Landscape Pattern- Ridge, Slough, and Tree Island Mosaics
(Cooperative Agreement #: W912HZ-20-2-0038)
Cycle-3: Year 3 Report
(2020-2023)

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General Background

The Water Resources Development Act (WRDA) of 2000 authorized the Comprehensive Everglades Restoration Plan (CERP) as a framework for modifications and operational changes to the Central and Southern Florida Project needed to restore the South Florida ecosystems. Provisions within WRDA 2000 provide for specific authorization for an adaptive assessment and monitoring program. A CERP Monitoring and Assessment Plan (MAP; RECOVER 2004, 2006, 2009) has been developed as the primary tool to assess the system-wide performance of the CERP by the Restoration Coordination and Verification (RECOVER) program. The MAP presents the monitoring and supporting research needed to measure the responses of the South Florida ecosystem to CERP implementation.

The general goals of restoration are to stem and possibly reverse the degradation of the ridge-slough-tree island landscape by redirecting flows to coastal waters across the surface of this landscape (USACE and SFWMD 1999). The CERP MAP, Parts 1 and 2, presented the overarching monitoring framework for guiding restoration efforts throughout the entire process (RECOVER 2004, 2006). This required the then ongoing monitoring and evaluation through the process that would aid the implementing agencies in optimizing operational procedures and project designs. The work described below represents the system-wide landscape monitoring project entitled "*Landscape Pattern - Ridge, Slough, and Tree Island Mosaics*," initiated in 2009 with funding from the US Army Corps of Engineers (USACE). Until 2012, the study was led by Dr. James Heffernan (PI) and then by Dr. Michael Ross for the next three years (2012-2015) (Heffernan et al. 2009; Ross et al. 2016). Since the Fall of 2015 (Cooperative Agreement # W912HZ-15-2-0027 (2015-2020), and W912HZ-20-2-0038 (2020-Present)), the study has been led by Dr. Jay Sah (PI), with Dr. Michael Ross as a Co-PI, and Dr. James Heffernan (Duke University) as a collaborator in the study.

This monitoring effort supports the Greater Everglades Wetlands module of the MAP and is designed to address the needs identified in the Greater Everglades wetlands performance measures: (1) GE 15: Wetland Landscape Patterns – Ridge-Slough Community Sustainability, and 2) Total System Performance Measures - Slough Vegetation (RECOVER 2011). This study addresses explicitly the Greater Everglades Wetland Landscape and Plant Community Dynamics hypotheses: (1) ridge and slough microtopography in relation to organic soil accretion and loss; (2) ridge and slough landscape pattern in relation to microtopography; and (3) plant community dynamics in ridge-slough peatlands along elevation gradients as water depths and hydroperiods change (RECOVER 2006). The working hypothesis is, '*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 primary objective of this monitoring project is to assess the condition of wetlands within the historic distribution of the ridge and slough (R&S) landscape and to provide baseline data and ongoing changes/trends in the patterns in microtopography and vegetation communities in response to water management operations, restoration initiatives and episodic events such as droughts, fire, and hurricanes. The specific objectives of the study are:

- To determine extant reference conditions for each performance measure described above (including variability of those measures in time and space).
- To establish a present status of landscape performance measures throughout the central Everglades, particularly in historic ridge-slough landscape patterning areas, identify spatial and temporal trends of those performance measures, and quantify their relationships to the present hydrologic regime.
- To detect unanticipated changes in ecosystem structure and processes that result from hydrologic management or manipulation, CERP restoration activities, or climatic variation.
- To provide data supporting scientific studies of inter-relationships among vegetation, microtopography, and hydrologic regime that may provide insight into the causes of unanticipated ecosystem responses.

This study took advantage of the Generalized Random-Tessellation Stratified sampling network (GRTS), an established framework for representative sampling of the entire Everglades landscape (Philippi 2007). The sampling framework divides the Everglades landscape into a grid of 2x5 km landscape blocks (primary sample units; PSUs) of which the 5 km edge is parallel to the historic water flow. Initially, a spatially stratified random sample of 80 PSUs were selected for sampling over a 5-year period (n=16 per year) (Philippi 2007; Heffernan et al. 2009). Those 80 PSUs were drawn to achieve a spatially balanced sample of the modern Everglades compartments (Everglades National Park (ENP), Water Conservation Area 3A North (WCA3AN), Water

Conservation Area 3A South (WCA3AS), Water Conservation Area 3B (WCA3B), Water Conservation Area 2 (WCA2), and Water Conservation Area 1 (WCA1)/the Loxahatchee National Wildlife Refuge (LNWR). However, once the project was launched in 2009, after three years (2009-2011) of sampling, because of budget constraints since FY 2012 (Cycle-1, Year 3), the number of PSUs and the number of sites within each PSU sampled in successive years were adjusted. Some PSUs that either were not within the historic R&S landscape or were dominated by woody components were later dropped. During Years 3 and 4, monitoring efforts also included additional PSUs or modified primary sample units (M-PSUs) outside the original sampling scheme, with the purpose of documenting pre-restoration reference conditions within wetlands influenced by the construction/ implementation of the DECOMP Physical Model and two Tamiami Bridges. The purpose for such an addition was that the monitoring within the modified sampling units (M-PSUs) would provide ecosystem responses to those specific projects over time and thus would be useful for the adaptive management. Together with these modifications, over six years (2009-2015), including a pilot project year (2009), 67 PSUs, including 5 in the marl prairie landscape, were sampled. These PSUs represent the full range of contemporary hydrologic regimes, and their vegetative and microtopographic structure range from well-conserved to severely degraded R&S landscapes (Ross et al. 2016). During the next five years (2015-2020), 58 PSUs were re-surveyed, and the results were summarized in four annual reports and the final 5-year report (Sah et al. 2021).

With the initiation of the 3rd 5-year cycle (2020-Present) of monitoring in 2020, the study plan focuses on resampling the plots within the previously sampled 59 PSUs, 11 PSUs in Year-1, and 12 PSUs in each of four years after that. Since researchers have described that prairie and marsh vegetation may change in 3-5 years in response to hydrologic changes (Armentano et al. 2006; Zweig and Kitchens 2008), re-sampling the plots every five years has been expected to provide an opportunity to assess changes in microtopography and vegetation composition over time. The Cycle-3 Year-1 and Year-2 survey results have been summarized in Sah et al. (2023a, 2023b). This document summarizes collective results for PSUs surveyed in Year-1, 2 and 3 (Water Years: 2021, 2022 and 2023) of this five-year cycle (Cycle-3; 2020-2025) of the project. The report primarily focuses on the changes in topographic metrics (distribution of soil elevation variance) and community characteristics (community distinctness and the strength of elevation-vegetation associations) since the first survey.

1. Introduction

The Florida Everglades is a large subtropical wetland with diverse hydrologic, edaphic, and vegetative characteristics. Of the eight major historic landscapes that comprised the greater Everglades, the ridge and slough (R&S) landscape - a mosaic of sloughs, sawgrass ridges, and tree islands - encompassed slightly over 50% of the total extent (McVoy et al. 2011). Within this landscape, biotic communities occupied distinct elevational niches organized in a characteristic elongated pattern parallel to water flow. Ridges, comprised almost entirely of dense stands of sawgrass, were present in areas of higher topographic relief with shallow water depths, whereas sloughs containing white water lily (*Nymphaea odorata*) and other macrophytes, were at lower elevations with relatively deep water (Loveless 1959, McVoy et al. 2011). A transitional community, the wet prairie, was usually present at the boundary of ridges and sloughs, in areas of intermediate water depths (Loveless 1959, Ogden 2005).

As in all wetlands, the hydrologic regime is a critical factor influencing the distribution and composition of vegetation in the greater Everglades (Gunderson 1994, Ross et al. 2003, Armentano et al. 2006, Zweig and Kitchens 2008, Todd et al. 2010). Local variation in hydrologic conditions resulting from microtopographic differentiation is essential for the maintenance of the distinct vegetation community boundaries that were a feature of the pre-drainage R&S landscape. This landscape, however, has undergone dramatic structural, compositional, and functional changes since human modification of the hydrologic regime began in the early 20th century (Davis and Ogden 1994, Bernhardt and Willard 2009, Larsen et al. 2011, McVoy et al. 2011). Where hydroperiods have been reduced, ridges have invaded marsh areas (Ogden 2005), and much of the slough component of the landscape has been usurped by both wet prairie and ridge. Woody vegetation might have been uncommon in the ridge community prior to hydrologic modification, but wax myrtle (*Morella cerifera*) and coastal plain willow (*Salix caroliniana*) now frequently inhabit ridges in drained areas (McVoy et al. 2011).

Hydrologic modification, coupled with the flow of phosphorus-enriched water into the system, also had consequences for the landscape-scale structure of the R&S mosaic (Figure 1). Areas of reduced flow have lost the elongated R&S topography, while areas with excessively extended flooding have experienced a decline in the prevalence of ridges and tree islands (Sklar et al. 2004, Ogden 2005). The remaining ridges have lost rigidity, structure, and directionality (or anisotropy; Wu et al. 2006, Watts et al. 2010; Ross et al. 2016), and elevation differences between ridges and sloughs have become less distinct (Watts et al. 2010, Hefferenan et al. 2009; Ross et al. 2016). Moreover, nutrient enriched areas have become dominated by stands of *Typha* with little topographic relief (Newman et al. 1998).

