameters were soil bulk density and pH; total C, N, and P (TC, TN and TP); ash content, inorganic carbon (IC, based on ash %C), total organic carbon (TOC), soil enzyme activities (e.g. phosphatase and glucosidase activity), and total extractable phosphorous and carbon. Soil pH was determined in 1:1 (w/v) soil:water suspension using a pH meter. TC and TN were measured on a dry weight basis using a CHN analyzer (Perkin Elmer, Inc, Wellesley, Massachusetts, USA), and TP was determined colorimetrically following the method of EPA-365.1 after ashing-acid digestion (Solorzano and Sharp 1980). Total inorganic carbon (IC) was determined in ash (residual after combustion at 500\_C) and scaled as % IC to total dry weight. Then, total organic carbon (TOC) was determined by difference (TC - IC).

The remineralization of elements in soils is a function of liberating exoenzymes, therefore, soil enzyme (e.g. phosphatase and glucosidase) activity was determined using methylumbelliferyl substrates (MUF) on a slurry created from the soil (Sinsabaugh et al. 1997). Enzyme activity was determined from the difference between the amounts of fluorescent substrate liberated during the incubation time from the time zero. The µmole MUF-substrate liberated  $g^{-1}$  dry weight soil  $h^{-1}$  was determined by comparison to standard curves generated using known concentrations of MUF. Bioavailable P was assayed via a serial extraction procedure that involved analysis for water soluble P (WSP) and sodium bicarbonate-extracted phosphorus (NaHCO<sub>3</sub>-P) (Wright and Reddy, 2001a). Likewise, sodium bicarbonate-extracted total organic carbon (NaHCO<sub>3</sub>-TOC) was also determined following slightly modified methods of Amador and Jones (1982). In this method, samples were loaded in vials and purged with CO<sub>2</sub>-free air. Samples were then incubated in the dark at 25°C for 72 to 96 hours and analyzed on a gas chromatograph for CO<sub>2</sub>.

## Carbon isotope ( $\delta 13C$ ) analysis

Plant biomass samples were collected from  $0.25 \times 0.25 \text{ m}^2$  quadrat within the same  $1 \times 1 \text{ m}^2$  sub-plots in which soil samples were collected. Plant samples were then separated by species. All samples were then oven dried at 70° C for 72 hrs. and weighed to calculate the above ground plant biomass. Plant samples for four major species, sawgrass (*Cladium jamaicense*), muhly grass (*Muhlenbergia capillaris* ssp. *filipes*), spikerush (*Eleocharis cellulosa*) and beak rush (*Rhynchospora tracyii*), were sab-sampled. Sawgrass was present in most of plots along the gradient, whereas muhly grass was restricted to the marl prairie portion of the gradient. Beakrush was common in marl prairie and transition zone while spikerush in transition zone and ridge & slough portion of the gradient. The subsamples of these species were grinded in a coffee-grinder. After each use, the coffee grinder was washed and dried to avoid contamination. The powdered samples were then sieved with a mesh (# 10 sieve), weighed, and placed into 20 mL scintillation glass vials for further analysis.

For isotope analysis, sub-samples of soils were treated with 1N hydrochloric acid (HCL) for one hour to remove carbonate, rinsed with distilled water thoroughly, and filtered using suction filtration. The residues were then air-dried, powdered and sieved. Leaf and soil samples were then packed in individual tin capsules that were rolled into small balls. Each ball was then placed into an automated elemental analyzer connected to a continuous flow isotope ratio mass spectrometer (EA-IRMS). The stable carbon isotope ratio in plants and SOM was reported as:

$$\delta^{13}C(\%0) = \left(\frac{\left(\frac{1^{3}C}{1^{2}C}\right)_{sample}}{\left(\frac{1^{3}C}{1^{2}C}\right)_{standard}} - 1\right) * 1000(\%0)$$

#### 2.5 Data Analysis

#### 2.5.1 Hydroperiod and annual mean water depth

We used field water depth-derived elevation and EDEN water surface elevation data to estimate the hydrologic conditions at each sampling site. We calculated the ground elevation of each plot using mean water depth for the plot and EDEN estimates of water surface elevation at that point (center of the plot) for the same sampling date. Daily water levels for each plot were estimated based on ground elevation and the time series data of water surface elevation extracted from EDEN database. The hydroperiod, the number of days per year when a location had water depth >0cm, and mean annual water depth were calculated for each plot. We then averaged hydroperiod and mean annual water depth for the four water years (May1<sup>st</sup>–April 30<sup>th</sup>) prior to each sampling event to examine vegetation response to hydrologic changes.

#### 2.5.2 Fire frequency and Time since last fire

Fire geodatabase in which the records of fire events are catalogued from 1948 to 2012 was obtained from Everglades National Park (ENP). The shape files for 2013-2016 fires were also obtained from the Park, and were later added to the geodatabase. The database contains shape files of fires with other attributes such as type of fire (Natural, RX, incendiary, etc.), date of incidence, etc. The data were used to calculate fire frequency and time since last fire (TSLF) for vegetation monitoring sites along Transect 3 using ArcGIS 10.3.

### 2.5.3 Vegetation classification and ordination

We summarized species data by calculating the importance value (IV) of each species present in herb and shrub layers in each plot. We calculated species' importance value as: IV = (relative cover + relative frequency)/2. For calculating IV of the species that did not occur in any of 5 subplots but occurred in 5 x 5 m<sub>2</sub> plot, a frequency of 4% was assigned. The assumption was that the species would have occurred in at least one subplot, had all 25 1 x 1 m<sup>2</sup> subplots within a plot sampled. Vegetation types at all sites that were surveyed along the five transects between 2005 and 2008 had already been defined using a hierarchical agglomerative cluster analysis (Sah et al. 2013). In the analysis, Bray-Curtis dissimilarity was used as the distance measure, and the flexible beta method to calculate relatedness among groups and/or individual sites (McCune and Grace 2002). In the current year analysis, non-metric multidimensional scaling ordination (NMDS) was done to analyze the shift in species composition using trajectory analysis (see below sub-section 2.4.5)

#### 2.5.4 Biomass estimation

For the sites in the marl prairie portion of the gradient, vegetation structural measurements were summarized for each plot, and mean canopy height and total vegetative cover were used to estimate above ground plant biomass, using the allometric equation developed by Sah et al. (2007) for marl prairie vegetation within CSSS habitat. The equation for calculating biomass was as follows:

$$\sqrt{Biomass} = 6.708 + 15.607 * \arcsin \sqrt{Cover/100} + 0.095 * Ht$$

where Biomass = Total plant biomass  $(g/m^2)$ , Cover = Crown cover (%), and Ht = Mean crown height (cm).

### 2.5.5 Vegetation response to hydrology – Trajectory analysis

At the slough sites on Transects M1-M3, changes in vegetation composition since 1999/2000 were analyzed using trajectory analysis (Minchin et al. 2005; Sah et al. 2014), an ordination-based technique designed to test hypotheses about rates and directions of community change. In the NMDS ordination performed for trajectory analysis, we included vegetation data for prairie sites collected on all transects during the first sampling cycle (2005-2008), and for the ridge and slough sites, the data collected between 1999 and 2016. Prairies sites were included to cover the full range of hydrologic conditions on the transects. In the NMDS ordination, hydrology vector represented by mean annual water depth was defined through a vector fitting technique in DECODA (Kantvilas and Minchin 1989; Minchin 1998; Sah et al. 2014).

To quantify the degree and rate of change in vegetation composition along the reference vector, two statistics, delta ( $\Delta$ ) and slope were calculated (Minchin et al. 2005). Delta measures the total amount of change in the target direction. It was calculated as the difference between the projected score at the final time step and the initial time. Slope measures the mean rate of change in community composition along the target vector. The statistical significance of both delta ( $\Delta$ ) and slope was tested using Monte Carlo simulations with 10,000 permutations of the cover scores of species among sampling times within each trajectory, with the NMDS ordination and calculation of trajectory statistics repeated on each permuted data matrix.

# 2.5.6 Weighted averaging and Vegetation-inferred hydroperiod

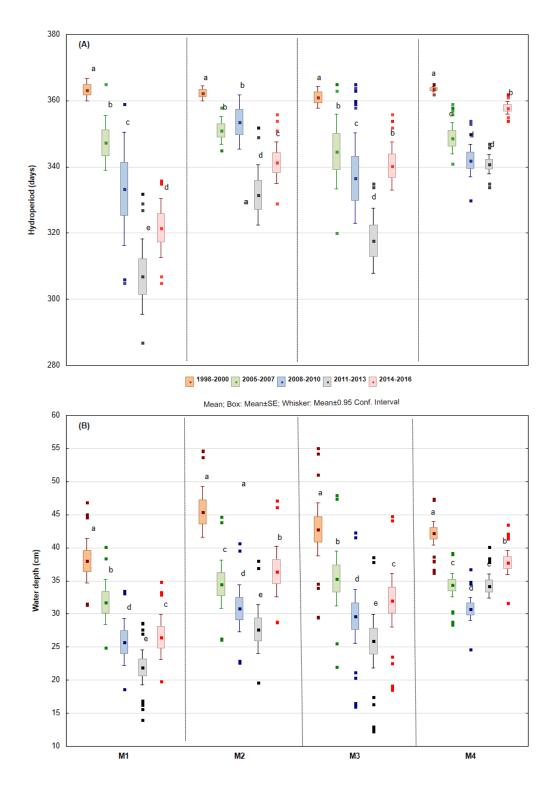
Vegetation change analysis in the marl prairie portion of the gradient, included calculation of vegetation-inferred hydroperiod, i.e., the hydroperiod for a site indicated from its vegetation composition using a weighted averaging partial least-square (WA-PLS) regression model. The training-data set with which we developed the WA-PLS regression model was the species cover data plus hydroperiod estimates from 291 plots on six topographically-surveyed transects within the Cape Sable seaside sparrow habitat (Ross et al. 2006). In developing the WA-PLS models, species cover were fourth square root transformed, which down-weights the influence of very dominant species. Mean hydroperiod was calculated across different time periods (i.e., years preceding vegetation sampling). The performance of the models was judged by the improvement in R2 value and RMSEP (root mean square error of prediction). RMSEP was estimated by a leave-one-out (jackknife) cross-validation procedure, in which a vegetation-hydroperiod model is developed from all samples except one, and consequently applied to predict the hydroperiod of the left-out point on the basis of its vegetation. We used the C2 program of Juggins (2003) to develop the WA-PLS model.

Finally, the best WA-PLS model was applied to the calibration data set, here the MP-S gradient data that included vegetation data at 107 sites on Transect M3. The predicted hydroperiods for those sites were termed 'vegetation-inferred hydroperiod'. A change in vegetation-inferred hydroperiod between successive samplings reflects the amount and direction of change in vegetation, expressed in units of days (0-365) along a gradient in hydroperiod.