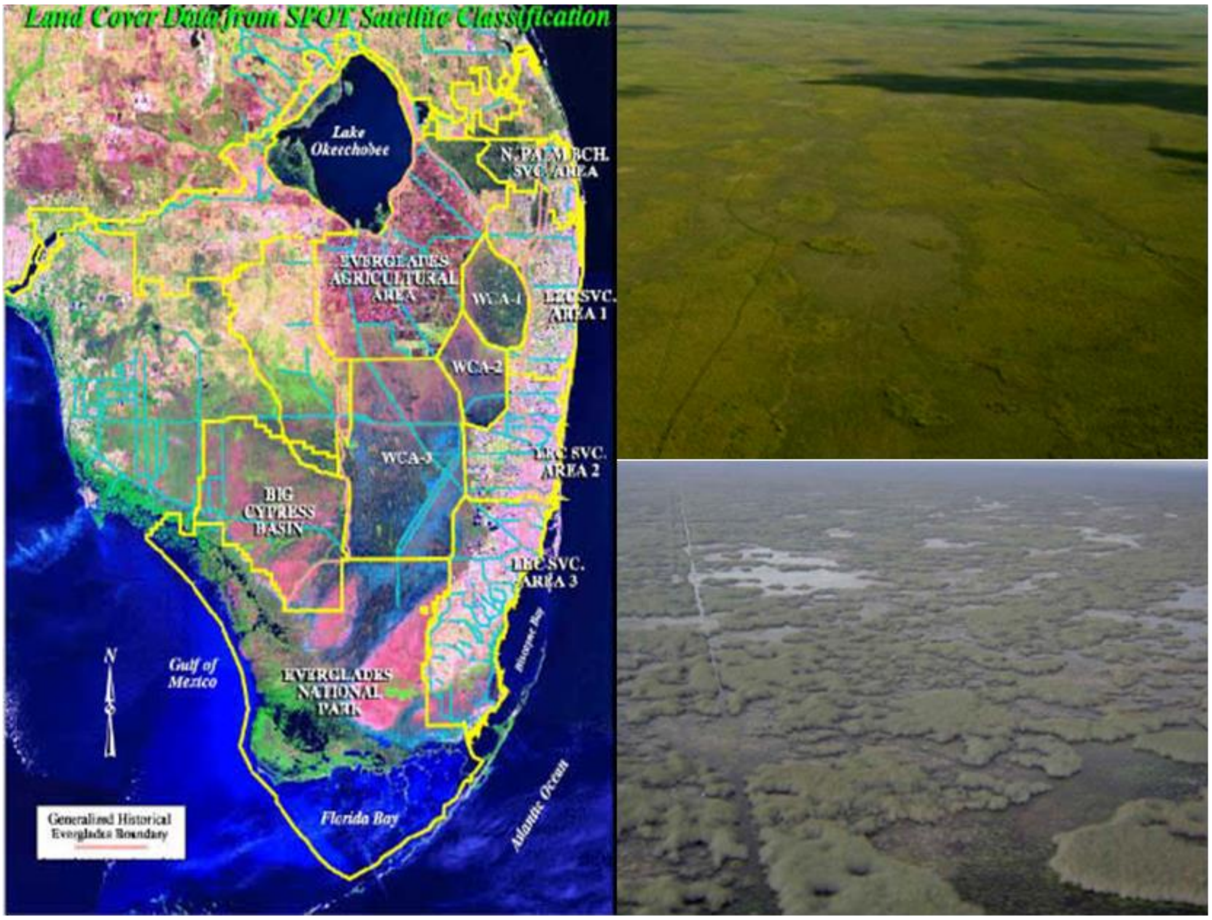

Figure 1: Present configuration of the greater Everglades and associated changes in ridge-slough structure (Ross et al. 2016). (Left) The contemporary Everglades, subdivided into distinct management basins subject to varied uses and management objectives. (Right top) Degraded R&S landscape in the area where hydrologic modification has reduced water levels and hydroperiod. (Right bottom) Degraded R&S landscape in the area where impoundment has raised water levels and lengthened hydroperiods.

The characteristic R&S mosaic has been theorized to be a self-organized landscape maintained by autogenic processes that balance ridge expansion and slough persistence (Watts et al. 2010, Larsen et al. 2011, Heffernan et al. 2013, Acharya et al. 2015). Decoupling of soil elevations from underlying bedrock topography in areas of relatively conserved landscape pattern suggests that historic microtopography and R&S landscape structure have arisen largely from internal feedback between vegetation, hydrology, and soil development. Whether local geologic features have acted as nucleation sites for ridge initiation remains unresolved. In either case, plant production provides raw material for peat development and may increase as increasing soil elevation allows for higher production of recalcitrant organic matter by sawgrass (Figure 2). Peat depth is maintained by the deposition of root biomass, while peat is lost through aerobic respiration. Ridges accumulate biomass faster than sloughs, but shallower water depths promote more rapid decomposition that roughly balances higher gross peat production (Larsen and Harvey 2010, Cohen et al. 2011). The production-respiration equilibrium is regulated within both community types at nearly equal rates over long time periods, keeping ridges and sloughs from forming mountains and valleys. Vegetation shifts in microtopographic range as the hydrologic regime changes may help maintain plant zonation, and thus potentially feedback on microtopographic structure (Larsen and Harvey 2010, Cohen et al. 2011). Zweig et al. (2018) suggest that once R&S pattern is established, decomposition is more important than production in maintaining the patterned microtopography and associated vegetation types in the Everglades R&S landscape, though local shifts between ridge and slough are sensitive to long-term hydrologic and edaphic factors (Zweig et al. 2020).

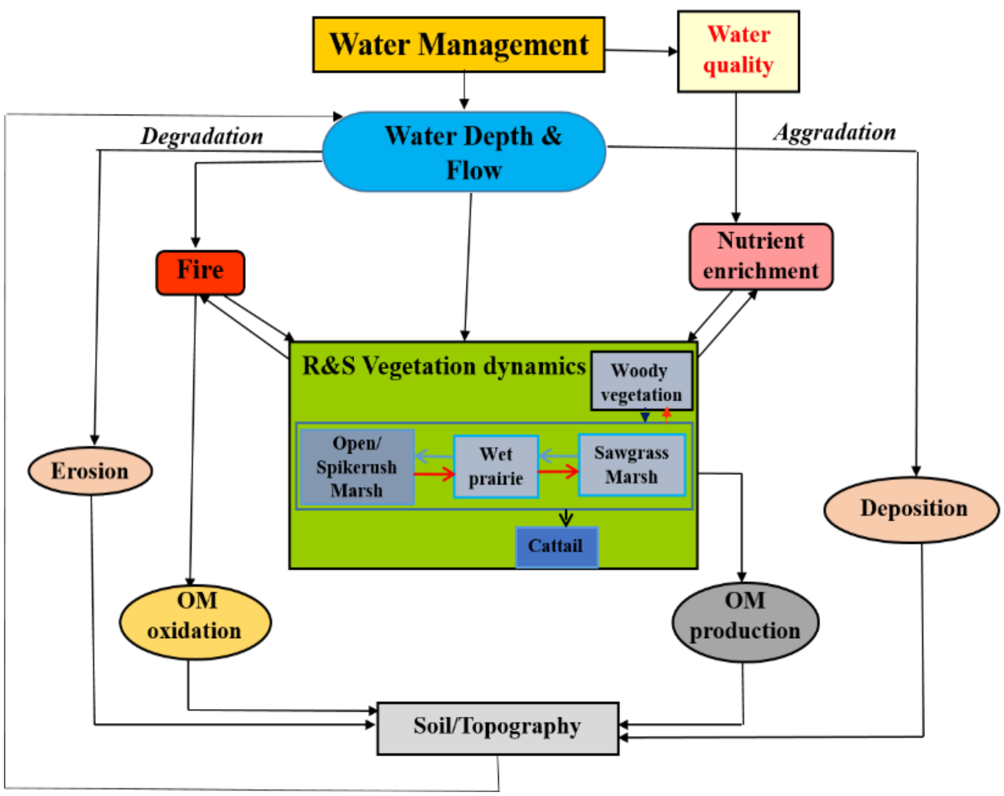

Figure 2: Conceptual model showing the relationships among causal factors such as soil microtopography, water regimes and disturbances (fire and nutrient enrichment) and vegetation dynamics within R&S landscape (Modified from Ross et al. (2006)).

The combination of microtopography, hydrology, vegetation composition and productivity, and their responses to hydrologic modification and other disturbances (fire and nutrient enrichment) create challenges in disentangling causal relationships and diagnosing trajectories of change. Therefore, one objective of our ongoing monitoring study has been to assess whether microtopographic structure, vegetation community composition, or relationships between these variables serve as leading indicators of change in other landscape characteristics. While it is known that altered microtopography affects vegetation structure after hydrologic modification (Ross et al. 2003, Givnish et al. 2008, Zweig and Kitchens 2008, 2009), vegetation changes may also influence microtopography (Cohen et al. 2011, Larsen et al. 2011, Casey et al. 2015, 2016).

A system-wide, simultaneous assessment of microtopographic structure and vegetation community composition over six years (2009-2015) suggests that while substantial portions of the R&S landscape are severely degraded (Heffernan et al. 2009, Ross et al. 2016), ground elevation changes often precede vegetation change during critical transitions from patterned to degraded landscape states in the drained landscapes. In contrast, vegetation change (reduction in vegetation distinctness) may be a leading indicator of landscape degradation in impounded conditions (Ross et al. 2016). This degradation process is expected to slow down or even reverse in response to restoration activities associated with the Everglades Restoration Plan (CERP). Nonetheless, the relative timescales of changing vegetation and topographic structure in R&S are not well understood yet.

In general, vegetation changes in the Everglades occur at different time scales. For instance, in the marl prairie of Taylor Slough, changes in the hydrologic regime over periods as brief as three to four years resulted in concurrent changes in vegetation composition (Armentano et al. 2006, Sah et al. 2014). In the R&S landscape within WCA3A, Zweig and Kitchens (2008, 2009) found that vegetation communities are influenced by both current and historic (up to four years) hydrologic conditions, though vegetation responses to hydrologic modification varied among species. Thus, the current system-wide monitoring of topographic structure and vegetation composition carried out at five-year intervals is expected to capture changes in the composition and spatial patterns of vegetation communities, and to some extent in microtopography, that occur as a result of water management operations, restoration initiatives, and episodic events such as high rainfall, droughts and fire within the Everglades R&S landscape.

2. Methods

2.1 Study Area

The study area includes the historical R&S landscape that currently exists in the Everglades. In general, the R&S landscape encompasses the deeper central portion of the Everglades and is a peat-dominated system. This landscape, however, has undergone dramatic structural changes since human modification of the hydrologic regime began in the early 20th century. The most obvious outcome of these changes was the compartmentalization of the landscape into discrete management areas, including Everglades National Park (ENP) and Water Conservation Areas (WCAs: 1, 2A, 2B, 3A and 3B), subjected to different water management, resulting in hydrologically independent systems that sharply differ in the hydrological conditions (Science Coordination Team 2003) (Figure 3).

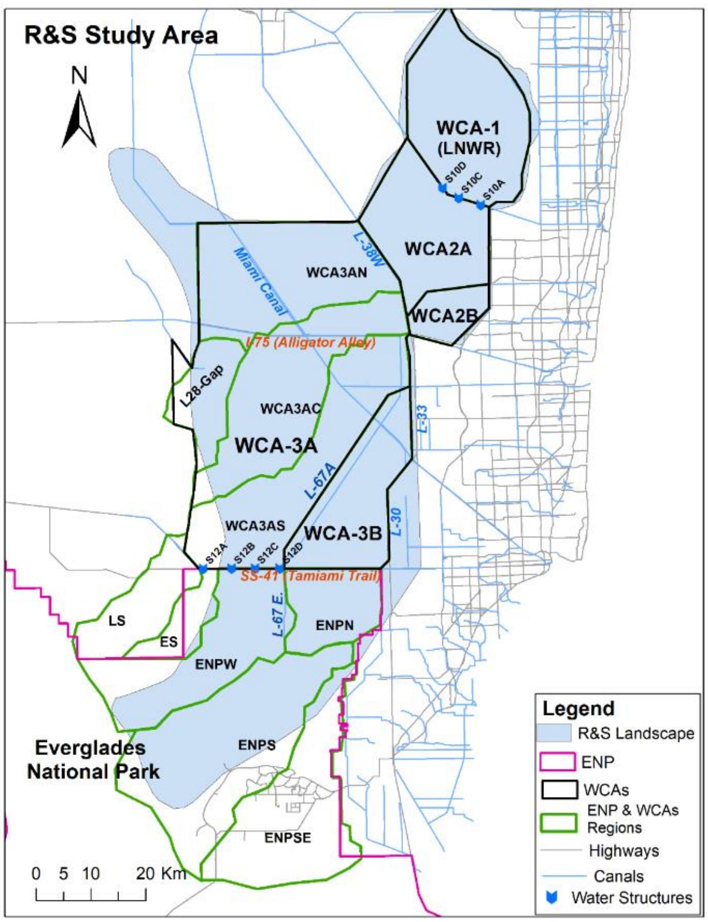

Figure 3: Study area showing the boundary of the remaining ridge and slough landscape system (as mentioned in Ogden 2005), Water Conservation Areas (WCA 1-3) and Everglades National Park. Regions in the ENP and the WCAs were named following RECOVER (2020).

In many parts of ENP and WCAs, prolonged flooding, drainage and/or phosphorus enrichment have led to the deterioration of the R&S landscape pattern (Larsen et al. 2011). For instance, WCA1, an enclosed area surrounded by canal dikes, has changed from a sheet-flow-driven system to an impounded marsh dotted with tree islands (Brandt et al. 2000). The WCA2A and WCA2B have also been impacted by different water management strategies (Light and Dineen 1994), and high phosphorus concentrations in water entering these areas have greatly contributed to the deterioration of landscape pattern. Currently, vegetation in WCA2A is a mosaic of sawgrass, cattails, wet prairies, and willows with deep sloughs in some areas (Gann and Richards 2015), while WCA2B has a relatively high percentage of sloughs.

Among WCAs, the WCA3A is the largest unit, and has four indicator zones or hydrologic regions (northern, central, southern and L28-Gap; Figure 3) that are used by some hydrological models to make predictions (RECOVER 2020). These zones differ in hydrologic conditions. For instance, northern WCA3A (WCA3AN), has been over-drained in recent years. Surface water flows in the central WCA3A (WCA3AC are also substantially lower than historic conditions and so are mean water levels (Science Coordination Team 2003, McVoy et al. 2011). In contrast, southern WCA3A (WCA3AS), has pooled water north of the roadway levee and restricts the surface flow into the southern Everglades. The impoundment in the WCA3AS and the relatively dry conditions in the upstream sections of the WCA3A have caused the fragmentation of ridges and loss of sloughs, respectively (Larsen et al. 2011, McVoy et al. 2011). Likewise, the low water level together with negligible flow in the WCA3B has resulted in loss of sloughs and expansion of sawgrass ridges. However, the recent changes, including degradation of some portions of the L-67 levees associated with Decompartmentalization Physical Model (DPM) project, have allowed water flow from WCA3A to the WCA3B.

Within ENP, the R&S landscape is mainly confined to the Shark River Slough (SRS) basin. Since the early 20th century, the water flow pattern through SRS has changed several times, mainly due to the changes in water management strategies. In summary, over more than half of the 20th century, the water flow regimes within the SRS remained deviated from its natural hydrologic conditions resulting in various degrees of deterioration of R&S landscape in different regions. However, under the recently adopted Combined Operation Plan (COP), water deliveries to the SRS are believed to improve, with an increase in flow to the NESRS (USACE 2020), which will have a significant impact on the R&S landscape.

2.2 Data Collection

The study continued using the sampling design that was used during the first and second 5-year cycles (2009-2015; 2015-2020) of the ongoing monitoring work within the R&S landscape. The details of the study are described in Ross et al. (2016) and Sah et al. (2021). In brief, the study design used a Generalized Random-Tessellation Stratified (GRTS) sampling network, an established framework for system-wide representative sampling within ENP and WCAs (Philippi 2007). It includes a grid of 2x5 km landscape blocks (primary sample units, PSUs), of which the 5 km edge is aligned parallel to the historic water flow. While in the beginning of the study, a spatially stratified random sample of 80 PSUs was selected for sampling over five years (n=16 per year) (Philippi 2007, Heffernan et al. 2009), after the first two years of the first cycle (2009-2011), the number of PSUs and the number of sites within each PSU have been adjusted (Ross et al. 2016). Elimination of PSUs from some areas together with the reduced number of plots in each PSU might have affected the balanced design by causing under-sampling of those areas such as WCA1, WCA2, and the eastern and southern portions of ENP, but the adjustment was necessary owing to the changes in available budgets.

Over six years, (2009-2015), including a pilot phase of the study (2009), 67 PSUs were sampled, though detailed data analyses focused on 62 PSUs that were within the historic distribution of the R&S landscape, and five PSUs, located within the marl prairie landscape in the ENP were excluded from the analysis (Ross et al. 2016). During the 2nd 5-year monitoring cycle (2015-2020), 58 PSUs were sampled (Sah et al. 2021). Ten PSUs, including five in marl prairies sampled in the first cycle were not sampled during Cycle-2. In contrast, one PSU in WCA3AN that was not sampled in Cycle-1 was sampled for the first time in Year-4 of the 2nd cycle.

Over three years (2020-2023) of the 3rd 5-year monitoring cycle (Cycle-3), we sampled 34 PSUs: 11 in the first year (2020/2021), 12 in the 2nd year (2021/2022), and 11 in the 3rd year (Figure 4). Those PSUs were from ENP (8), WCA3AN (6), WCA3AC (6), WCA3AS (4), WCA3B (5), WCA2 (3), and the WCA1/LNWR (2) (Table 1). Within the ENP, the sampled PSUs were from Northeast Shark River Slough or northern ENP (NESRS, hereafter 'ENP_N'), western region (ENP_W) and southern ENP (ENP_S). Regions in the ENP and the WCAs were named following RECOVER (2020) given in Figure 3 and Table 1.

During the Spring of 2023 (Option Year-2), while the field crew was sampling in PSU-39, an airboat accident happened on Jan 11, 2023, resulting in an injury to the field crew members involved in vegetation sampling. The crews were rescued from the field, and we followed the necessary post-incident procedures properly. However, due to circumstances following the incident and interruptions in the operation of FIU-owned airboats for the next two and half months, vegetation sampling in three PSUs (PSUs 39, 45 (partly) and DPM), scheduled for Year-3 (Option Year-2), were not completed during that season. Later, sampling in PSUs 39 and 45 was completed in the Fall of 2023, while sampling in the DPM area was delayed for the next season.

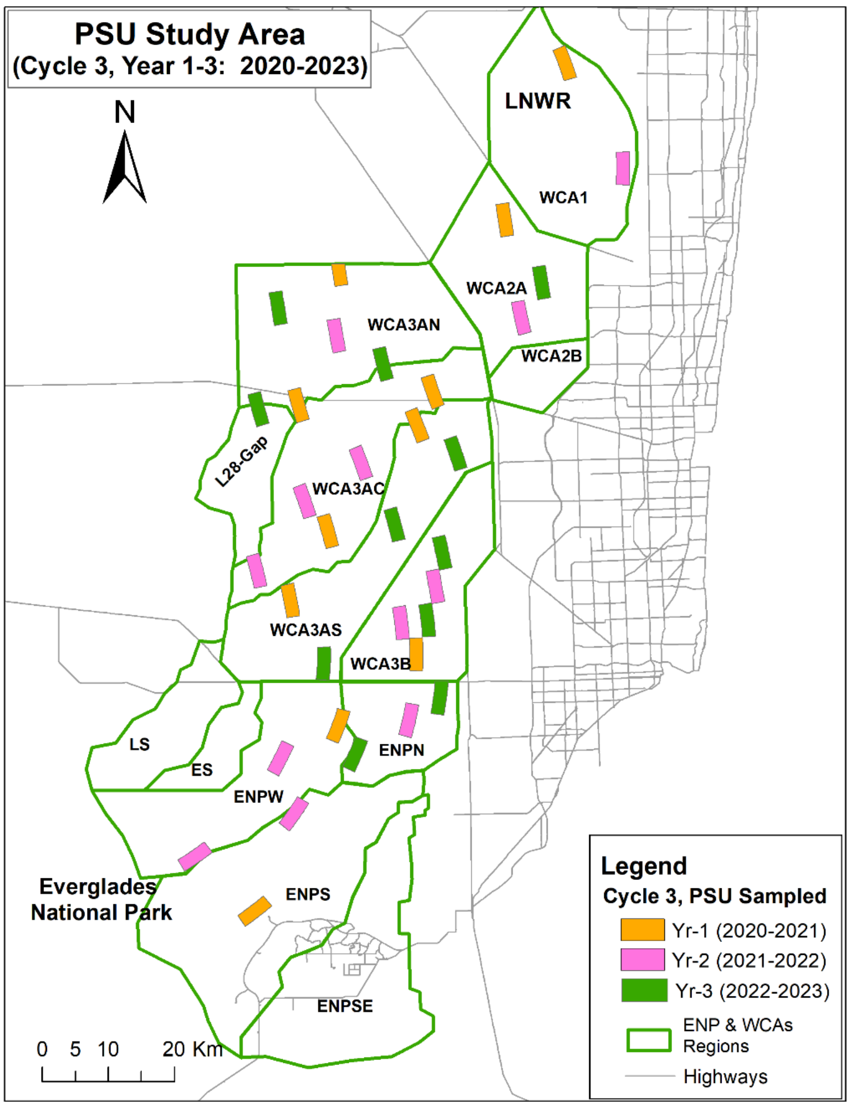

Figure 4: Map of PSUs for landscape sampling showing thirty-four PSUs sampled over three years (2020-2023) of the current sampling cycle. Colors indicate years for sampling of individual PSUs. National Park/Conservation area names: ENP = Everglades National Park, WCA1 = Loxahatchee National Wildlife Refuge (Water Conservation Area 1), WCA 2 = Water Conservation Area 2, WCA3AN, S = Water Conservation Area 3A North and South, WCA3B = Water Conservation Area 3B. The suffixes ‘C’, ‘N’, ‘S’ and ‘W’ after ENP and WCA3A represents central, northern, southern, and western regions of those management areas (RECOVER 2020).

Table 1: Characteristics of PSUs sampled in Year 1, 2, and 3 of the current 5-year project cycle (C3: 2020-2025).

PSU	Cycle	Cycle-3 Year	Cycle-1 Sampling Year (WYr)	Cycle-2 Sampling Year (WYr)	Cycle-3 Sampling Year (WYr)	Cycle-3 Sampling date	Region*	Historical R&S	X_UTM-NAD83	Y_UTM-NAD83	Cycle-3 No. of plots
0	3	1	2012	2016	2021, 2022	12/4, 12/9/2020, 11/17/2021	ENP_W	Y	532345.5	2842696.3	135
1	3	1	2010	2016	2021	10/30, 10/31/2020	WCA1	Y	566677.9	2942982.1	117
2	3	1	2010	2016	2021	12/12, 12/12/2020	WCA3AS	Y	525056.6	2861614.1	132
3	3	1	2010	2016	2021	1/23/2021	WCA3AN	Y	532505.3	2910966.9	89
4	3	1	2010	2016	2021, 2022	12/16, 12/28/2020, 12/12/2021	WCA3AC	Y	530756.4	2872127.6	132
6	3	1	2010	2016	2021, 2022	11/2, 12/2/2020, 12/22/2021	ENP_S	Y	519649.4	2814585.3	130
7	3	1	2010	2016	2021	1/13, 1/15/2021	WCA3AN	Y	526262.4	2891226.1	135
9	3	1	2010	2016	2021	1/25, 1/27/2021	WCA2A	Y	557549.6	2919280.2	120
11	3	1	2011	2016	2021, 2022	1/20, 1/21, 11/23/2021	WCA3AC	Y	546603.3	2893273.0	135
15	3	1	2011	2016	2021, 2022	1/8, 1/11, 11/23, 11/29/2021	WCA3AC	Y	544263.6	2888174.1	134
108	3	1	2011	2016	2021, 2022	12/30/2020, 1/6/2021, 1/26/2022	WCA3B	Y	544130.1	2853456.0	132
17	3	2	2010	2017	2022	10/6, 11/30, 12/26/2021	WCA1	Y	575467.5	2927079.8	131
18	3	2	2011	2017	2022	10/22, 10/27, 12/7/2021	ENP_W	Y	523582.5	2837739.8	99
19	3	2	2011	2018	2022	10/15, 11/24/2021	WCA3AN	Y	532020.9	2901747.8	114
20	3	2	2011	2017	2022	9/24, 9/27/2021	WCA3B	Y	541840.2	2858248.3	135
21	3	2	2010	2018	2022	9/17, 9/20/2021	WCA2A	Y	560020.3	2904486.4	135
22	3	2	2011		2022	11/1, 11/11, 11/17/2021	ENP_W	Y	510586.7	2822844.4	135
23	3	2	2012	2017	2022	9/1, 9/3/2021	WCA3AC	Y	527209.6	2876687.7	135
24	3	2	2012	2017	2022	9/8, 9/10/2021, 1/26/2022	ENP_N	Y	543033.6	2843539.1	133
26	3	2	2011	2017	2022	10/13, 11/9/2021	WCA3AC	Y	519957.4	2866106.0	135
28	3	2	2011	2017	2022	10/8, 11/3/2021	WCA3B	Y	547035.4	2863766.4	132
30	3	2	2012	2017	2022	11/8, 11/17/2021	ENP_S	Y	525597.5	2882440.9	121
31	3	2	2012	2017	2022	10/11, 10/29/2021	WCA3AC	Y	535763.3	2882440.9	132
32	2	3	2013	2018	2023	9/16, 9/20/2022	ENP_N	Y	534894.8	2838347.8	135
34	2	3	2013	2018	2023	9/14, 9/21/2023	WCA3AS	Y	530097.7	2852094.7	138
35	2	3	2013	2018	2023	12/21/2022, 1/6/2023	WCA3AN	Y	523207.3	2905898.8	135