# 3. Results

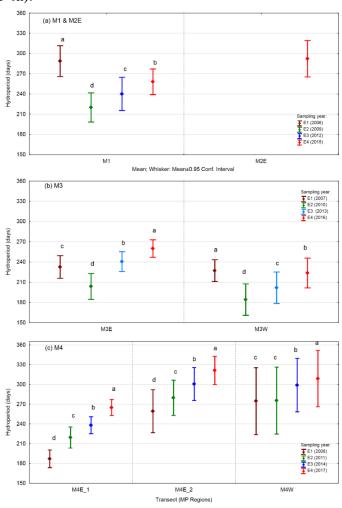
# 3.1 Hydrologic pattern (1999-2017)

In the slough portion of transects (M1-M4), the hydroperiod and mean annual water depth averaged over four years prior to vegetation sampling varied over the study period. In the late 1990s, i.e. four years before the 1999 (E0) vegetation sampling, mean hydroperiod on all four transects were >360 days, and mean ( $\pm$  SD) annual water depths were  $38.0 \pm 6.8$ ,  $45.4 \pm 7.7$ ,  $42.8 \pm 10.3$  cm and  $42.2 \pm 5.3$  cm on Transects M1, M2, M3 and M4, respectively (**Figure 2**). At the slough sites on those transects, mean hydroperiod and annual water depth were lower during three subsequent sampling events (2005/2006 (E1), 2008/2009 (E2) and 2011/2012 (E3)) than during the initial; period. However, between 2014-2017 (E4) sampling, sites in the slough were wetter than the sampling done three years ago (**Figure 2**). During 2014-2017 sampling period, four-year average hydroperiod at the slough sites on M1, M2, M3, and M4 were 315, 337, 338 and 355 days, and annual mean water depth were lower than late 1990s. The hydroperiod was 22-42 days shorter and mean water depth 6-12 cm less than the years before 1999 sampling.

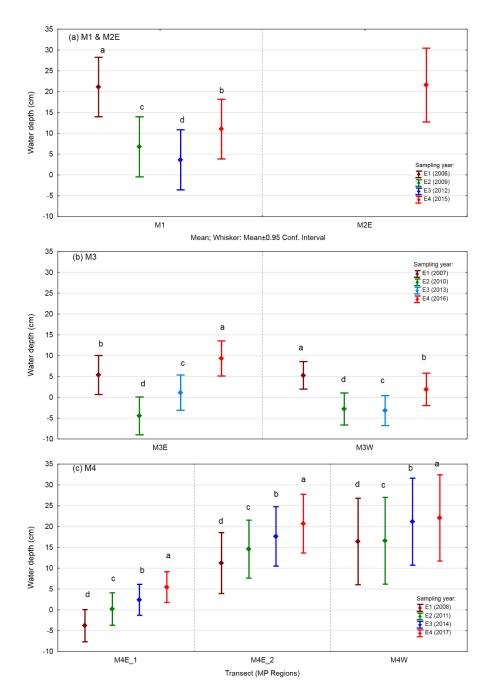


**Figure 2:** Box Plots showing (A) hydroperiod and (B) mean annual water depth averaged over four years prior to vegetation sampling in the Shark Slough portions of MAP transects M1, M2, M3 and M4. Different letters represent significant (pairwise t-test; p < 0.05) difference in (A) hydroperiod, and (B) mean annual water depth among years on individual transects.

Hydrologic conditions in the marl prairie portion of the transect M1 varied among sampling events. Mean hydroperiod, averaged over four years before E2 census was 60 days shorter than in the years before E1 (Figure 3a). The hydrologic conditions in subsequent years, i.e. after E2 became wetter. The mean 4-year average hydroperiod before E3 was 18 days longer than the hydroperiod before E2, and the trend continued during the sampling event E4. In contrast to the hydroperiod, 4-year average annual mean water level was lowest during E3 (Figure 4a). However, both the hydroperiod and mean annual water level before 2015 spring sampling (E4) was higher than E2 and E3, but was still shorter or less than the years before E1. The differences in hydrologic conditions between sampling events were mostly due to extreme events. The prolonged dry period between 2006 and 2008, i.e. the period before the 2<sup>nd</sup> census (E2) saw water levels dip far below the ground level (Figure 5). The water level in the spring of 2011, i.e. just before the 3<sup>rd</sup> census (E3) was also much lower than ground level. In the southern portion of NESRS, where Transect M2 was extended in 2015, four-year average hydroperiod was  $292 \pm 55$  days (Figure 3a), the value ranged from 161 days at the easternmost site to 346 days at the west end of the extended transect (M2E). Likewise, the four-year average annual mean water depth was  $21.6 \pm 17.8$  cm, and it ranged between -15.5 cm to 45 cm (Figure 4a).



**Figure 3:** Mean ( $\pm 95\%$  CI) hydroperiod averaged over four years prior to vegetation sampling in the marl prairie portions of MAP transects M1, M2E, and M3. Different letters represent significant (pairwise t-test; p < 0.05) difference in hydroperiod between sampling period on individual transects or a section of the transect.



**Figure 4:** Mean ( $\pm 95\%$  CI) annual water depth averaged over four years prior to vegetation sampling in the marl prairie portions of MAP transects M1, M2E and M3. Transect M3 has marl prairies both sides (East & West) of Shark Slough. Different letters represent significant (pairwise t-test; p < 0.05) difference in hydroperiod between sampling period on individual transects or a section of the transect

Transects M3 and M4 are unique, as the hydrologic conditions in the marl prairie portion of these two transects differ between eastern and western marl prairies. On the Transect 3, when average over all the sampling events, water condition was wetter in the eastern prairies than the western prairies. However, in the prairies on both sides of the slough, it was much drier during E2 than E1.

In contrast, an increasing trend in both four-year average hydroperiod and mean annual water depth was observed during the next two sampling events, E3 and E4 (**Figure 3b, 4b**). On the both sides of the slough, the four-year average hydroperiod showed its highest value during the last sampling year 2016 (**Figure 3b**), but mean annual water depth was highest in 2016 only in eastern sites (**Figure 4b**). It was important to note that, at the western prairie sites, despite unusually high water conditions in spring of 2016, the four-year mean annual water depth associated with the 2016 sampling was still significantly lower than during 2007 (E1) sampling. In 2016, the four-year average hydroperiod in eastern and western prairies were  $260 \pm 41$  days and  $224 \pm 59$  days, respectively. Likewise, the mean annual water depths were  $9.3 \pm 13.3$  cm and  $1.9 \pm 10.4$  cm, respectively.

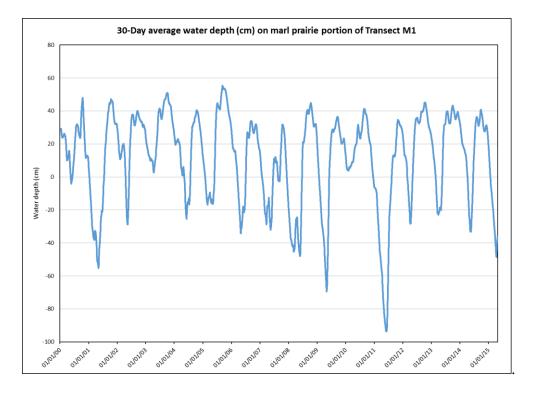


Figure 5: 30-day average mean annual water depth on the marl prairie portion of the transect M1.

The hydrologic conditions in the marl prairie portion of the Transect 4 also differ between eastern and western marl prairies (**Figure 3c, 4c**). Across all the sampling events, water condition was wetter in the eastern prairies than the western prairies. In this region of marl prairie landscape, the main park road also affects the hydrologic conditions. The sites to the southeast of the road (M4E\_1) are much drier than the sites in northwestern portion (M4E\_2) of marl prairie. However, in the prairies on both sides of the slough, it was drier during E1 than any other sampling events. In all three portions of the prairies of this transect, both the four-year average hydroperiod and mean annual water depth increased during the next three sampling events. The increase in water depth across the four sampling periods was less in the prairies west of slough (~5 cm) than in the eastern marl prairies (~10 cm) (**Figure 4c**). In 2017, the four-year average hydroperiod in M4E\_1, M4E\_2 and M4W portions of this transect were 264, 321 and 309 days, respectively (**Figure 3c**). Likewise, the mean annual water depths were 5.5, 21 and 22 cm, respectively (**Figure 4c**).

## 3.2 Fire frequency and time since last fire

Several sites on the MP-S gradient transects have burned frequently in the past. Between 1990 and 2005, the period that included vegetation sampling (1999/2000) at the slough sites, there was not many fires within the area, except M4 on which four sites were burned in 1999 and four in 2003. Nevertheless, since 2005, when vegetation monitoring began at regular interval on the transects, both prairie and slough sites on Transects M1, M2, M3 & M4 burned due to either prescribed burns (Rx), human-caused fire or wild fires (**Table 2**). Time elapsed between the burned-year and sampling events, defined as time since last fire (TSLF), have affected vegetation composition observed at these sites.

**Table 2:** Vegetation sampling sites burned over the sampling period (2005-2016). The fire attributes were obtained from the Fire database of Everglades National Park.

Fire Name	Year	M1	M2	M3	M4
L67 Rx	2005	0	1	0	0
Airboat	2006	18	4	7	0
U Road Rx	2007	0	10	0	0
Coptic	2007	1	0	0	0
West L67 WFU	2007	0	1	0	0
Mustang Corner	2008	11	1	44	0
Shark Valley Tram Rx	2009	0	0	1	0
ROG NE Rx	2012	0	12	31	0
EE 1 Rx	2012	18	13	0	0
Branch	2014	0	0	0	1

## **3.3 Soil characteristics**

Surface soil characteristics vary along MP-S gradient represented by the Transect M3. In general, soil bulk density (BD) showed a decreasing trend from marl prairie to slough portions of the transect (**Figure 6**), whereas total carbon (TC), organic carbon (OC) and total nitrogen (TN) showed an increasing trend (**Figure 7**). The mean ( $\pm$ ) bulk density varied between 0.076 ( $\pm$  0.009) g cm<sup>-3</sup> and 0.547 ( $\pm$  0.043) g cm<sup>-3</sup> (**Table 3**). The mean ( $\pm$ ) TC content ranged from 138.1  $\pm$  15.4 mg g<sup>-1</sup> at a prairie site near the ENP boundary to 444.1  $\pm$  15.1 mg g<sup>-1</sup> at a site (M3-18300) in the slough portion of the transect. The mean OC ranged from 22.7  $\pm$  5.58 mg g<sup>-1</sup> to 404.0  $\pm$  13.2 mg g<sup>-1</sup>, and mean TN varied between 2.79  $\pm$  0.99 mg g<sup>-1</sup> and 32.54  $\pm$  0.43 mg g<sup>-1</sup>.

Unlike TC and OC, the mean IC showed a decreasing trend from prairie to slough. Soil IC ranged from the maximum of  $116.2 \pm 2.5 \text{ mg g}^{-1}$  at a prairie site to  $4.5 \pm 2.7 \text{ mg g}^{-1}$  at a slough site (**Table 3**). Soil total phosphorous (TP) did not show strong trend. However, in general, the prairie sites had lower soil phosphorous (dry weight basis) than the ridge and slough sites (**Figure 8**), but the

variability is very high in the transition zone (**Table 3**). Mean ( $\pm$  SD) TP ranged between 100.5  $\pm$  20.9 µg g<sup>-1</sup> and 367.4  $\pm$  25.1 µg g<sup>-1</sup>.