PSU	Cycle	Cycle-3 Year	Cycle-1 Sampling Year (WYr)	Cycle-2 Sampling Year (WYr)	Cycle-3 Sampling Year (WYr)	Cycle-3 Sampling date	Region*	Historical R&S	X_UTM-NAD83	Y_UTM-NAD83	Cycle-3 No. of plots
36	2	3	2013	2018	2023	11/1/2022, 1/9/2023	WCA3AS	Y	540859.6	2873130.6	135
37	2	3	2013	2018	2023	9/7, 9/30/2022	WCA2A	Y	563108.3	2909792.2	129
39	2	3	2013	2018	2024	9/15, 9/18/2023	WCA3AN	Y	520196.3	2890623.0	135
43	2	3	2013	2018	2023	8/31, 9/2/2022	WCA3AN	Y	539077.4	2897449.3	135
44	2	3	2013	2018	2023, 2024	9/12, 9/13/2022, 9/12/2023	WCA3B	Y	545823.9	2858632.9	135
45	2	3	2013	2018	2023, 2024	12/14/2022, 10/4, 10/6/2023	WCA3AS	N	550107.7	2883908.2	135
220	2	4	2014	2019	2023	11/7/2022	WCA3B	Y	548070.8	2868866.4	135
513	2	3	2013	2018	2023	10/24, 10/25,2022	ENP_N	Y	547619.4	2846243.2	135

* ENP = Everglades National Park, WCA1 = Loxahatchee National Wildlife Refuge (Water Conservation Area 1), WCA 2 = Water Conservation Area 2, WCA3AN, S = Water Conservation Area 3A North and South, WCA3B = Water Conservation Area 3B. The suffixes ‘C’, ‘N’, ‘S’ and ‘W’ after ENP and WCA3A represents central, northern, southern, and western regions of those management areas (RECOVER 2020).

2.2.1 *Field Survey*

The approach for field sampling adopted during the Cycle 3 (Year 1, 2 & 3) study was the same as during Cycle 2, described in Sah et al (2021). In the beginning of the first cycle of the study (2009-2015), the 2x5 km area in each PSU was subdivided in 80 equal area zones (250 m x 500 m) and a sampling cluster was located at a random location in those grid cells (Figure 5). At each cluster, samples were collected using a 1m^2 quadrat, placed at the center and at two randomly selected distances between 3 and 35 m in two cardinal directions, east and north. Thus, there were 240 sample quadrats in each PSU. However, after the 2012 water year (i.e., two years of the first cycle), the number of clusters for sampling was reduced to 45 clusters, resulting in a maximum of 135 quadrats in each PSU, the sampling scheme that continued during Cycle 2 too. Therefore, in Year-1 and 2 of the current (3rd) cycle, we sampled the sites at a maximum of 45 clusters (i.e., 135 quadrats) in each PSU. However, in some cases, when the sites were sampled outside the PSU boundary during the first cycle and maintained the same in the 2nd cycle, the sampling clusters were randomly selected within the boundary, and sampling was done in new plots.

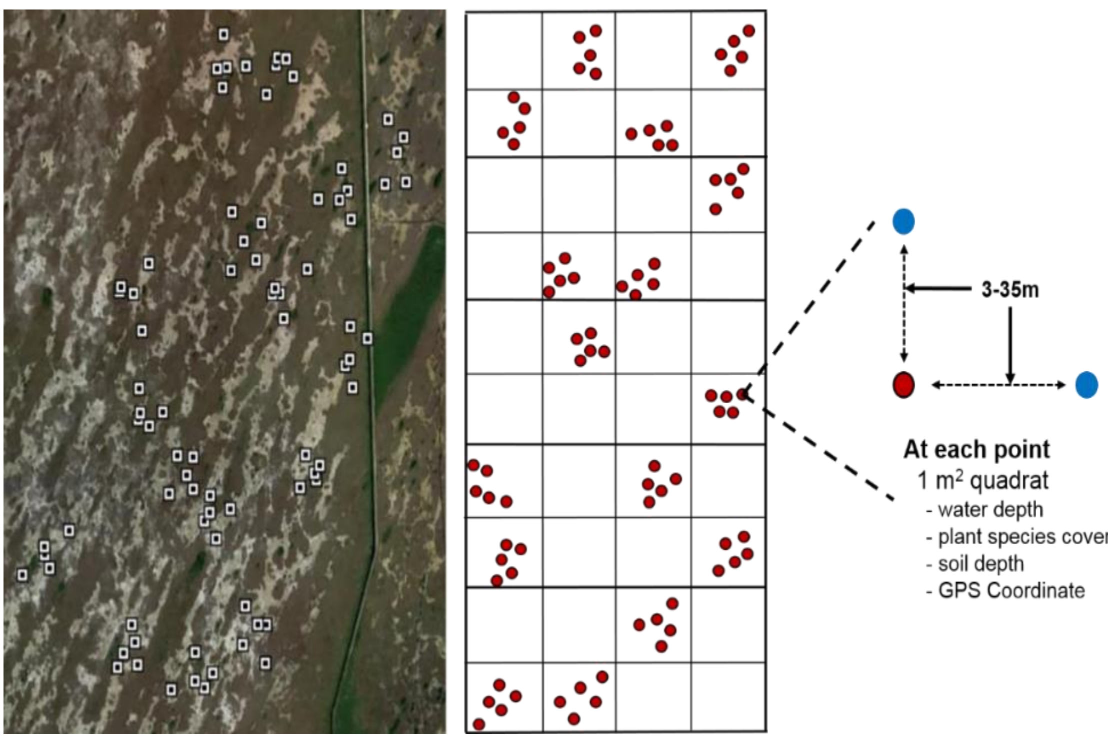

Figure 5: Locations of sampling clusters (red dots) within 2x5 km primary sampling units (PSUs); the location of clusters within 500 x 500 m zone is assigned randomly. At each cluster, 3 sampling locations are visited; sites are situated at the center of each cluster and at a random distance between 3 and 35 m in the direction of the PSU azimuth and the orthogonal direction.

Within each quadrat, water depth was measured using a meter stick. Field training of sampling personnel ensured that a standardized amount of pressure was applied so that the measurement of water depth was uniform across time and space. Water depths were measured with a precision of 0.5 cm. In addition, we determined soil depth, i.e., depth to bedrock at each node, using a 1 cm diameter metal rod. At some sites, however, the soil depth was much deeper than the metal rod we used, and thus we were not able to reach bedrock. Soil depth at those sites was recorded as >210, >254 and >371 cm, i.e., the effective length of the metal rod used at the time.

Vegetation characterization within each quadrat consisted of identifying all taxa present to the species level and estimating the abundance of each species as a percentage cover of the plot area, in either 1%, 5%, or 10% intervals. Based on visual observation associated with these vegetation measurements, the vegetation within a 25 m radius of each sampling location was assigned to a community category (ridge, slough, tree island vegetation, wet prairie, and cattail). Where study sites span a transition from one community type to another, we assigned points to mixed categories (e.g., ridge/wet prairie or transition). The field classifications of vegetation type were also adjusted so that they are better and more directly related to community classifications adopted by Rutchey et al. (2006) and Sah et al. (2010), and the types recently used in mapping from aerial imagery (Ruiz et al. 2017).

Field sampling of the ridge-slough landscape was done via airboat when sufficient water was present to obtain a reliable measure of water depth at all locations. As such, no dry weather sampling was conducted. For PSUs situated in Everglades National Park, sites were accessed by airboat or helicopter, as allowed by permitting and budgetary constraints.

2.2.2 *Fire Data*

To quantify fire frequency (FF) and time since last fire (TSLF), we obtained fire data for the Park from 1948 to 2021 (Source: ENP), and for WCAs from 1997 to 2021 (Source: US Fish and Wildlife Commission), and a comprehensive fire history geodatabase detailing the location and attributes of fires was created. However, for consistency purposes, only fire data between the years 1997 and 2021 were used for both areas. The shapefiles for each year were merged into one fire history dataset, resulting in overlapping polygons from different years whilst maintaining the spatial integrity and attributes of all original fire data. The sampled plot point layer was overlaid on the fire data layer, and information about which years the plots burned were extracted using

‘Intersect’ command in ArcMap. In fact, within a fire boundary, not all the areas burn uniformly. For this study, however, when plot was located within a fire boundary, it was assumed burned. For each sampled plot, fire frequency per decade (FF) and time since last fire (TSLF) were calculated. For the PSU level, we used the Fire Frequency Index (FF Index) calculated for each PSU using fire data from 1997 and 2019 (Sah et al. 2021).

2.3 Data analysis

2.3.1 *Site/Point Hydrology*

Since water depths in the field were measured over several months in different hydrological conditions, we established site hydrologic conditions by coupling our synoptic measurements of water depths with daily water surface elevation (WSE) obtained from Everglades Depth Estimation Network (EDEN) based on the geographic location of sampling plots. We first determined soil (ground) elevation from EDEN estimates of water elevation on the day of sampling and water depth measurements. Then, using the daily water surface elevation data, we calculated mean water depth and inundation frequency for each plot over the preceding 5 and 20 years (i.e., 5 and 20 years before the water year when the sites were samples). Because vegetation composition in ridge and slough may change in 3-5 years in response to hydrologic changes (Zweig and Kitchens 2008) but a change in topography takes much longer time, we used hydrologic variables derived from 5-year prior to sampling year as a predictor of vegetation condition, while 20-year hydrologic record as predictors of landscape (PSU and regions) scale vegetative and microtopographic condition (Ross et al. 2016).