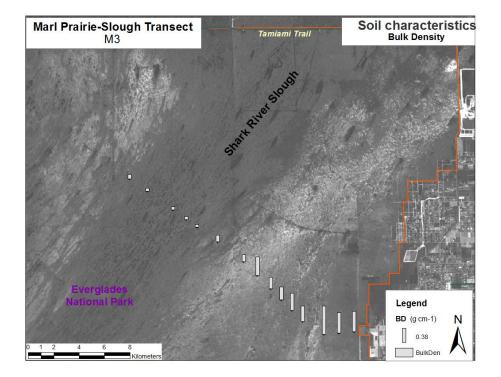


Figure 6: Soil bulk density at sites along marl prairie-slough gradient on Transect M3

Site ID	Bulk	TN (mg g-1)	TC (mg g-1)	OC (mg g-1)	IC (mg g-1)	TP (µg g-1)
M3-00300	$\frac{\text{Density}}{0.547 \pm 0.043}$	$6.7 \pm 0.66$	$138.1 \pm 15.4$	$44.9 \pm 2.8$	$93.2 \pm 14.3$	$208.9 \pm 34$
M3-01500	$0.544 \pm 0.073$	$6.1 \pm 2.51$	147.7 ± 13.9	$43.5 \pm 20.1$	$104.1 \pm 7$	$163.4 \pm 54.8$
<b>M3-02700</b>	$0.760\pm0.099$	$2.8 \pm 0.99$	$138.8\pm5.3$	$22.7\pm5.6$	$116.2 \pm 2.5$	$100.5\pm20.9$
M3-04800	$0.410\pm0.046$	$7.3 \pm 1.9$	$155.2\pm4.4$	$51.9\pm8.9$	$103.3\pm5.7$	$197.6\pm73$
<b>M3-06000</b>	$0.472\pm0.098$	$9.2\pm2.74$	$141.9\pm9.5$	$72.4\pm23.8$	$69.5\pm30.5$	$197.8\pm35.8$
M3-07200	$0.343\pm0.027$	$13.4\pm2.14$	$150.8 \pm 14.9$	$138.8\pm20.6$	$12\pm16.4$	$337.8 \pm 104.3$
<b>M3-08400</b>	$0.315\pm0.057$	$14.1\pm5.47$	$170.3\pm61.9$	$164.9\pm60.8$	$5.4 \pm 8.2$	$310 \pm 111.2$
M3-09900	$0.497 \pm 0.115$	$7.6\pm4.73$	$157.2 \pm 19.8$	$81.5\pm19.2$	$75.8\pm0.5$	$261.9 \pm 191.8$
M3-11400	$0.184\pm0.003$	$18.9 \pm 10.08$	$270.8\pm59.9$	$209.7 \pm 114.9$	$61.1\pm55$	$357.7 \pm 164.4$
M3-14000	$0.177\pm0.043$	$10.6\pm2.3$	$226.9\pm28.6$	$119.5\pm32.9$	$107.4\pm4.3$	$177.3\pm24.3$
M3-16000	$0.076\pm0.015$	$21.8 \pm 12.84$	$321.4 \pm 173.1$	$292.5\pm176.9$	$28.9\pm5.8$	$347.1\pm25.5$
M3-17000	$0.087\pm0.021$	$28.1\pm2.11$	$408.5\pm17.8$	$381\pm9.1$	$27.4\pm9$	$269.9 \pm 16.9$
M3-18300	$0.088 \pm 0.009$	$32.5\pm0.43$	$444.1\pm9.1$	$392.2\pm19.2$	$51.8\pm24.4$	$262.1\pm25.1$
M3-20800	$0.085\pm0.026$	$28.5\pm2.05$	$441.3 \pm 10.1$	$399 \pm 23.8$	$42.2\pm16.3$	$367.4\pm56$
M3-22500	$0.118\pm0.033$	$31.9 \pm 1.31$	$408.5\pm15.1$	$404 \pm 13$	$4.5\pm2.7$	$339 \pm 29.2$

Table 3: Mean (± 1 SD) soil properties at the selected sites along MP-S gradient on the Transect M3.

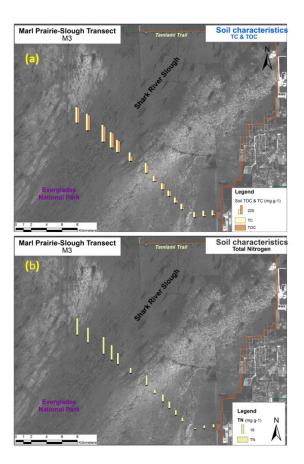


Figure 7: Soil (a) total carbon (TC), organic carbon (OC, and (b) Total nitrogen (TN) content at sites along marl prairie-slough gradient on Transect M3

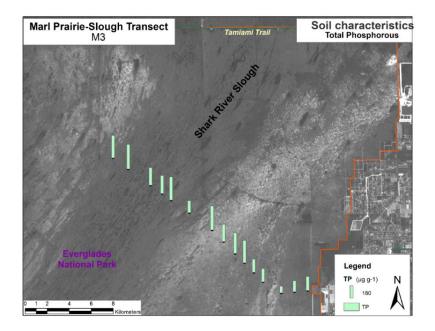
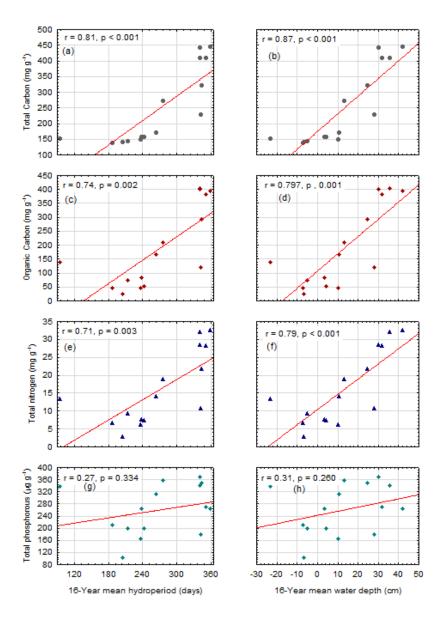


Figure 8: Soil total phosphorus (TP) content at sites along marl prairie-slough gradient on Transect M3.

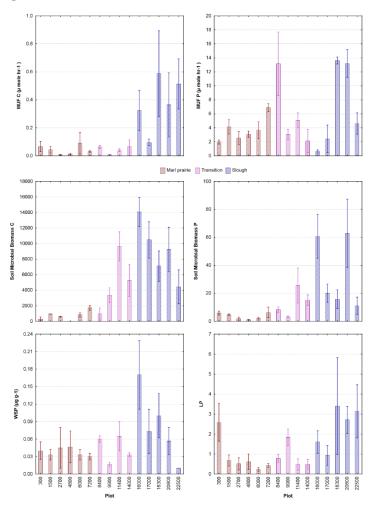
Along the MP-S gradient, TC, OC and TN, were strongly and positively correlated (r > 0.7; p = 0.001) with hydroperiod and mean annual water depth, both averaged over 16 years (**Figure 9a-f**). Soil TP also increased with the two hydrologic variables. However, the correlation between soil TP and both variables were not significant in this study (**Figure 9g, h**). Unlike the TC, OC, TN and TP, soil IC decreased with the wetness of the sites, though the relationship was weak.



**Figure 9:** Relationships between hydrologic variables and soil TC, OC, TN and TP at the sites along marl prairieslough gradient on the transect M3.

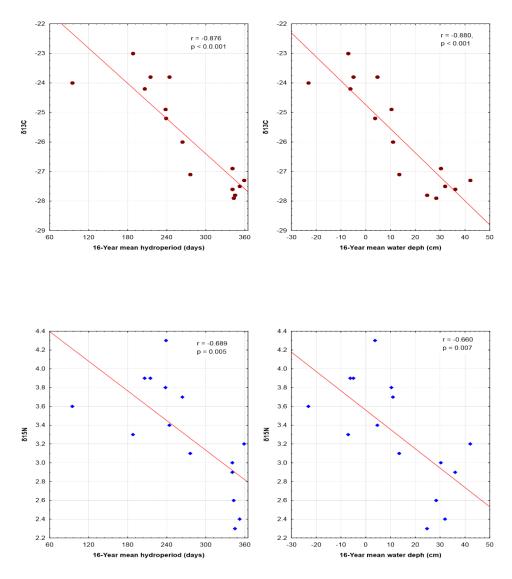
In this study, physicochemical measurements that included enzyme (phosphatase and glucosidase) activities and microbial biomass (as were also determined. Additionally, bioavailable P was assayed via a serial extraction procedure that involved analysis for water soluble P (WSP) and NaHCO3-P.

Mean ( $\pm$  SD ) methylumbelliferyl-glucosidase (MUF-C) ranged between 0.007  $\pm$  0.003 and 0.583  $\pm$  0.533  $\mu$  mole hr<sup>-1</sup>, and the values were higher at the sough sites than at marl prairies sites (**Figure 10a**). However, mean methylumbelliferyl-phosphatase (MUF-P) did not show significant (Nonparametric, M-W Test: p = 0.395) difference between slough and prairie sites. In contrast, both soil microbial biomass carbon (biomass-C) and phosphorous (biomass-P) showed an increasing trend along marl prairie-slough gradient (**Figure 10c, d**). Along the gradient, mean ( $\pm$  SD) microbial biomass-C increased by several magnitude, ranging from 35.6 to 14069.6  $\mu$ g g<sup>-1</sup> (dry weight). Mean microbial biomass-P ranged between 1.13  $\mu$ g g<sup>-1</sup> and 68.01  $\mu$ g g<sup>-1</sup>. The increase in MUF-C (glucosidase enzyme activity) and both microbial biomass C and P values along the MP-S gradient were similar to the trend observed in organic TC, OC and TN, and they were positively correlated with 16-year average hydroperiod and annual water depth. Nonetheless, the increasing trend in water-soluble phosphorus (WSP) and labile phosphorous (LP) along the MP-S gradient was not so strong (**Figure 10e, f**).



**Figure 10:** Soil characteristics at sites along marl prairie-slough gradient on Transect M3. (a) Methylumbelliferylglucosidase (MUF-C,  $\mu$  mole hr<sup>-1</sup>) (b) Methylumbelliferyl-phosphatase (MUF-P,  $\mu$  mole hr<sup>-1</sup>), (c) Soil microbial biomass carbon (biomass-C,  $\mu$ g g<sup>-1</sup>), (d) Soil microbial biomass phosphorous (biomass-P,  $\mu$ g g<sup>-1</sup>), (e) Water soluble phosphorus (WSP,  $\mu$ g g<sup>-1</sup>), and (f) Labile phosphorous (LP).

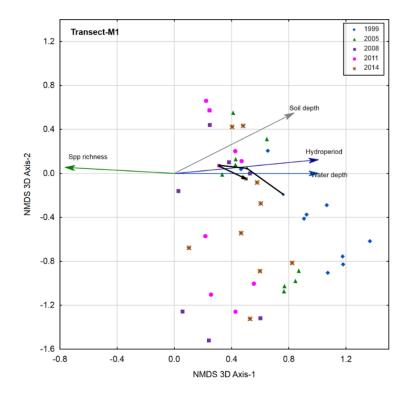
Surface soils along MP-S gradient varied in Carbon-13 ( $\delta$ 13C) and Nitrogen-15 ( $\delta$ 15N) values. The  $\delta$ 13C values in surface soil organic matter (SOM) were more negative in sloughs than in marl prairies, and had significant negative correlation (r = -0.88, p < 0.001) with both 16-year average hydroperiod and mean annual water depth (**Figure 11a, b**). The mean  $\delta$ 13C values in SOM ranged from -23.0 ± 1.22 % o at a marl prairie site to -27.9 ± 0.50 % o at a site within the R&S landscape. The mean  $\delta$ 15N values ranged between 2.34 ± 0.09 % o and 4.32 ± 0.17 % o, and the values were significantly (Nonparametric M-W Test: p < 0.001) lower at the slough sites than marl prairie sites. The  $\delta$ 15N values were negatively correlated (r = -0.66, p < 0.01) with wetness of the sites (**Figure 11c, d**).



**Figure 11:** Relationships between hydrologic variables (hydroperiod and mean annual water depth) and soil  $\delta$ 13C and  $\delta$ 15N values along marl prairie-slough gradient on the Transect M3.

#### 3.4 Shark River Slough vegetation change (1999-2016)

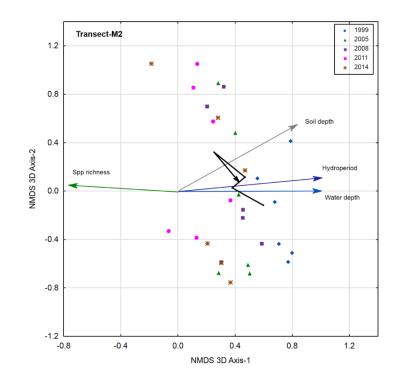
Between 1999 and 2016, marsh vegetation on all four transects (M1-M4), showed a shift in relative abundance of species indicative of sensitivity to the hydrologic changes. However, the direction and rate of vegetation change was not uniform throughout the sampling period. Trajectory analysis revealed that in the slough portion of M1 and M2 sampled at 3-6 year intervals between 1999 and 2014, species composition continued to shift towards drier vegetation types until 2011 (**Figures 12, 13**), but between 2011 and 2014, species composition shifted in the opposite direction, i.e. towards wetter vegetation types. In the slough portion of transects M3, the direction of vegetation shift was similar to that observed on M1 and M2 through 2009 (**Figure 14**). However, during the next two sampling periods, its vegetation trajectory was opposite in direction to those observed on M1 and M2; between 2009 and 2012, the vegetation on M3 slough sites shifted towards a wetter type, whereas between 2012 and 2015 the sites showed a drying trend. In contrast, in the slough portion of M4, the direction of vegetation shift towards drier type was only until 2007 (E1). During the subsequent sampling, i.e. between 2007 and 2010, vegetation shifted towards wetter type, and the trend roughly continued through 2016 sampling (**Figure 15**).



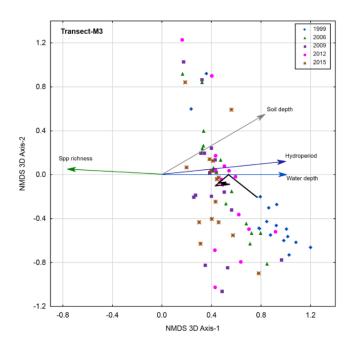
**Figure 12:** NMDS ordination bi-plots of site scores, the environmental vectors fitted in the ordination space, and the trajectory of centroid. The ordination is based on species abundance data collected five times between 1999 and 2014 in the Shark Slough portion of the Transect M1. Only the sites that showed significant ( $p \le 0.1$ ) rate of change in species composition along the hydrology gradient are shown. Initial point and the end of the trajectory represent the 1999 and 2014 sampling event, respectively

The percentage of sites that showed a drying or wetting trend in vegetation varied among transects, and in different portions of Transect 2. When summarized over the sampling period (1999-2015), most of sites during the E4 sampling still showed drier vegetation types when

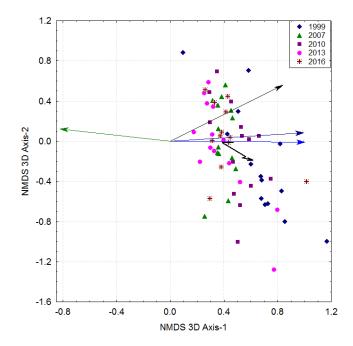
compared to the conditions in 1999. Trajectory analysis revealed that the percent of sites with a significant shift towards dry vegetation was higher on M1 (50.0%) and M3 (53.6%) than M2 (33.3%) and M4 (33.3%), and east of the L67 levee than west of the levee on M2. On the three transects (M1 to M3), the shift towards drier vegetation was greatest between the first two sampling events, E0 (1999) and E1 (2005). However, during subsequent periods, the vegetation change pattern differed among transects. Between E1 and E2 samplings, the shift towards dry vegetation continued on M1 and M3. In contrast, sites on M2 did not show a significant shift along the hydrologic gradient during that period (**Figure 13**). The transect, M4 differed from all other three transects. Between E1 (2007) and E2 (2010), vegetation composition at many sites shifted significantly towards wetter type, and the similar trend was observed between E3 (2013) and E4 (2016) (**Figure 15**).



**Figure 13:** NMDS ordination bi-plots of site scores, the environmental vectors fitted in the ordination space, and the trajectory of centroid. The ordination is based on species abundance data collected in the Shark Slough portion of the Transect M2. Only the sites that showed significant ( $p \le 0.1$ ) rate of change in species composition along the hydrology gradient are shown. Initial point and the end of the trajectory represent the 1999 and 2014 sampling event, respectively.



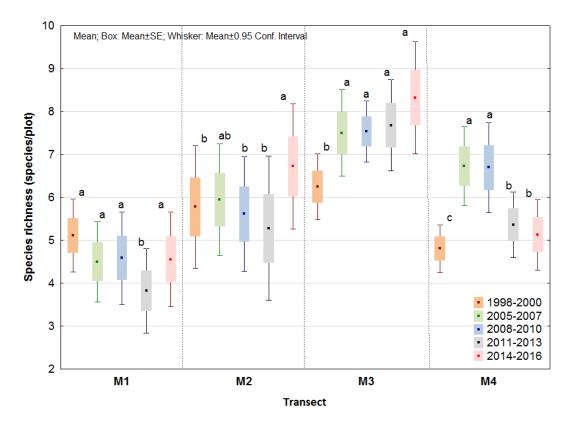
**Figure 14:** NMDS ordination bi-plots of site scores, the environmental vectors fitted in the ordination space, and the trajectory of centroid. The ordination is based on species abundance data collected in the Shark Slough portion of the Transect M3. Only the sites that showed significant ( $p \le 0.1$ ) rate of change in species composition along the hydrology gradient are shown. Initial point and the end of the trajectory represent the 1999 and 2015 sampling event, respectively.



**Figure 15:** NMDS ordination bi-plots of site scores, the environmental vectors fitted in the ordination space, and the trajectory of centroid. The ordination is based on species abundance data collected in the Shark Slough portion of the Transect M4. Only the sites that showed significant ( $p \le 0.1$ ) rate of change in species composition along the hydrology gradient are shown. Initial point and the end of the trajectory represent the 1999 and 2016 sampling event, respectively

### Species richness

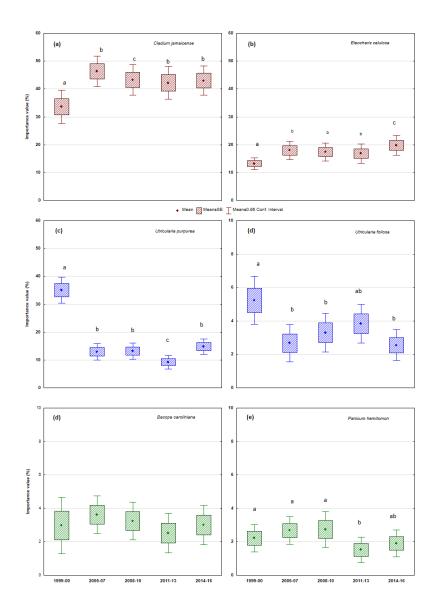
Between E3 (2011-2013) and E4 (2014-2016), significant changes in species richness and relative abundance of some major species accompanied the trajectories described above. Mean species richness in the slough portion of M1 in 2014 (E4) was significantly higher than in 2011 (E3), but was similar to that in other sampling years (**Figure 16**). In the slough portion of M2, mean species richness in 2014 was significantly higher than any previous sampling years, except in 2005 (E1). During the E4 (2015) sampling, mean species richness at the M3 sites was also higher than other sampling years, but the difference was significant only between E0 and E4. In contrast, species richness at the M4 marsh sites in 2016 (E4) was similar to 2013 (E3), but lower than both E1 (2007) and E2 (2010) samplings. Nevertheless, the species richness on this transect in 2016 (E4) was similar to 1999 (E0) sampling (**Figure 16**).



**Figure 16:** Plant species richness (species/plot) at the sites in slough portion of the transects M1, M2, M3 and M4. Different letters represent significant (pairwise t-test; p < 0.05) difference in species richness between sampling period on individual transects.

## Change in major species cover

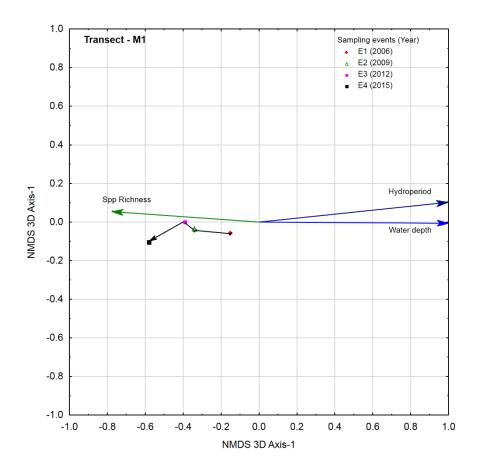
When averaged over all four transects, the total plant cover at the slough sites did not change much. However, relative abundance (Importance Value) of some of most abundant species (Mean Importance Value >2.0) changed significantly. In the previous sampling years, the drying trend was supplemented by an increase in relative abundance of sawgrass (*Cladium mariscus* ssp. *jamaicense*) and spikerush (*Eleocharis cellulosa*) and a decrease in abundance of bladderworts (*Utricularia* spp.) (Figure 17). However, during the sampling period (2011-2016), opposite trend was observed. Relative abundance of sawgrass did not differ between E3 and E4 samplings (Figure 17a). The mean abundance of spikerush significantly increased (Paired t-test; p = 0.28) from 17.2% in E3 to 19.7% in E4 sampling (Figure 17b). In contrast, within the same period the relative abundance of eastern purple bladderwort (*Utricularia purpurea*) increased by more than 50%, from 9.6% to 14.8% (Figure 17c). Interestingly, mean relative abundance of the other species of bladderwort (*Utricularia foliosa*) did not show significant change during the same period (Figure 17d). As in previous sampling years, changes in the relative abundance of lemon bacopa (*Bacopa caroliniana*) was not significant (Figure 17e), while the relative abundance of maidencane (*Panicum hemotomon*) was less in E3 and E4 than in E1 and E4 (Figure 17f).



**Figure** 17: Box-plots of major species' importance value (IV) in the slough portion of transects, averaged across all four (M1-M4) transects for each sampling period. Different letters represent significant (pairwise t-test; p < 0.05) difference in species cover between sampling period.

#### 3.5 Vegetation change in Marl Prairie (2005-2017)

In contrast to the vegetation change pattern observed in slough portion of the transect M1, trajectory analysis revealed that between 2012 and 2015 sampling events, species composition on prairie portion of this transect continued to shift towards a drier type (**Figure 18**). Likewise, more than 50% of sites showed that vegetation-inferred hydroperiod decreased. However, the magnitude of such changes in vegetation-inferred hydroperiod was much less (<30 days) than what was observed between 2006 and 2012 (>30 days) (**Figure 19**). Instead, during the latest sampling event there were more sites showing an increase in inferred hydroperid than the sites before 2012. When averaged over all the sites, the mean reduction in inferred hydroperiod between 2006 and 2012 was 20 days, whereas between 2012 and 2015, it was only one day. Continued drying trend of some sites on M1 observed in 2014, resulted in a significant (t-test, p<0.05) increase in relative abundance (IV) of Muhly grass (*Muhlenbergia capillaris* ssp. *filipes*), *Centella asiatica*, but a decrease in relative abundance of sawgrass and spikerush (Appendix 1). In contrast, relative abundance of some hydric species, such as Beakrush, (*Rhynchospora tracyi*) and Red Bacopa (*Bacopa caroliniana*) increased during the same period.