2.3.2 *Microtopography*

To assess microtopographic variation and hydrologic regime, we calculated summary statistics of soil elevation and water level, including mean, standard deviation, skewness, and kurtosis following Heffernan et al. (2009). The standard deviation of water level describes the temporal variability of water level, while the standard deviation of water depth (or soil elevation) describes the magnitude of spatial variation in microtopography. To test for bimodality in the peat elevation distributions, we used the R package 'mclust' to assess goodness-of-fit between the observed histogram of peat elevations, and 1) a single normal, and 2) a mixture of two normal distributions:

$$P_s = N(\mu_i, \sigma_i) \tag{1}$$

$$P_m = q \cdot N(\mu_1, \sigma_1) + (1 - q) \cdot N(\mu_2, \sigma_2) \tag{2}$$

where q represents the probability of falling within the first normal distribution, and N is a normal distribution with mean μ_i and standard deviation σ_i. Model goodness of fit was compared using Bayes' information criterion (BIC). The best-fit model was considered to have the lowest BIC score. Moreover, to evaluate how microtopographic structure responds to hydrologic regime, we examined the relationship between mean annual water depth and the elevation difference between modes of bimodal distributions, where present.

2.3.3 *Vegetation structure and composition*

In the R&S landscape, vegetation communities are generally separated in ridge and slough by clear topographic boundaries in areas with relatively well-maintained hydrologic regimes. However, as the hydrologic regime degrades, this patterning is lost. We assessed variation in community distinctness in response to hydrologic and topographic changes using dissimilarity between R&S vegetation community composition, defined as the distance (in multivariate space) between two vegetation clusters (Isherwood 2013). First, using the species cover data from all PSUs sampled over three years (Years 1-3) of the current cycles, we generated a Nonmetric multidimensional scaling (NMDS) ordination plot. This single global NMDS ordination plot enabled us to; 1) obtain a global estimate of the clustering of sampling points containing a set of species among all PSUs, and 2) standardize the among-PSU data. For the global NMDS ordination, we decided to retain three dimensions (3-d) solution, which was different from Cycle-1 and Cycle-2, during which 5-d or 4-d solutions were retained (Ross et al. 2016; Sah et al. 2021). Each PSU was then isolated from the global NMDS ordination plot and coerced into two distinct clusters using k-means clustering. The sum of squares distance between the two cluster centres (BSS) based on their Voronoi sets was calculated for each PSU to obtain a test statistic that we used as a description of vegetation community distinctness (Isherwood 2013). A higher BSS value (greater distance between the two clusters) indicated a more distinct vegetation community structure, whereas more overlapping clusters (smaller BSS) would indicate less distinctness between sites, and a more degraded landscape structure (Isherwood 2013, Ross et al. 2016).

Since the sample points in the ordination space were artificially grouped into only two clusters, rather than allowing them for multiple clusters, several approaches were used to assess the rationality of using R&S community distinctness (Isherwood 2013, Ross et al. 2016). Those included analysis of the distribution of key indicator taxa (*Cladium, Eleocharis*, *Nymphaea*, and *Utricularia* species) in the two global clusters, agreement between cluster assignments in the global analysis and within individual PSUs, analysis of the covariation among characteristic species of each community in NMDS space, and the distribution of sample points along individual axes of the global NMDS. The rationale for using these approaches and detailed interpretation is given in Isherwood (2013) and Ross et al. (2016). The global NMDS plot was created using the 'metaMDS' function in the *vegan* package (Oksanen et al. 2020). All the statistical analyses, including k-means clustering, were performed using the R program (R Core Team 2022).

Landscape-scale co-variation between elevation and vegetation community composition was assessed by different metrics: bivariate regression between sawgrass abundance and elevation within each PSU, a Mantel test between matrices of between-site dissimilarities in elevation and in community composition, and the difference in elevation between points assigned to the two clusters in the k-means analysis (Isherwood 2013; Ross et al. 2016). This suite of measures provides a more integrated view of the vegetative and microtopographic structure of R&S landscapes.

Species richness was calculated at a plot level, whereas diversity measures, including species richness, evenness, and beta diversity, were calculated at the PSU level. At both plot and PSU level, we analysed the effects of Long-Term Mean Water Depth (LTMWD), the standard deviation of mean long-term water depth (LTMWD_SD), FF, and TSLF on species richness using Generalized Linear Models (GLM). However, at the PSU level, we analysed the effects of LTMWD, LTMWD_SD and FF Index on species richness using Generalized Linear Models, and on beta diversity and evenness, both continuous variables, with General Linear Models. These analyses were run in R v.4.3.1 (R core team 2022).

Finally, we examined the changes in both topographic and community metrics between Cycle-1 and Cycle-3 and between Cycle-2 and Cycle-3 across the 34 PSUs studied over three years (2020-2023) and assessed the relationship between those changes and hydrologic conditions using both linear and non-linear regressions.

3. Results

3.1 Hydrologic conditions & Microtopography

In the PSUs sampled during the first three years (2020-2023) of Cycle-3, both 5-year and long-term (20-year average) mean water depth (averaged over 5 and 20 water years before sampling year across all points sampled within each PSU) varied across different regions of R&S landscape (Table 2; Figure 6a, b). The 5-year mean water depths (hereafter, 5-Yr_WD) ranged between -11.6 (±3.3) cm in PSU-3 and 83.0 (±11.8) cm in PSU-45. Likewise, 20-year mean water depths (hereafter termed as 'LTMWD') varied from 7.6 (±3.6) cm in PSU-3 to 82.9 (±11.8) cm in PSU-45. The lowest water depths were in PSUs within the northern water conservation area 3A (WCA3AN) and the northern portion of WCA1 and WCA2A, whereas moderately high to high water depths were in the central, southern, and northeastern portions of WCA3A. In some regions, e.g., WCA3B, ENP_N, and ENP_S, the 5-Yr_WD values were higher than LTMWD, suggesting that those areas have become wetter in the last five years than before.

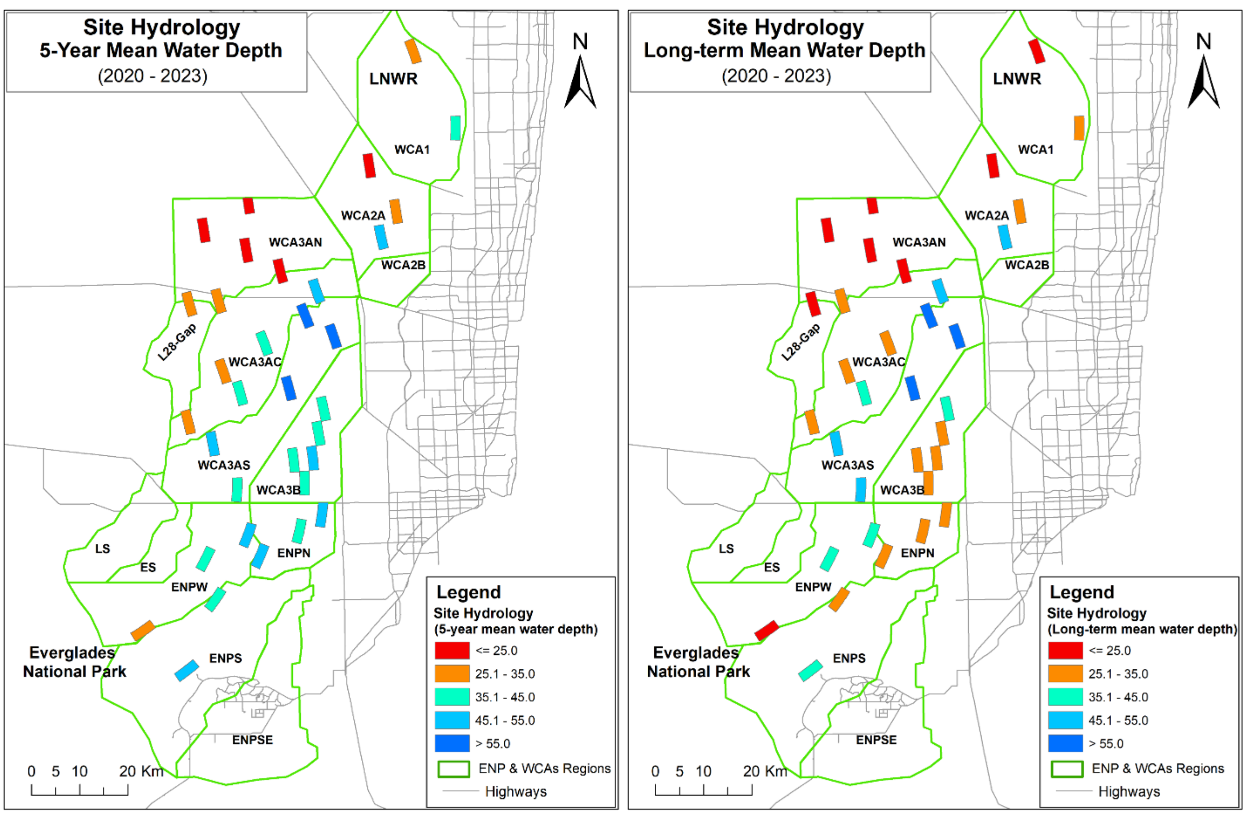

Figure 6: Spatial patterns in 5-year and long-term (20 years average; LTMWD) mean water depth in 34 PSUs sampled over three years (Year 1-3; 2020-2023) of the current five-year cycle (C3). Daily mean water depth across all sampled plots within a PSU were averaged over 5- and 20-year (Water Years) prior to vegetation sampling year.

Table 2: Hydrologic and microtopographic characteristics of Cycle-3 Year 1-3 (2000-2023) PSUs. Additional hydrologic descriptors at the point scale for each PSU are included in the dataset in Excel format.