**Figure 18:** NMDS ordination bi-plots of the trajectory of centroid and the environmental vectors fitted in the ordination space. The ordination is based on species abundance data collected four times between 2006 and 2015 in the prairie portion of the Transect M1. Initial point and the end of the trajectory represent the 2006 and 2015 sampling event, respectively

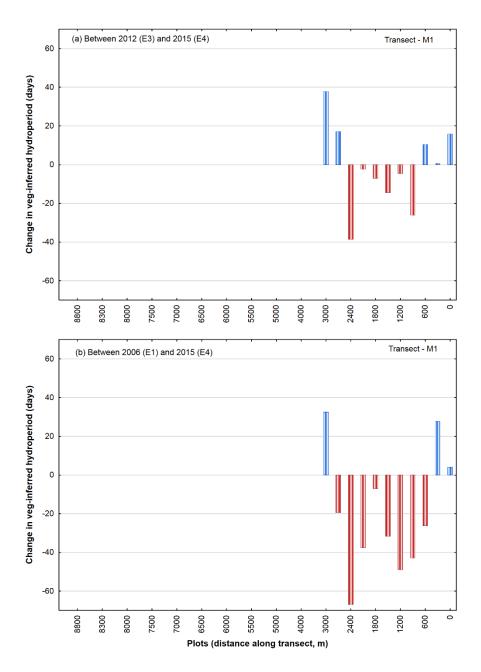
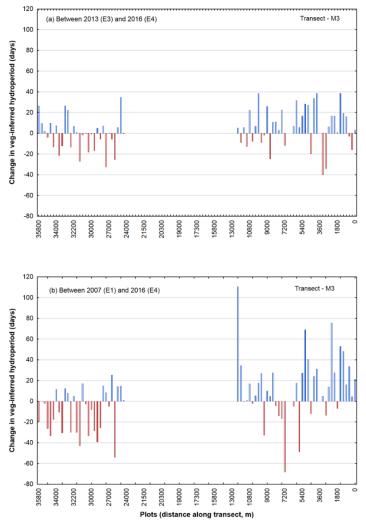


Figure 19: Change in vegetation-inferred hydroperiod between different samplings at the vegetation monitoring plots on the marl prairie portion of the Transect M1.

On M3, which has marl prairie sites located both sides of SRS, vegetation change pattern differed between eastern and western prairies. While species composition in western prairies shifted towards a drier type, as evidenced in an increase in the abundance of *Schizachyrium rhizomatum*, the direction of change in vegetation composition in eastern prairie sites showed a mixed pattern (**Appendix 1**). Several sites at the distal portions of the transect, especially close to the eastern Park boundary, exhibited an increase in inferred-hydroperiod, suggesting that species composition at these sites shifted toward a wetter type between 2007 and 2016 (**Figure 20**).

However, between the E3 (2013) and E4 (2016) samplings, the magnitude of change toward wetter vegetation type along the eastern boundary of ENP was less than the shift over 10 year sampling period, between E1 (2007) and E4 (2016). Surprisingly, in recent years, the abundance of representatives of both prairie and hydric species were observed to increase on this section of the transect. Mean relative abundance of both sawgrass (*Cladium mariscus* ssp. *jamaicense*) and muhly grass (*Muhlenbergia capillaris* ssp. *filipes*) doubled in three years, from 2013 and 2016. The relative abundance of these species in 2016 was similar to the values in 2007.



**Figure 20:** Change in vegetation-inferred hydroperiod between different samplings at the vegetation monitoring plots on the marl prairie portion of the Transect M3.

On M4, which also has marl prairie sites located both sides of SRS, vegetation change pattern in three years, between 2014 and 2017 did not differ much. During this period, vegetation composition at majority of sites showed drying trend (**Figure 21**). However, this shift in vegetation composition differed from the long-term trend observed on this transect, mainly on the eastern portion of the transect. Over ten years, from 2007 to 2017, eastern and western prairies showed different pattern. Vegetation at the sites to the west of SRS (M4W sites) shifted towards drier type, whereas vegetation at the eastern portion of the transect, especially west of the main park road (sites at >3000 m from the beginning of the transect), shifted towards wetter type.

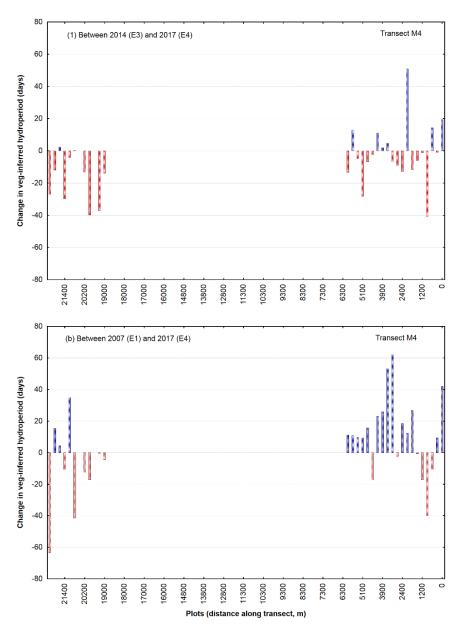
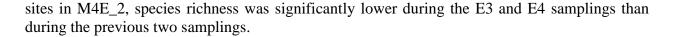
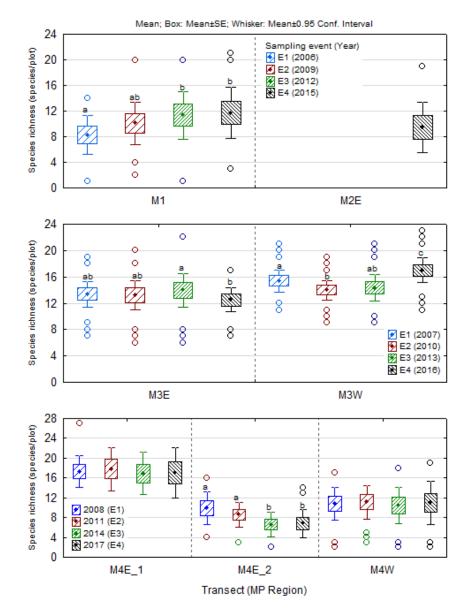


Figure 21: Change in vegetation-inferred hydroperiod between different samplings at the vegetation monitoring plots on the marl prairie portion of the Transect M4

#### Species richness in marl prairies

The shift in vegetation composition observed over 10 years at the marl prairie portion M1 and M3 transects resulted in changes in species richness and plant biomass. Mean species richness increased significantly (Pairwise t-test) on M1 and the eastern portion of M3W, where a drying trend was observed over 13 years. In contrast, on the western portion of M3E, species richness was significantly lower during the E4 than during any previous sampling years (**Figure 22**). On M4, species richness was almost the same over the decade (2007-2017), except in M4E\_2 portion of the transect (east of main park road), where the sites were much wetter in recent years. At the





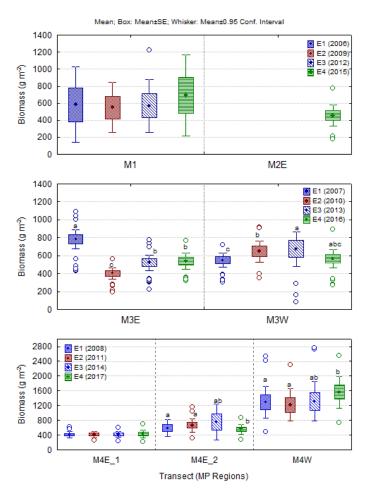
**Figure 22:** Plant species richness (species/plot) at the sites in marl prairie portion of the transects M1, M2E and M3. Transect M3 extends within the marl prairies both sides (M3E and M3W) of Shark River Slough. Different letters represent significant (pairwise t-test; p < 0.05) difference in species richness between sampling period on individual transects or sections of a transect.

#### Change in aboveground plant biomass

Mean plant biomass did not change on M1 (**Figure 23**), but did change on the eastern portion of M3 (M3E), where all but three plots burned in Mustang Fire in 2008. In the eastern marl prairie, above ground biomass during the  $2^{nd}$  sampling (E2), two years after the fire, was only half of what it was during E1. Mean (± SD) aboveground biomass during E1 and E2 was 768 ± 332 g m<sup>-1</sup> and

 $403 \pm 197$  g m<sup>-1</sup>, respectively. In this portion of M3, biomass recovered in three years, but at the time of E3, it was still only two-thirds of the initial biomass. In next three years, biomass increased slightly, but the mean biomass during E4 sampling ( $541 \pm 273$  g m<sup>-1</sup>) was not significantly different (pairwise t-test; df = 38, p = 0.428) from the biomass during E3 ( $514 \pm 267$  g m<sup>-1</sup>).

Biomass on the transect M4 was more or less the same during the first three sampling events (E1-E3). However, biomass during the 2017 sampling varied spatially and across compartments (Figure 23). For instance, at the sites west of the main park road (M4E\_2), where hydrologic conditions in 2017 were wetter than previous sampling years, aboveground biomass during the 2017 (E4) sampling was significantly (Paired t-test: p < 0.001) less than the biomass during the 2007 (E1) and 2010 (E2) samplings. In contrast, at the sites on the western portion of the transect (M4W), aboveground biomass was significantly higher than the first two samplings, E1 and E2. In general, mean ( $\pm$  SD) aboveground biomass at sites west of Shark River Slough was 2-3 times higher than biomass in the eastern prairies. In 2017, the mean ( $\pm$  SD) aboveground biomass was 1,560  $\pm$  604 g m<sup>-1</sup>, 432  $\pm$  145 g m<sup>-1</sup> and 557  $\pm$  176 g m<sup>-1</sup> on M4E\_1, M4E\_2 and MW portions of the transect M4, respectively.



**Figure 23:** Above ground plant biomass (g m-2) at the sites in marl prairie portion of the transects M1, M2E, M3 and M4. Two transects, M3 and M4, extend within the marl prairies both sides of Shark River Slough. Different letters represent significant (pairwise t-test; p < 0.05) difference in aboveground biomass between sampling periods on individual transects or sections of a transect.

### 4. Discussion & Conclusion

In the Greater Everglades, the relationship between hydrologic regime and vegetation distribution is dynamic. Along the marl prairie-slough gradient, vegetation shift on individual transects was also influenced by year-to-year variation in water conditions, possibly caused by both rainfall and water management activities. For instance, while water level was above the thirty-year average during the mid-to-late 1990s, and continuously for three years prior to the 2005 sampling in northern SRS, water level was at or below the 30-year benchmark during the next four years. Moreover, in subsequent years, including the 2011 drought, mean annual water level varied greatly. However, the four-year average water level before E4 (2014-2016) sampling was higher than previous two (2008 & 2011) samplings (Figure 2). In concurrence with the hydrologic shift during the last sampling, vegetation composition in slough portions of M1, M2 and M4 shifted toward a more hydric type (Figures 12, 13 & 15). In contrast, vegetation change on M3 was towards a drier type. Since not all transects were sampled in the same year, the annual variation in water conditions might have also affected the magnitude and direction of vegetation change on these transects. For instance, conditions in 2014, the year before the 2015 sampling on Transect M3, were very dry, which might have caused an aberrant shift in vegetation on M3 compared to other transects. In general, the sensitivity of vegetation to short-term variation in hydrologic conditions observed in this study supports earlier findings that in Everglades prairies and marshes, discernible change in species composition can occur in periods as short as 3-4 years (Armentano et al. 2006; Zweig and Kitchens 2008; Sah et al. 2014).

In the Everglades, the relative abundance of sawgrass and other hydric species such as spikerush, bladderwort and water lilies are considered as an indicator of water conditions in ridge and slough landscape (Ross et al. 2003; Zweig and Kitchens 2008; Ross et al. 2016). In this study, mean sawgrass cover showed an increasing trend until the 2010 sampling, while its cover when averaged over slough sites on all four transects slightly decreased in next six years (Figure 17). However, the sawgrass cover during E4 (2014-2016) sampling was still much higher than during the 1999 sampling. In contrast, mean cover of bladderworts showed an opposite trend. The changes in sawgrass cover during the last 16 years in SRS support the longer-term dynamics, described for the post-drainage era in the Everglades by Bernhardt and Willard (2009). Other researchers also have reported expansion of sawgrass and other emergent species, such as spikerush, in the R&S landscape, primarily due to decrease in water levels (Busch et al., 1998; Zweig and Kitchens, 2008, 2009; Nungesser 2011) and flow velocities (Larsen et al. 2011). Such expansion may occur within 3-4 years, especially when a minimum water level is maintained beneath the peat surface of the sloughs for three consecutive dry seasons (Zweig and Kitchens 2009). While the extensive expansion of sawgrass could be a step towards succession toward woody vegetation, especially when it occurs on elevated ground that experiences prolonged dry conditions, the extended wet seasons that occur intermittently in some years would reverse the process. In the slough portion of all four transects (M1-M4), both four-year average hydroperiod and mean annual water depth before E4 sampling was higher than before E3 (Figure 2). Results suggest that an increase of even 5-10 cm mean annual water depth in SRS can rapidly shift the vegetation towards one more characteristic of slough, as was evidenced in overall decrease in sawgrass and increase in bladderworts in recent years, between E3 and E4 sampling events.

The deviation in trajectories of vegetation shift observed in the slough portion of transects is also affected by fire. Several sites on Transects M1-M3 burned between 2006 and 2012. The Mustang Corner fire that occurred in May 2008, following almost two years of drought and at a time when water level was 65 cm below the surface (Ruiz et al. 2013), may have consumed significant amounts of peat on the SRS portion of Transect M1. The vegetation at five burned sites on M1, where the mean cover was 32% in 1999, was very sparse (cover 11.2%) and comprised mostly of hydric species during 2011 sampling. However, over the next three years, vegetation cover recovered, and mean species richness increased. Fire-induced elevation loss may also have contributed to compositional shift toward wetter vegetation at several locations on this Transect. In comparison to dry season fire, wet season fire seems to have less impact on the vegetation cover. All 18 slough sites on M1, and 25 sites on M2 were burned in two different prescribed fires in 2012 (**Table 2**), but the mean total cover in 2014, two and half years after fire, was already 65% of pre-burn cover. Only one site on Transect M4 burned in 2014, and its effects on vegetation were minimal.

Short-hydroperiod marl prairies in the Everglades are flooded annually for varying periods, while remaining dry for extended portions of the year. Generally, in seasonally-flooded ecosystems similar to the Everglades marl prairies, differences in optimum flooding tolerances of species present in the vegetation mosaic form the basis for variation in vegetation composition (Ross et al. 2006). Hence, the change in vegetation-inferred hydroperiod on the prairie portion of M1, M3 and M4 reflects the amount and direction of change in vegetation (Armentano et al. 2006). In this study, the observed vegetation shift on M1 toward a drier type was not a surprise, especially considering that most years after the E1 sampling event in Spring 2006 were relatively dry. This trend will likely be reversed with the implementation of the MOD Water Delivery Project, which will send more water into the Park through NESRS.

Management-driven water conditions were also responsible for the discrepancy in vegetation change patterns observed in the eastern and western prairies on M3, and to some extent on M4. Water conditions in the prairies west of SRS are influenced by the regulatory schedules for the S-12 structures along Tamiami Trail, implemented under the operational objectives of Interim Structural and Operation Plan (ISOP)/Interim Operational Plan (IOP). In concurrence with management efforts to regulate water deliveries from the S-12 structures, a consistently low water level has been maintained at water recorder NP-205. This has caused the vegetation composition to shift toward a drier type in recent years. However, such a shift towards drier vegetation was less prominent during E4 (2016) sampling, primarily because of the unusually high water conditions in the spring of 2016. These conditions were brought on by high rainfall due to the very strong El Niño, which necessitated the 2016 Temporary Emergency Deviation operated by USACE. The four-year average water depth during E4 sampling also was about 5 cm higher than the previous two samplings. The effect of spring 2016 flooding will likely be intensified and the drying trend will be reversed if the water conditions in subsequent years continue to be high. In the past, dry season flooding followed by years of high water conditions have also caused significant change in habitat conditions. For instance, the management-driven spring season flooding of 1993, followed by high rains in subsequent years, caused short-hydroperiod wet prairie vegetation to change to long-hydroperiod sawgrass marsh that was less suitable habitat for CSSS (Nott et al. 1998). However, under the current water management goal of moving water from west to east, these conditions are unlikely to persist. Recent modeling carried out using Regional

Simulation Model (RSM) tool to evaluate the potential impact of Everglades Restoration Transition Plan (ERTP) also has shown that marl prairies to the northeast of current CSSS habitat will be relatively dry (USACE 2011, 2014; USFWS 2016) resulting in a major change in vegetation composition in the transition zone.

Management-driven water condition has also been a driver of the vegetation shift observed on the eastern portion of Transect M3. In this region, water pump structures at S332B and S332C deliver water from the L31N canal into a series of inter-connected detention ponds. These ponds have a large fixed-crest weir on the western levee that allows water from the pond to enter ENP marl prairies. In addition, water may also enter ENP through subsurface flow. The purpose of operating pump stations along the L-31N canal includes lowering canal and groundwater levels, but creating a continuous hydraulic ridge to control seepage back to the canal while protecting the marl prairie (sparrow habitat) from further deterioration (USACE 2006). Pumping through S332B and S332C serves the management goal of re-hydrating the marl prairies of the Rocky Glades. Thus, a shift in vegetation towards wetter types indicates that the management goal is being achieved, at least in part. However, regular monitoring is essential to detect a signal that inputs of water from the ponds continue to cause a shift in vegetation from marl-dominated wet prairie to marsh types.

In addition to a positive outcome of the operations of water pumps and detention ponds along eastern boarder of the Park, the impact of such management efforts on prairie vegetation needs to be interpreted cautiously, because water flow from detention ponds towards prairies in the Park may have adverse consequences as well. For instance, periphyton near inflow structures had elevated phosphorus in comparison to adjacent marl prairie sites to the west, suggesting an increase in P-loading due to long-term exposure of the canal-side sites to seepage (Gaiser et al. 2008; 2014). Sah et al. (2014) also concluded that vegetation in upper Taylor Slough basin showed significant trajectory along the vector representing the phosphorus gradient, possibly due to the influence of seepage water from the detention ponds. In this study, while soil TC, OC and TN increased along marl prairie-slough gradient as expected (Figure 7), soil phosphorous at the site nearest to (300m) the detention pond levee was higher than at several sites 1200-2400 m from levee (Figure 8). In areas of lower P enrichment, physicochemical measurements that include "bioavailable" P as estimated through fractionation schemes, enzyme (glucosidase, MUF-C and phosphatase, MUF-P) activities and microbial biomass have been used to characterize P-limitation (Wright and Reddy, 2001a,b). In this study, while we observed an increase in MUF-C and microbial biomass-C and P along the marl prairie-slough gradient, higher values of MUF-C, microbial biomass-P and labile-P were observed in soil at the site near the levee than at other adjacent marl prairie sites (Figure 11), suggesting an increase in soil P at that site. If water from the detention ponds continues to influence vegetation in the adjacent prairies, the water quality issue also needs to be addressed so that the affected marl prairies do not shift to another stable state more adapted to P-enriched soil (Hagerthey et al. 2008).

Contrary to management-induced changes in water conditions followed by a shift in vegetation composition observed on Transect M3, the hydrologic changes together with the vegetation shift on southern Transects M4 are more likely rain-driven. In the prairie portion of these transects, vegetation was first sampled in spring 2008, an extremely dry year, and similar conditions prevailed during the previous two years. Subsequently, water conditions varied, though the mean annual water level was above 30-year average for five of the next eight years, probably causing

vegetation on this transect to shift toward a wetter type. However, differences in direction and magnitude of vegetation shift observed between M4E\_1 and M4E\_2, eastern and western sides of the main park road, respectively, were obvious. A shift in vegetation towards wetter type at most sites northwest of the road suggests that main park road acts as the barrier to the natural flow of the water from north to south, resulting in an impounding effect in that region. In fact, mean hydroperiod, averaged over four years was 60 days longer and mean annual water depth was 15 cm higher at the M4E\_2 sites than the M4E\_1 sites. A number of culverts are placed along the road to facilitate the natural flow of water. However, while the culverts along the east-west road in the Pinelands allow the water to flow southward, in the area south of Pay-hay-okee there is a predominant westward flow of water (Steward et al. 2002).

Despite the prevalence of more nature-driven hydrologic condition, an influence of water management activities on the western section of M4, cannot be ruled out. In the western part of the prairie west of Shark River Slough, relatively high water level persisted in mid- to late 2000s, mainly because the hydrologic conditions in this area are influenced by flows through the culvert and bridges on Tamiami Trail and the Loop Road (Kotun et al. 2009). However, the current water management goal of moving water from west to east seems to reverse the trend to some extent.

In summary, regional differences in hydrologic regimes resulting from alternative management strategies have caused variation in species composition within individual landscapes and have also brought on temporal change in vegetation composition in Shark River Slough and adjacent marl prairies. The occurrence of these changes coincided with changes in hydrologic regimes during the past 18 years. Our results provide feedback for the adaptive management of Everglades wetland ecosystems along the marl prairie-slough gradient.

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**Appendix 1:** Importance value index (IV) of species present at the marl prairie sites of Transect M1, M2E, M3 (M3E and M3W), and M4 (M4E\_1, M4E\_2 and M4W) that were sampled four times between 2006 and 2016. Marl prairie sites on Transect M3 and M4 are on both sides of the Shark River Slough.