PSU-Identification			Elevation/Water Depth Statistics								Elevation Cluster Analysis						*Best Model	Notes
			Elevation		*Water depth*						*Mode 1*			*Mode 2*				
PSU	Cycle	Cycle-3 Year	Mean (cm asl)	St. Dev. (cm)	5Y-MWD	5Y-WD-SD	LMWD (cm)	SD (cm)	Kurtosis	Skew	Depth (cm)	†St. Dev. (cm)	††Mode Wt (q)	Depth (cm asl)	†St. Dev. (cm)	††Mode Wt (q)		
0	3	1	133.6	11.3	50.5	9.1	44.1	8.7	2.74	-0.33	44.07	8.64	1	-	-	-	1	
1	3	1	430.5	25.9	28.5	6.0	22.7	5.9	2.85	0.46	22.71	5.87	1	-	-	-	1	
2	3	1	201.8	12.2	52.9	12.0	49.7	12.0	4.52	-1.17	24.46	7.92	0.11	52.89	-	0.89	2	q<0.25
3	3	1	285.9	3.7	11.6	3.3	7.6	3.6	4.61	0.59	7.64	3.56	1	-	-	-	1	
4	3	1	220.5	16.1	43.3	15.6	39.1	15.5	6.93	0.53	30.87	5.87	0.38	44.06	17.24	0.62	2	
6	3	1	3.9	8.5	46.2	6.5	35.3	6.6	2.89	-0.72	32.58	6.89	0.62	39.78	1.98	0.38	2	
7	3	1	253.5	7.7	29.9	7.1	26.2	7.2	5.87	1.09	21.37	3.14	0.44	29.97	7.12	0.56	2	
9	3	1	338.5	5.5	21.3	5.3	14.0	5.2	2.27	0.04	13.97	5.16	1	-	-	-	1	
11	3	1	219.4	8.4	50.2	8.0	46.2	8.0	15.32	2.08	46.24	7.93	1	-	-	-	1	
15	3	1	202.2	10.0	65.5	9.3	62.0	9.4	2.50	0.51	56.59	5.44	0.66	72.81	-	0.34	2	
108	3	1	144.8	9.8	42.6	9.4	32.0	9.4	17.62	3.01	30.90	5.79	0.98	80.67	4.33	0.02	2	q<0.25
17	3	2	423.4	14.4	35.6	14.4	26.6	14.3	4.56	0.78	19.01	2.41	0.39	31.35	16.45	0.61	2	
18	3	2	121.8	7.0	41.9	6.8	36.7	6.9	2.26	-0.19	36.65	6.85	1	-	-	-	1	
19	3	2	270.1	5.8	20.1	5.5	18.6	5.6	3.15	0.19	18.60	5.53	1	-	-	-	1	
20	3	2	151.8	5.6	43.9	5.4	33.7	5.4	6.71	-1.53	18.21	3.97	0.05	34.5	-	0.95	2	q<0.25,
21	3	2	280.5	15.8	51.4	15.1	46.0	15.0	2.26	0.73	37.05	6.46	0.7	66.35	-	0.3	2	
22	3	2	6.7	8.8	29.4	5.4	22.5	5.3	2.40	-0.01	22.50	5.32	1	-	-	-	1	
23	3	2	234.3	11.0	32.4	11.0	30.7	11.2	1.74	0.27	22.53	5.13	0.6	42.77	-	0.4	2	
24	3	2	131.6	6.0	42.5	6.3	27.8	6.3	6.37	-1.16	1.37	5.42	0.02	28.21	-	0.98	2	q<0.25
26	3	2	222.5	10.2	34.7	10.3	34.3	10.3	1.87	0.02	29.05	7.35	0.7	46.64	3.23	0.3	2	
28	3	2	152.6	5.9	42.8	5.4	32.6	5.3	5.99	-1.33	17.63	4.05	0.05	33.41	-	0.95	2	q<0.25
30	3	2	93.1	11.3	36.1	9.0	28.6	9.0	2.99	0.03	28.59	8.93	1	-	-	-	1	
31	3	2	228.7	9.6	37.1	8.3	34.0	8.2	2.21	-0.03	33.98	8.17	1	-	-	-	1	
32	3	3	127.9	7.7	45.2	8.2	34.8	7.6	3.18	-0.44	34.80	7.62	1	-	-	-	1	
34	3	3	198.6	12.2	40.5	12.0	47.9	12.1	2.86	0.19	47.90	12.10	1	-	-	-	1	
35	3	3	297.5	5.4	14.3	5.3	12.1	5.3	3.27	-0.88	2.99	3.22	0.17	13.96	-	0.83	2	q<0.25
36	3	3	176.6	15.6	71.8	15.2	75.2	15.2	2.84	0.23	75.19	15.15	1	-	-	-	1	

PSU-Identification			Elevation/Water Depth Statistics								Elevation Cluster Analysis						*Best Model	Notes
			Elevation		*Water depth*						*Mode 1*			*Mode 2*				
PSU	**Cycle**	**Cycle-3 Year**	**Mean (cm asl)**	**St. Dev. (cm)**	**5Y-MWD**	**5Y-WD-SD**	**LMWD (cm)**	**SD (cm)**	**Kurtosis**	**Skew**	**Depth (cm)**	**†St. Dev. (cm)**	**††Mode Wt (q)**	**Depth (cm asl)**	**†St. Dev. (cm)**	**††Mode Wt (q)**		
37	3	3	308.6	15.2	32.2	15.1	29.3	15.1	14.66	0.15	26.76	5.10	0.81	40.45	30.87	0.19	2	
39	3	3	262.6	6.5	27.1	5.9	24.0	6.0	2.71	0.22	23.97	6.02	1	-	-	-	1	
43	3	3	252.3	4.3	19.1	4.2	21.5	4.3	25.60	2.30	21.77	2.35	0.92	18.47	12.89	0.08	2	q<0.25
44	3	3	147.9	5.4	46.7	5.6	34.3	6.0	3.61	-0.71	19.89	4.95	0.05	35.08	-	0.95	2	q<0.25
45	3	3	174.8	11.9	83.0	11.8	82.9	11.8	8.83	-1.39	17.35	10.25	0.01	83.36	-	0.99	2	q<0.25
220	3	3	152.9	4.2	42.4	4.2	36.1	4.2	2.72	-0.23	36.06	4.20	1	-	-	-	1	
513	3	3	126.1	9.0	54.9	5.1	34.3	5.1	2.78	-0.51	34.32	5.05	1	-	-	-	1	

†Standard Deviation of water depth describes the spatial variability of soil elevation across all points sampled within each PSU.
†† Mode weight describes the proportion of data that occur within each mode, allowing for imbalance in mode prevalence
* Best fit model selected based on Bayes' Information Criterion; number refers to the number of modes
'q' represents the weight of the modes of water depth (or soil elevation), and so reflects the relative prevalence of the high- and low-elevation points within the landscape. When q<0.25 was in any of two modes, unimodal distribution is preferred (see Table 3).

In the surveyed PSUs, while the 5-Yr_WD were significantly different (Friedman ANOVA; n=34, df=2, Chi-square = 33.7, p <0.001) among three sampling periods (Figure 7a, b), LTMWD were reasonably consistent across three cycles (Figure 7c, d). Though, the average LTMWD during Cycle-3 was 2.8 cm higher than average LTMWD during the Cycle-1 sampling. Averaged over 34 PSUs, the mean 5-Yr_WD during Cycle-3 was 4.3 cm and 11.0 cm higher than Cycle-2 and Cycle-1, respectively (Figure 7). Moreover, differences in 5-Yr_WD among the sampling periods were much higher in PSUs located within ENP than in any other regions, more so between Cycle-1 and Cycle-3 (Mean difference = 23.1; Figure 7a) than between Cycle-2 and Cycle-3 (Mean difference = 12.0; Figure 7b).

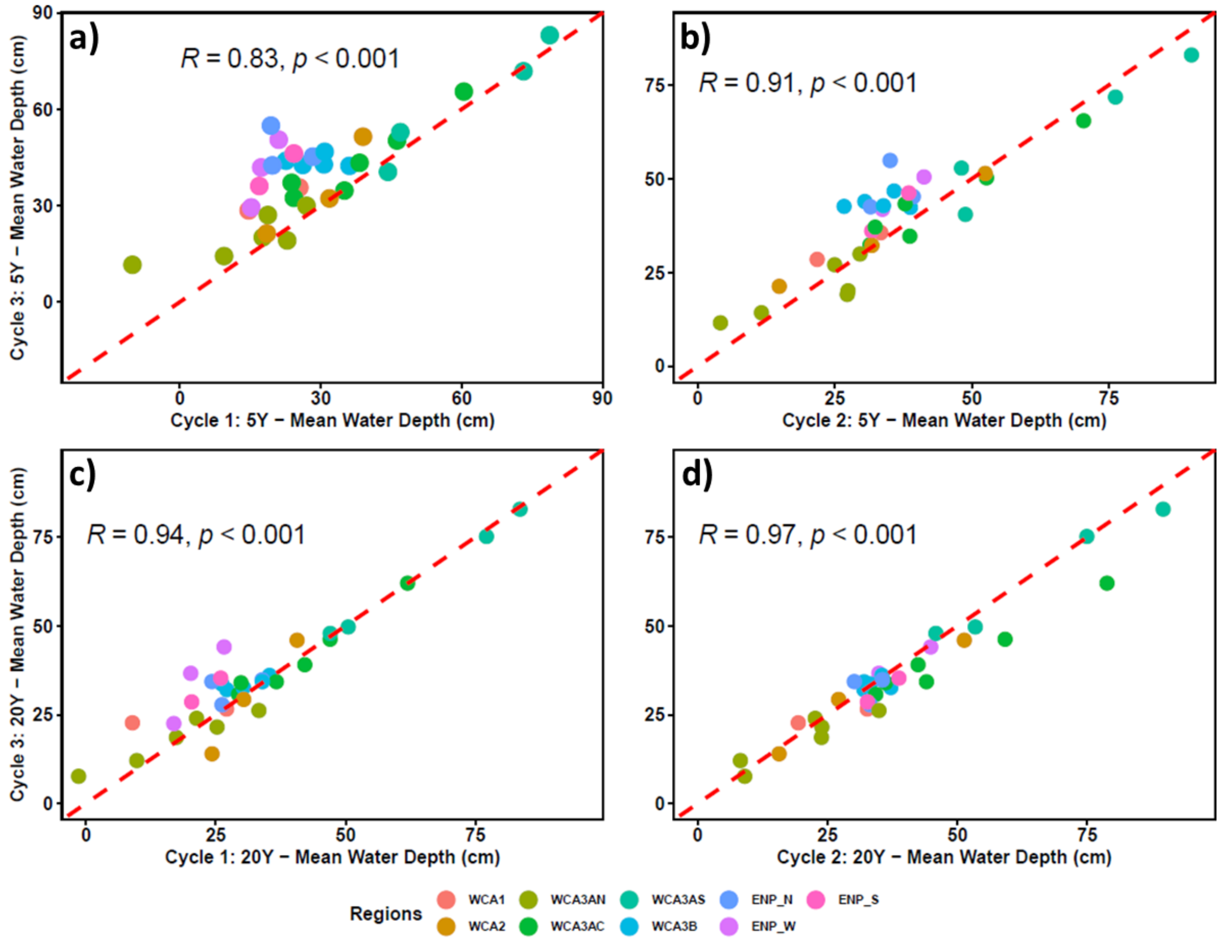

Figure 7: Relationships between 5-year and 20-year (LTMWD) mean water depth (cm) in the 34 PSUs between Cycle-1 and Cycle-3, and in 33 PSUs between Cycle-2 and Cycle-3. PSU-22 was not sampled during the Cycle-2 sampling. PSU-220 was sampled in Year-4 of Cycle-1 and Cycle-2, but it was included in the analysis.

The magnitude and structure of microtopographic relief, measured as the standard deviation of LTMWD, also varied considerably among 34 PSUs (Figure 8). Standard deviations of water depth ranged from 3.6 cm in PSU-3 to 15.5 cm in PSU-4 (Table 2), with most values falling between 5.0 and 10.0 cm (Figure 8). Like the pattern seen during Cycle-1 and Cycle-2 (Ross et al. 2016; Sah et al. 2021), the magnitude of topographic relief during Cycle-3 was generally highest in PSUs in the central and southern WCA3A and southern WCA2A. In contrast, almost all PSUs in WCA3B and ENP had low (<10 cm) topographic variation, and the PSU-3 in northern WCA3A had the least topographic relief.

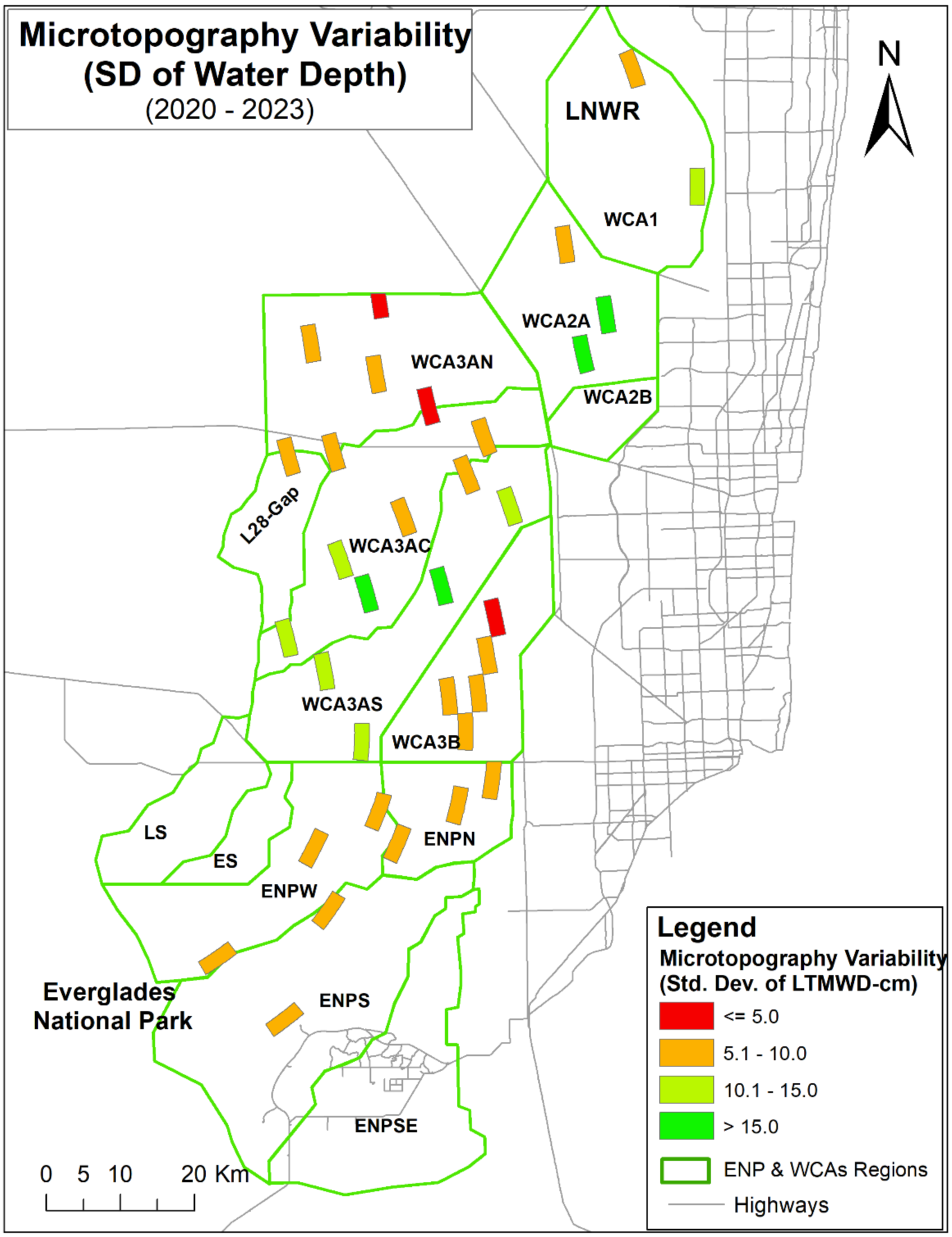

Figure 8: Spatial patterns of elevation variance across historic ridge-slough landscape represented by 34 PSUs sampled over three years (Year 1-3: 2020-2023) of the sampling Cycle-3. Colors indicate the amount of microtopographic relief (measured as the standard deviation of elevation within each PSU).

In general, the number of PSUs that exhibited statistically significant bimodality of soil elevation in Cycle-3 was the same as observed in Cycle-1 and Cycle-2 (Table 3). However, more PSUs in Cycle-3 had also the q<0.25 or >0.75 than in Cycle-1. The parameter q represents the weight of the modes of water depth (or soil elevation), and so reflects the relative prevalence of the high- and low-elevation points within the landscape. Because the historic and conserved ridge-slough landscape has an approximately equal proportion of ridges and sloughs (McVoy et al. 2011), the PSUs with q<0.25 or >0.75 were not considered to exhibit conserved microtopography, even if water depth distributions were best fit statistically with a bimodal rather than a unimodal model. When the PSUs with q<0.25 or >0.75 were discounted, an almost equal number of PSUs had the bimodality fit in first three years of both the 2nd and 3rd cycles (Table 3).

Table 3: Summary of difference in mean elevation (water depth) between two modes for the PSUs which were sampled during Cycle-1, Cycle-2, and Cycle-3.

PSU	**Area†**	**Bi-modal in Cycle-1**	**Elevation Difference between two modes (cm)**	**Bi-modal in Cycle-2**	**Elevation Difference between two modes (cm)**	**Bi-modal in Cycle-3**	**Elevation Difference between two modes (cm)**
0	ENP_W	Yes	14.74	No	-	No	-
1	WCA1	No	-	No	-	No	-
2	WCA3AS	No*	-	Yes	15.24	No*	-
3	WCA3AN	Yes	6.69	Yes	6.61	No	-
4	WCA3AC	Yes	20.56	No	-	Yes	13.19
6	ENP_S	No	-	No*	-	Yes	7.20
7	WCA3AN	No	-	Yes	10.12	Yes	8.60
9	WCA2	No	-	Yes	13.71	No	-
11	WCA3AC	No	-	Yes	11.46	No	-
15	WCA3AC	No	-	No	-	Yes	16.22
108	WCA3B	No	-	No	-	No*	-
17	WCA1	Yes	13.17	Yes	19.32	Yes	12.34
18	ENP_W	Yes	12.95	No	-	No	-
19	WCA3AN	Yes	13.74	No*	-	No	-
20	WCA3B	No*	-	No*	-	No*	-
21	WCA2	Yes	16.40	Yes	16.91	Yes	29.30
**22	ENP_S	No	-	-	-	No	-
23	WCA3AC	Yes	17.98	Yes	18.98	Yes	20.24
24	ENP_N	No	-	No*	-	No*	-
26	WCA3AC	Yes	18.13	Yes	15.79	Yes	17.59
28	WCA3B	No	-	No*	-	No*	-
30	ENP_S	No	-	No	-	No	-

PSU	Area†	Bi-modal in Cycle-1	Elevation Difference between two modes (cm)	Bi-modal in Cycle-2	Elevation Difference between two modes (cm)	Bi-modal in Cycle-3	Elevation Difference between two modes (cm)
31	WCA3AC	No	-	No	-	No	-
32	ENP_N	No	-	Yes	16.76	No	-
34	WCA3AS	No*	-	No*	-	No	-
35	WCA3AN	No	-	No	-	No*	-
36	WCA3AS	Yes	10.63	Yes	14.36	No	-
37	WCA2A	Yes	17.23	Yes	12.02	Yes	13.69
39	WCA3AN	No	-	Yes	10.3	No	-
43	WCA3AN	No	-	No	-	No*	-
44	WCA3B	No*	-	No	-	No*	-
45	WCA3AS	No	-	No	-	No*	-
220	WCA3B	No	-	No	-	No	-
513	ENP_N	No	-	No	-	No	-

* Indicates high unevenness in cluster weight (q<0.25 was in any of two modes: See Table 2), on which basis a unimodal model was deemed the more appropriate fit.
** this PSU was not sampled in Cycle-2.
'-' Not available, as unimodal fit was considered more appropriate fit.

The PSUs with bimodality fit were not all the same across the three cycles. Seven of nine PSUs in which strong bimodality was observed during Cycle-3 sampling also had conserved topography either in Cycle-1, Cycle-2, or both (Table 3). Among PSUs in which bimodality was detected in all three cycles, elevation differences between the two modes were similar, generally around 12.3 – 29.3 cm. However, the PSUs in which bimodality was observed either in Cycle-1, Cycle-2, or both, but not in Cycle-3, generally had relatively small mode elevation differences (6.6 – 15.2 cm). In contrast, two PSUs that had bimodal soil elevations in Cycle-3, after exhibiting a unimodal distribution in Cycle-1 and Cycle-2, had elevation differences of 7.2 cm (PSU-6) and 16.2 cm (PSU-15).

3.2 Soil depth

Soil depth varied greatly among 34 PSUs sampled throughout the R&S landscape during the sampling period of Cycle-3 (Year 1, 2 & 3: 2020-2023). Mean (± SD) soil depth ranged between 28.8 (±21.1) cm in PSU-17 and 318.9 (±55.1) cm in PSU-1. In general, soils are much deeper in WCA1 (LNWR) than in other areas, whereas most of the PSUs in northern WCA3A had shallow soil depths (Figure 9; Appendix 1).

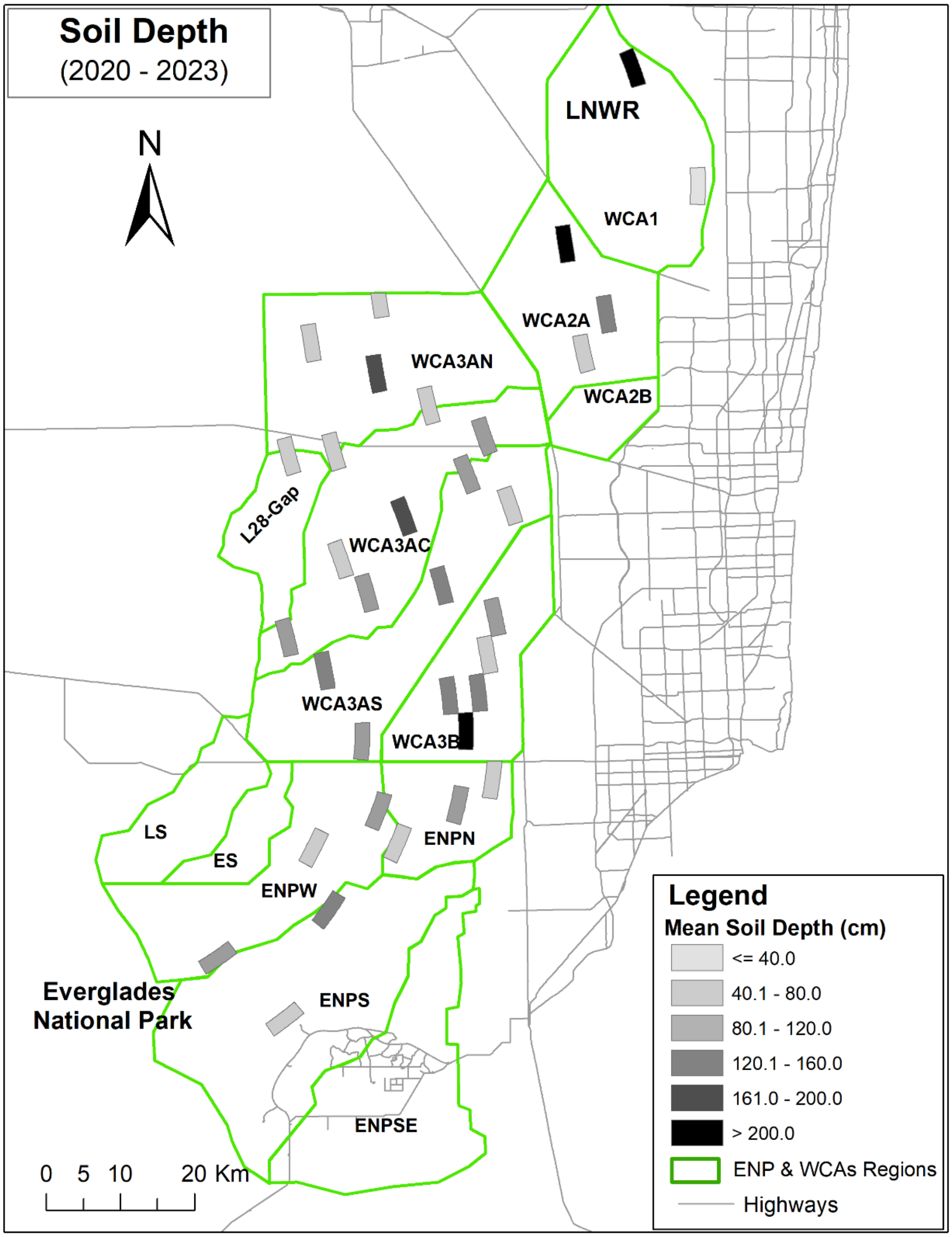

Figure 9: Spatial patterns of mean soil depth in 34 PSUs surveyed over three years (Year 1-3) of the Cycle-3 sampling.