			Ν	<b>T</b> 1		M2E				Μ	3E			
S.NO	Species		14.	11				Μ	3E			M	3W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
1	Aeschynomene pratensis	0.81	1.52	0.06	2.19	0.49		0.01		0.12	0.79	0.56	0.09	1.29
2	Agalinis linifolia		0.46					0.20	0.02	0.27	0.15	0.07	0.11	0.87
3	Agalinis sp.								0.00					
4	Aletris bracteata							0.02	0.01	0.05				
5	Ambrosia artemisiifolia									0.04				
6	Andropogon glomeratus var. glomeratus						0.01			0.05				
7	Andropogon virginicus	0.69	1.05	2.43	0.24	0.14	0.02	0.46	0.37	0.26	0.34	0.46	0.02	0.23
8	Anemia adiantifolia						0.11							
9	Angadenia berteroi						0.10	0.05	0.00	0.08				
10	Annona glabra	0.42		0.18	0.15	0.45	0.08	0.06	0.06	0.06	3.58	1.96	0.60	0.78
11	Ardisia escallonioides							0.01	0.00	0.02				
12	Aristida purpurascens			0.38		0.77	0.04	0.95	0.74	0.94	0.02	0.13	0.56	0.29
13	Aristida stricta						0.02							
14	Asclepias lanceolata					0.03	0.16	0.07	0.00	0.04	0.03	0.16	0.08	0.23
15	Asclepias longifolia												0.04	0.04
16	Symphyotrichum adnatum					0.07								
17	Symphyotrichum bracei				2.94	0.92				2.05	0.33			1.19
18	Symphyotrichum dumosum				0.47		0.01	0.08	0.02	0.38	0.64	2.23	0.32	1.04
19	Symphyotrichum subulatum						0.92							
20	Symphyotrichum tenuifolium	1.75	1.17	3.09				4.99	0.66	0.11	0.06	2.54	0.59	0.36
21	Aster sp.						0.08							
22	Bacopa caroliniana	3.94	1.14	1.50	4.95	4.65	2.27	1.95	0.95	1.21	4.64	4.43	1.37	3.11
23	Baccharis glomeruliflora									0.13				
24	Baccharis halimifolia		0.04				0.11		0.08					
25	Bacopa monnieri										0.04	0.48		
26	Blechnum serrulatum					0.07	0.59	0.10	0.30	0.27				
27	Buchnera americana						0.01			0.04				0.11

			N	<b>I</b> 1		M2E				Μ	3E			
S.NO	Species		10	11		NIZE		Μ	3E			M,	3W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
28	Capraria biflora								0.00					
29	Cassytha filiformis						0.70	0.29	0.05	0.19	3.81	0.59	0.71	1.37
30	Centella asiatica	2.77	3.00	3.56	4.63	1.54	3.27	0.70	0.40	0.16	3.04	4.64	2.49	2.85
31	Cephalanthus occidentalis				0.17				0.00		0.01		0.00	0.01
32	Chiococca parvifolia					0.29	0.04			0.05				
33	Chrysobalanus icaco									0.07				
34	Cirsium horridulum							0.05	0.00	0.01				
35	Cladium mariscus ssp. jamaicense	40.94	41.35	37.42	27.65	34.49	47.57	38.03	22.79	42.34	20.38	22.39	24.44	25.86
36	Coelorachis rugosa											0.01		
37	Conoclinium coelestinum							0.42						
38	Crinum americanum		0.31	0.54	0.33	0.23				0.01	1.72	1.03	0.53	2.37
39	Cyperus haspan			0.06			0.01	0.14	0.01					
40	Cyperus sp.							0.08						
41	Dichanthelium aciculare			0.04		0.11			0.03	0.15				
42	Dichanthelium dichotomum				0.16	0.06	1.11	1.64	0.01	1.76				0.13
43	Dichanthelium sp.					0.33								
44	Diodia virginiana								0.00					
45	Dyschoriste angusta					0.11							0.07	
46	Echinochloa sp.										0.01			
47	Eleocharis baldwinii			0.04					0.03					
48	Eleocharis cellulosa	9.88	13.72	9.91	7.14	27.15	3.25	4.94	3.59	3.59	2.35	2.08	1.28	2.43
49	Eleocharis elongata							0.08						
50	Eleocharis geniculata				0.06									
51	Eragrostis elliottii	0.27		0.84	0.16	0.07	0.87	0.81	0.03	0.32	0.20	0.32	0.15	0.37
52	Eriocaulon compressum											0.15		0.04
53	Erigeron quercifolius						0.07	0.12	0.01		0.06			
54	Eugenia axillaris						0.08		0.00					
55	Eupatorium capillifolium		0.36	0.04			1.30							
56	Eupatorium leptophyllum				0.26			0.49	0.16	0.37				
57	Eupatorium mikanioides		0.29	0.04	0.21		0.18	0.11	0.00	0.02	0.16	0.24	0.08	0.53

			Ν	<b>f</b> 1		M2E				Μ	3E			
S.NO	Species		10.	11		WIZE		Μ	3E			M	3W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
58	Eustachys petraea						0.11		0.12				0.04	
59	Flaveria linearis						0.36							
60	Fuirena breviseta	0.07		0.21	0.03	0.06	0.01	0.59	0.15	0.12	0.01			0.07
61	Habenaria repens											0.07		
62	Helenium pinnatifidum											0.01		0.11
63	Heliotropium polyphyllum					0.20	0.15	0.09	0.00	0.11				
64	Hibiscus grandiflorus						0.01			0.05	0.07	0.01		0.08
65	Hydrolea corymbosa								0.00					
66	Hymenocallis latifolia													0.66
67	Hymenocallis palmeri						0.17	0.07	0.05	0.41	1.16	1.18	0.62	1.46
68	Hyptis alata						0.38	0.25	0.04	0.27	0.04	0.10		0.05
69	Hypericum cistifolium							0.07						
70	Hypericum hypericoides								0.00					
71	Ipomoea sagittata		1.20	0.03	0.71	0.48	0.27	0.15	0.16	0.62	0.37	0.34	0.10	0.57
72	Iva microcephala			0.59	0.77	0.40	0.14	0.46	0.08	0.12				
73	Juncus megacephalus													0.01
74	Justicia angusta	0.90	0.96	1.33	1.44	0.47	0.27	1.02	0.08	1.16	0.96	1.56	0.47	1.50
75	Kosteletzkya virginica							0.03	0.10				0.02	
76	Leersia hexandra	0.21	0.78	0.35	0.19			0.16			0.23	0.70	0.31	1.44
77	Linum medium						0.01	0.02					0.03	0.11
78	Lobelia glandulosa							0.03	0.01		0.14			0.16
79	Ludwigia alata				0.33	0.06	0.03	0.34	0.10	0.12			0.00	
80	Ludwigia curtissii				0.30	0.03				0.36				
81	Ludwigia microcarpa	0.22	0.06	0.93	0.04		0.19	0.63	0.11	0.10	0.06	0.80	0.07	0.19
82	Ludwigia repens		1.38			0.06	1.20	0.81	0.08		0.32	0.05		
83	Ludwigia sp.									0.44				
84	Melaleuca quinquenervia		0.22											
85	Metopium toxiferum						0.20							
86	Mikania scandens	0.27		0.18		0.07	1.55	1.84	0.09	1.06	0.14	0.14		0.05
87	Mitreola petiolata			0.23		0.20	0.71	0.08	0.00	0.39	0.81	0.28	0.18	2.04

			Ν	<b>F1</b>		M2E				Μ	3E			
S.NO	Species		10.	11		WIZE		M	3E			M	8W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
88	Muhlenbergia capillaris var. filipes	2.46	5.42	4.32	7.90	3.17	7.49	7.74	3.55	7.07	0.61	0.39	0.18	0.17
89	Morella cerifera	0.12			0.07	0.15	0.62	0.01	0.30	0.67	0.06	0.17	0.03	0.09
90	Myrsine floridana						0.14			0.05				
91	Nymphoides aquatica						0.01	0.03			0.07	0.20	0.00	0.18
92	Nymphaea odorata											0.05		
93	Oxypolis filiformis	0.50	0.14	0.09	0.09	0.04	0.11	0.48	0.04	0.10	0.16	0.18	0.17	0.21
94	Panicum dichotomiflorum							1.57						
95	Panicum hemitomon	0.21		0.07	1.16	1.38	0.22	0.93	0.20	0.14	2.15	1.54	0.20	0.60
96	Panicum rigidulum						0.04	0.13	0.18		0.04	0.14	0.00	
97	Panicum tenerum	4.38	4.35	5.74	3.65	1.45	3.05	5.43	2.22	5.06	3.37	6.77	1.75	4.95
98	Panicum virgatum		0.61	0.03	0.33	0.41	0.61	0.15	0.06	0.59	5.59	7.57	5.06	6.58
99	Panicum sp.							0.11						
100	Parthenocissus quinquefolia								0.00	0.15				
101	Paspalidium geminatum				0.17	1.55	0.05		0.11	0.12	0.14	0.61	0.02	0.28
102	Paspalum monostachyum							0.05	0.06	0.21	4.29	8.47	5.60	5.02
103	Passiflora suberosa							0.01	0.02					
104	Peltandra virginica	0.74	1.16	0.21	0.06		0.11	0.06	0.01	0.27	0.01		0.12	0.06
105	Persea borbonia						0.20	0.10	0.38	0.75	0.03	0.13	0.00	0.03
106	Phytolacca americana								0.01					
107	Phyllanthus caroliniensis					0.05	0.05							
108	Phyla nodiflora	1.39	0.51	0.44	2.66	1.08	3.77	0.89	0.58	0.60	0.10	0.09		
109	Phyla stoechadifolia		0.74	0.47	0.89	0.60	0.70	0.10	0.04	0.32				
110	Phyllanthus sp.												0.02	
111	Pinguicula pumila						0.01							
112	Piriqueta cistoides ssp. caroliniana												0.02	
113	Pluchea odorata									0.03				
114	Pluchea rosea	1.61	2.67	1.55	2.78	1.09	4.10	4.50	1.02	4.51	3.40	3.20	1.00	2.22
115	Polygala balduinii					0.07	0.01							
116	Polygala grandiflora						0.10	0.04	0.00	0.04			0.03	0.13
117	Polygonum hydropiperoides		0.79	0.75	0.06	0.19	0.31	0.15	0.06	0.09	0.17	0.09	0.02	

			N	<b>1</b> 1		M2E				Μ	3E			
S.NO	Species		IV	11		NIZE		Μ	3E			M	3W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
118	Pontederia cordata	0.27	0.58	0.27	0.33			0.35	0.00		0.42	0.41	0.02	0.06
119	Proserpinaca palustris		0.72	0.19	0.07		0.75	0.54	0.19	0.24	0.30	0.06	0.00	0.16
120	Psychotria nervosa						0.35			0.01				
121	Pteridium aquilinum						0.58	0.34	0.25	0.20				
122	Quercus sp.													0.01
123	Randia aculeata						0.38	0.01	0.08	0.26				
124	Rhus copallinum								0.25	0.09				
125	Rhynchospora colorata						0.01	0.15	0.03	0.01	0.13	0.06	0.00	0.07
126	Rhynchospora divergens				0.03	1.11		0.85	0.86	0.01				0.09
127	Rhynchospora inundata	0.25		1.12	1.35	0.65	0.12			0.09	4.66	1.70	0.53	1.31
128	Rhynchospora microcarpa	0.05		1.35	0.44	1.76	0.69	1.38	0.71	1.81	9.21	3.00	2.71	3.16
129	Rhynchospora miliacea		1.56											
130	Rhynchospora tracyi	11.80	9.08	13.12	15.89	9.57	0.61	4.54	1.08	2.70	10.18	4.70	4.93	5.77
131	Rhynchospora sp.						0.02							
132	Sabatia grandiflora						0.06	0.14		0.04	0.12			0.29
133	Sabal palmetto					0.03	0.01	0.02			0.01			
134	Saccharum giganteum		0.18	0.22	0.60		0.18	0.06	0.01	0.21	0.01	0.07	0.00	0.18
135	Sagittaria lancifolia				0.15	0.16	0.61	0.59	0.12	0.53	0.77	1.04	0.15	0.58
136	Salix caroliniana	0.21	0.22	0.32	0.33		0.08		0.28	0.03	0.12		0.16	0.36
137	Samolus ebracteatus					0.05	0.37	0.23	0.05	0.16				
138	Funastrum clausum										0.18	0.38		
139	Schoenolirion albiflorum											0.19	0.05	
140	Schoenus nigricans						0.01	0.01	0.05	0.01	1.24	1.73	2.80	2.21
141	Schizachyrium rhizomatum		0.15	0.24		0.11	2.97	2.72	1.85	5.32	4.64	5.71	4.17	8.78
142	Schinus terebinthifolius					0.01	0.08		0.01					
143	Scirpus sp.										0.09			
144	Setaria parviflora			0.69	1.58	0.37	0.74	0.08		0.65	0.01	0.22		
145	Sideroxylon salicifolium						0.01	0.05	0.00					
146	Smilax laurifolia						0.02							
147	Solidago fistulosa							0.16						

			N	<b>I</b> 1		M2E				M	3E			
S.NO	Species		IV	11		NIZE		Μ	3E			M.	3W	
		2006	2009	2012	2015	2015	2007	2010	2013	2016	2007	2010	2013	2016
148	Solidago stricta		0.03	0.13		0.39	0.29	0.82	0.08	0.25	0.17		0.02	0.18
149	Taxodium distichum var. imbricrium										0.05			
150	Teucrium canadense					0.01		0.29	0.04	0.04		0.11		
151	Thalia geniculata							0.03			0.01	0.12	0.02	
152	Thelypteris kunthii						0.01							
153	Toxicodendron radicans						0.39	0.06	0.21	0.65				
154	Trema micrantha							0.22	0.07	0.02				
155	Typha domingensis	6.34	0.94	1.84	3.82						0.35	0.67	1.17	0.38
156	Unknown grass									0.05	0.03			0.05
159	Unknown sp01										0.06			
160	Unknown sp02								0.00		0.14			
161	Unknown sp03								0.00		0.04			
162	Unknown sp04										0.08			
163	Unknown sp05										0.05			
164	Unknown sp06							0.10						
165	Unknown sp07											0.04		
166	Unknown sp08										0.05			
157	Unknown sp16					0.21								
158	Unknown sp17									0.02				
167	Utricularia cornuta					0.34							0.02	
168	Utricularia foliosa	0.05	0.09	0.51			0.08	0.01	0.00	0.96		0.02		0.56
169	Utricularia purpurea	6.49	1.04	2.36				0.09	1.43	4.32		0.42		1.28
170	Utricularia subulata											0.01		
171	Utricularia sp.							0.01						
172	Vernonia blodgettii							0.16	0.00	0.02				
173	Vitis rotundifolia						0.16			0.04				

Appendix 1: Contd. (Transect M4 (M4E\_1, M4E2, MW)

							N	<b>1</b> 4					
S. SO.	Species		M4	E_1			M4	E_2			M	4W	
		2008	2011	2014	2017	2008	2011	2014	2017	2008	2011	2014	2017
1	Aeschynomene pratensis	0.00				0.47	0.41		0.72	2.88	1.77	1.29	0.48
2	Agalinis linifolia		0.18	0.18	0.46	1.12	0.79	0.26	0.32		0.29		
3	Aletris bracteata		0.02	0.02	0.15								
4	Anemia adiantifolia	0.02		0.11	0.15								
5	Angadenia berteroi	0.23	0.02	0.28	0.31								
6	Annona glabra	0.17				0.18				1.05		0.09	0.03
7	Aristida purpurascens	0.29	0.40		0.57								
8	Asclepias lanceolata	0.03	0.03	0.34							0.14	0.02	
9	Asclepias longifolia		0.20										
10	Symphyotrichum bracei	2.27		3.75	0.15	0.12						0.14	
11	Symphyotrichum dumosum	1.78	2.40	1.07	2.35	0.20		0.23		0.48	0.76	0.72	0.70
12	Symphyotrichum tenuifolium		3.51		3.90		0.26				0.52	0.34	0.38
13	Bacopa caroliniana					4.89	7.22	6.32	6.25	5.11	4.11	7.13	3.80
14	Bacopa monnieri						1.69				1.87		
15	Boehmeria cylindrica										0.11		
16	Cassytha filiformis	1.93	2.11	1.18	1.05					4.00	5.59	4.80	3.63
17	Centella asiatica	0.55	0.21	0.61	0.27	0.23				1.49	1.85	1.03	0.65
18	Cephalanthus occidentalis										0.35		
19	Chiococca parvifolia	0.14	0.21	0.40									
20	Cirsium horridulum Cladium mariscus ssp.		0.02	0.14									
21	jamaicense	23.76	24.94	20.56	33.17	41.77	37.09	42.91	42.96	44.82	35.27	41.15	53.62
22	Conocarpus erectus	5.40	1.01	2.29	0.08								
23	Crinum americanum									0.92	2.63	2.78	1.30
24	Cyperus haspan									0.05		0.03	
25	Dichanthelium aciculare				0.16								
26	Dichanthelium dichotomum	0.57	0.35	0.25	0.60	0.45							

							Ν	<b>1</b> 4					
S. SO.	Species		M4	E_1			M4	E_2			M	łW	
		2008	2011	2014	2017	2008	2011	2014	2017	2008	2011	2014	2017
27	Dyschoriste angusta	1.55	3.76	0.93	1.00								0.59
28	Eleocharis cellulosa					12.48	24.20	22.99	27.69	2.62	4.44	5.79	5.23
29	Eragrostis elliottii		0.33	0.14	0.31					0.03	0.42		
30	Eriocaulon compressum								0.25				
31	Eupatorium mikanioides	0.39	0.48	0.45	0.29					0.02	0.33	0.11	0.24
32	Evolvulus sericeus		0.14										
33	Ficus aurea									0.05			
34	Fuirena breviseta	0.02				0.51		0.09				0.03	
35	Fuirena scirpoidea						0.29						
36	Galactia volubilis									0.23			
37	Helenium pinnatifidum		0.40	0.37	0.34	0.76	0.26	0.35	0.04	0.22	0.05	0.12	0.11
38	Heliotropium polyphyllum	0.80	0.76	1.20	1.37								
39	Hydrolea corymbosa									1.46	1.24	0.05	1.11
40	Hymenocallis palmeri	0.17	1.09	0.86	0.51	0.30	0.71	0.08	1.22	0.51	0.31	0.40	1.27
41	Hyptis alata	0.37	0.53	0.14	0.44					0.33	0.20		
42	Ilex cassine		0.50	0.14									
43	Ipomoea sagittata	1.01	0.61	1.40	1.18	0.04		0.09		0.71	0.36	1.80	0.98
44	Iva microcephala	1.10	0.46	1.02	0.94								
45	Jacquemontia curtissii			0.14	0.02								
46	Justicia angusta	0.04			0.17		0.67	0.97	1.65	1.49	2.68	1.77	1.70
47	Kosteletzkya virginica									0.05			
48	Leersia hexandra					0.23	0.39			0.76	0.03	0.16	0.31
49	Ludwigia alata									0.56	1.12		0.76
50	Ludwigia curtissii				0.15								
51	Ludwigia microcarpa	0.18	0.78										
52	Ludwigia repens					0.48				1.31	3.48	0.80	0.38
53	Ludwigia sp.											0.23	
54	Magnolia virginiana	0.03				0.04							
55	Melanthera nivea	0.22	1.40	0.33	0.66								

							Μ	4					
S. SO.	Species		M4	E_1			M41	E_2			M	4W	
		2008	2011	2014	2017	2008	2011	2014	2017	2008	2011	2014	2017
56	Metopium toxiferum				0.06								
57	Mikania scandens	2.01	2.18	2.55	2.45			0.09	0.28			0.73	2.39
58	Mitreola petiolata Muhlenbergia capillaris var.					0.49	0.27	0.05	0.04			0.11	
59	filipes	10.43	14.48	11.41	11.38							0.14	0.46
60	Morella cerifera		0.12	0.36	0.19								
61	Myrsine floridana	0.31		0.26	0.02								
62	Nymphoides aquatica							0.20					
63	Nymphaea odorata					0.04					0.14		
64	Oxypolis filiformis	0.03		0.21	0.15	0.17	0.49	0.26	1.19			0.04	0.12
65	Panicum hemitomon					0.91	0.91		0.40	1.68	2.37	0.90	0.51
66	Panicum rigidulum					0.06							
67	Panicum tenerum	3.63	2.82	1.14	2.37	0.49		0.30	0.04	1.12	0.86	1.56	1.61
68	Panicum virgatum	1.85	3.78	3.00	3.47	0.04		0.82	0.04	2.42	3.23	1.47	1.60
69	Paspalidium geminatum				0.17			0.41	0.25				
70	Paspalum monostachyum	2.33	2.38	4.60	1.71					0.12			
71	Passiflora suberosa		0.02										
72	Peltandra virginica					0.23		0.54	0.41	0.28	0.04	0.27	0.44
73	Persea borbonia	0.06				0.04	0.09						
74	Phyla nodiflora	6.00	4.00	5.26	4.74	0.45	0.06	0.26		0.16			
75	Phyla stoechadifolia	1.17	0.28	0.36	0.45								
76	Phyllanthus sp. Piriqueta cistoides ssp.	0.26											
77	caroliniana		0.02		0.32								
78	Pluchea rosea	6.32	5.98	4.42	4.80	1.17	1.28	1.58	1.06	2.54	1.27	1.31	1.37
79	Polygala grandiflora	0.30	0.14		0.49								
80	Polygonum hydropiperoides					0.05				0.37		0.33	
81	Pontederia cordata					1.06	0.29	0.09	1.42	0.51	2.35	1.47	1.14
82	Proserpinaca palustris	0.03	0.37			0.23	0.88	0.09		0.21	0.74	0.98	0.18

							M	[4					
S. SO.	Species		M4	E_1			M4	E_2			M4	łW	
		2008	2011	2014	2017	2008	2011	2014	2017	2008	2011	2014	2017
83	Rhynchospora colorata	0.02	0.14	0.14									
84	Rhynchospora inundata					0.68		1.23	0.28	0.46	1.53	1.25	0.92
85	Rhynchospora microcarpa	0.64	2.64	2.82	2.09	0.82	0.69		0.21	0.62	0.39	0.65	1.69
86	Rhynchospora tracyi	0.44	0.35	0.48	2.24	8.40	3.05	3.88	5.70	4.94	1.68	1.42	1.75
87	Sabal palmetto		0.23	0.03									
88	Sagittaria lancifolia					1.80	2.35	1.13	1.22	0.68	0.82	0.81	1.59
89	Samolus ebracteatus	0.12	0.02		0.15								
90	Funastrum clausum									0.05		0.06	
91	Schoenus nigricans									1.49	1.35	0.91	1.58
92	Schizachyrium rhizomatum	9.17	7.40	7.55	6.83					3.47	3.60	4.28	1.57
93	Setaria parviflora				0.16								
94	Smilax sp.		0.02										
95	Solidago stricta	0.98	0.29	0.70	0.32	0.20							
96	Spartina bakeri Taxodium distichum var.	1.42	0.31	1.20									
97	imbricrium	9.27	3.26	13.18	3.77	9.87	6.75	12.97	5.78				
98	Teucrium canadense	0.12	1.84	1.83	0.47		0.35						
99	Thalia geniculata									0.42	1.75	3.37	1.33
100	Tillandsia balbisiana								0.05				
101	Tillandsia flexuosa					0.04							
102	Tillandsia paucifolia	0.07	0.08			0.33	0.11		0.11				
103	Typha domingensis									6.94	7.70	6.39	2.46
104	Unknown gr02				0.15								
105	Unknown sp11						0.39						
106	Unknown sp15			0.21									
107	Unknown sp19				0.15								
108	Unknown sp20				0.15								
109	Unknown sp09									0.11			
110	Utricularia cornuta					0.41							

							Μ	[4					
S. SO.	Species		M4	E_1			M4]	E_2			M4	łW	
		2008	2011	2014	2017	2008	2011	2014	2017	2008	2011	2014	2017
111	Utricularia foliosa						3.68	1.10	0.41	0.05	0.26	0.79	
112	Utricularia gibba					4.16				0.02	0.05		
113	Utricularia purpurea					3.60	4.40	0.71		0.22			
114	Vitis shuttleworthii					0.04							