3.3 Vegetation characteristics

3.3.1 *Vegetation composition and community distinctness*

Vegetation composition varied greatly within and across the PSUs sampled during the first three years (2020-2023) of Cycle-3 (Table 4). Sawgrass (*Cladium jamaicense*) was present in all the sampled PSUs, and its relative cover ranged between 12.3% in PSU-1 (WCA1) and 86.8% in PSU-9 (WCA2). Water lily (*Nymphaea* spp.) was also recorded in all but 7 PSUs. However, its relative cover was <5% in about 47% of PSUs in which it was recorded. Relative cover of two major species (*C. jamaicense*, and *Utricularia* spp.) were significantly ($p < 0.05$) correlated between two sampling periods, Cycle-2 and Cycle-3 (Figure 10). In contrast, relative cover of other two major species, spikerush (*Eleocharis spp.*) and water lily (*Nymphaea odorata*), varied greatly ($R^2 = 0.063$; $p = 0.17$, and $R^2 = 0.11$; $p = 0.12$) between those two surveys. Change pattern in relative cover of major taxa since the first survey (Cycle 1) varied by species and among regions. For instance, mean relative cover of water lily (*Nymphaea* spp.), bladderworts (*Utricularia* spp.) and spikerush (*Eleocharis* spp.) was higher during the Cycle-3 than in the Cycle-1 and Cycle-2. Interestingly, such an increase in relative cover of water lily was noticeable (>5% increase) in PSUs mostly in WCA3AC and WCA1, where 5-year average water depth increased up to only 7 cm or 15 cm since Cycle-2 and Cycle-1, respectively. In contrast, in WCA3B and ENP areas, where an increase in water depth was relatively high, up to 19.8 cm or 35.5 cm since Cycle-2 and Cycle-1, respectively, the relative cover of water lily either increased minimally or did not change at all (Figure 11).

Relative cover of bladderworts (*Utricularia* spp.) also increased in more than half of the sampled PSUs. However, across all the sampled PSUs, difference in median bladderworts relative cover between Cycle-2 and Cycle-3 was not significant (Wilcoxon Pair-Test: $n = 33$, $Z = 1.56$, p-value = 0.117). While relative cover of sawgrass increased in some PSUs within WCA3AC and WCA3B areas, the sawgrass cover decreased in the majority (61.7%) of PSUs, especially those within ENP and WCA3AN. Hence, across all the sampled PSUs, difference in sawgrass median relative cover between Cycle-2 and Cycle-3 was not significant (Wilcoxon Pair-Test: $n = 33$, $Z = 0.97$, p-value = 0.330). Relative cover of spikerush increased in 50% of surveyed PSUs, while it deceased in other half of the surveyed PSUs.

Table 4: Vegetation characteristics of 34 PSUs sampled over three years (2020-2023) of the sampling Cycle-3 (2020-2025).

PSU-Identification			Vegetation characteristics					Elevation-Composition Relationships		
			Species Mean Relative Cover (%)				Community Distinctness (cluster distance)	k-means WD difference (cm)	Mantel's r	r2 Cladium-WD
PSU	**Cycle**	**Cycle (Year)**	***Cladium jamaicense***	***Nymphaea spp.***	***Utricularia spp.***	***Eleocharis spp.***				
0	3	1	50.66	0.12	21.22	19.03	0.22	3.23	0.02	0.278
1	3	1	12.34	21.62	22.58	25.06	0.32	3.37	0.09	0.078
2	3	1	27.41	40.73	20.00	1.10	0.67	13.53	0.31	0.308
3	3	1	57.94	0.00	5.01	0.30	0.43	3.77	0.39	0.238
4	3	1	44.39	32.21	12.52	1.88	0.70	14.13	0.41	0.237
6	3	1	44.47	0.00	25.56	22.32	0.26	-0.26	0.03	0.033
7	3	1	48.30	14.25	6.83	10.16	0.61	10.48	0.46	0.326
9	3	1	86.80	7.50	1.58	0.74	0.10	6.71	0.12	0.214
11	3	1	30.24	38.11	10.45	0.27	0.57	4.72	0.17	0.122
15	3	1	23.62	43.81	23.85	0.34	0.34	5.41	0.05	0.053
108	3	1	66.69	10.71	6.46	3.74	0.36	3.28	0.06	0.082
17	3	2	37.40	29.06	8.21	4.31	0.63	13.32	0.36	0.298
18	3	2	20.67	0.00	20.69	43.28	0.32	4.14	0.09	0.050
19	3	2	25.71	0.16	3.62	7.05	0.33	5.42	0.08	0.134
20	3	2	84.97	3.16	0.97	6.36	0.13	2.63	0.01	0.083
21	3	2	52.28	0.00	0.98	32.06	0.32	16.17	0.30	0.453
22	3	2	21.36	0.16	13.99	28.59	0.30	-3.30	0.11	0.023
23	3	2	41.64	28.54	7.90	3.89	0.85	18.62	0.72	0.621
24	3	2	52.53	0.00	24.78	13.35	0.15	-1.22	0.17	0.014
26	3	2	40.34	25.09	10.49	2.74	0.84	16.19	0.63	0.513
28	3	2	72.28	13.02	2.99	4.51	0.30	2.49	0.07	0.016
30	3	2	65.50	2.01	14.03	8.10	0.31	12.94	0.39	0.409
31	3	2	43.28	36.85	3.98	4.81	0.57	6.68	0.21	0.159
32	3	3	63.96	5.31	14.79	7.65	0.29	9.46	0.17	0.361
34	3	3	43.10	29.50	3.43	5.23	0.77	13.25	0.31	0.228
35	3	3	23.58	0.00	1.70	34.00	0.50	0.29	0.27	0.003

PSU-Identification			Vegetation characteristics					Elevation-Composition Relationships		
			Species Mean Relative Cover (%)				Community Distinctness (cluster distance)	k-means WD difference (cm)	Mantel's r	r2 Cladium-WD
PSU	**Cycle**	**Cycle (Year)**	***Cladium jamaicense***	***Nymphaea spp.***	***Utricularia spp.***	***Eleocharis spp.***				
36	3	3	14.46	37.30	32.57	2.24	0.32	15.28	0.14	0.120
37	3	3	49.04	4.73	4.99	7.61	0.32	17.78	0.41	0.078
39	3	3	35.42	0.71	7.81	20.63	0.34	3.94	0.22	0.231
43	3	3	67.72	0.27	0.10	6.83	0.21	-3.69	0.19	0.003
44	3	3	64.24	7.73	10.12	10.58	0.24	1.90	0.03	0.000
45	3	3	23.48	0.17	0.23	18.93	0.50	2.52	-0.07	0.003
220	3	3	66.81	12.27	12.87	2.46	0.17	-2.47	0.10	0.001
513	3	3	61.30	0.00	16.52	15.62	0.14	2.71	0.10	0.195

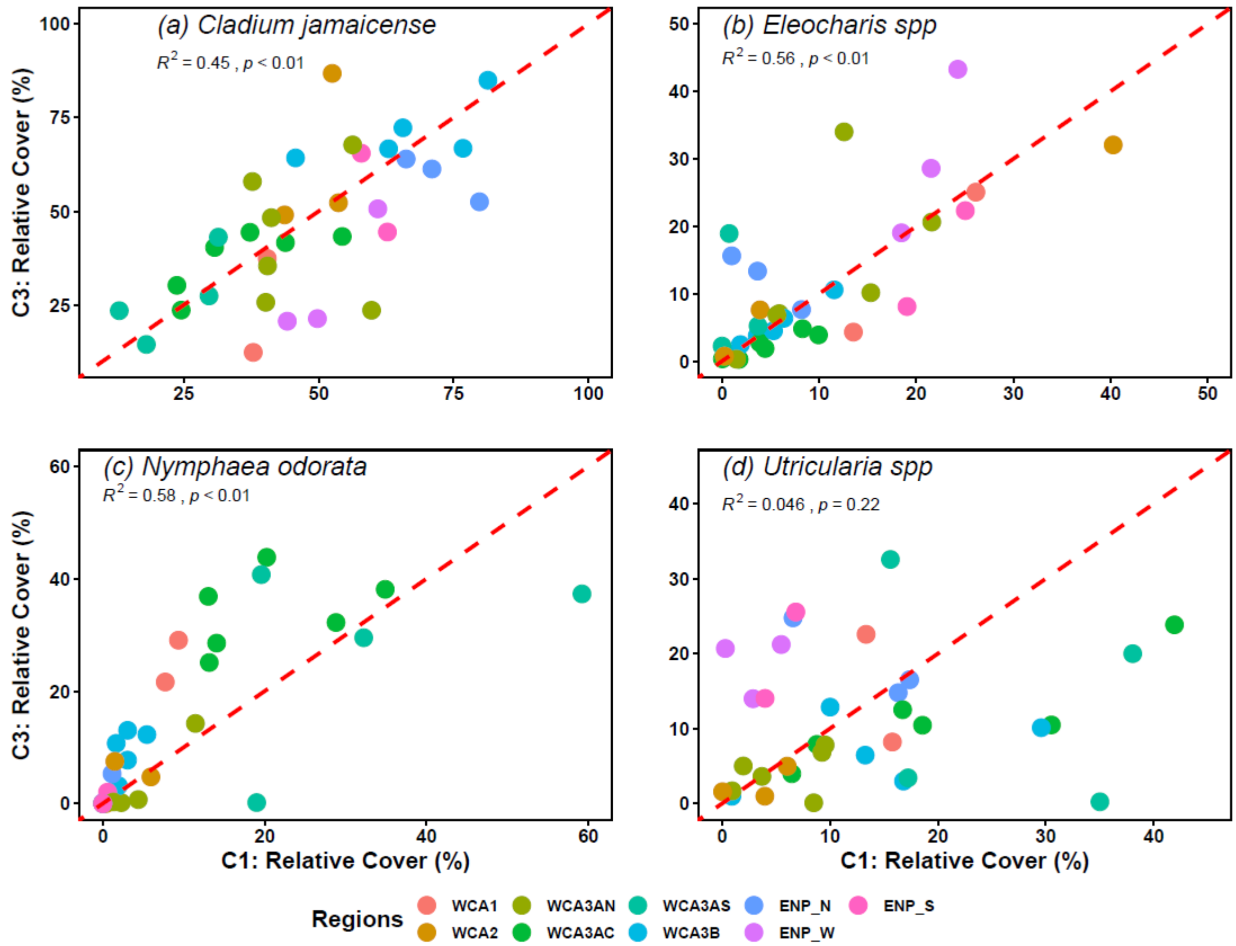

Figure 10: PSU level major species relative cover in 33 PSUs sampled over three years (Year 1-3; 2020-2023) in both Cycle 2 and 3. PSU-22 in ENP-W was only sampled during Cycle-3 and thus was excluded from this analysis. PSU-220 was sampled in Year-4 of Cycle-1 and Cycle-2, but it was included in the analysis.

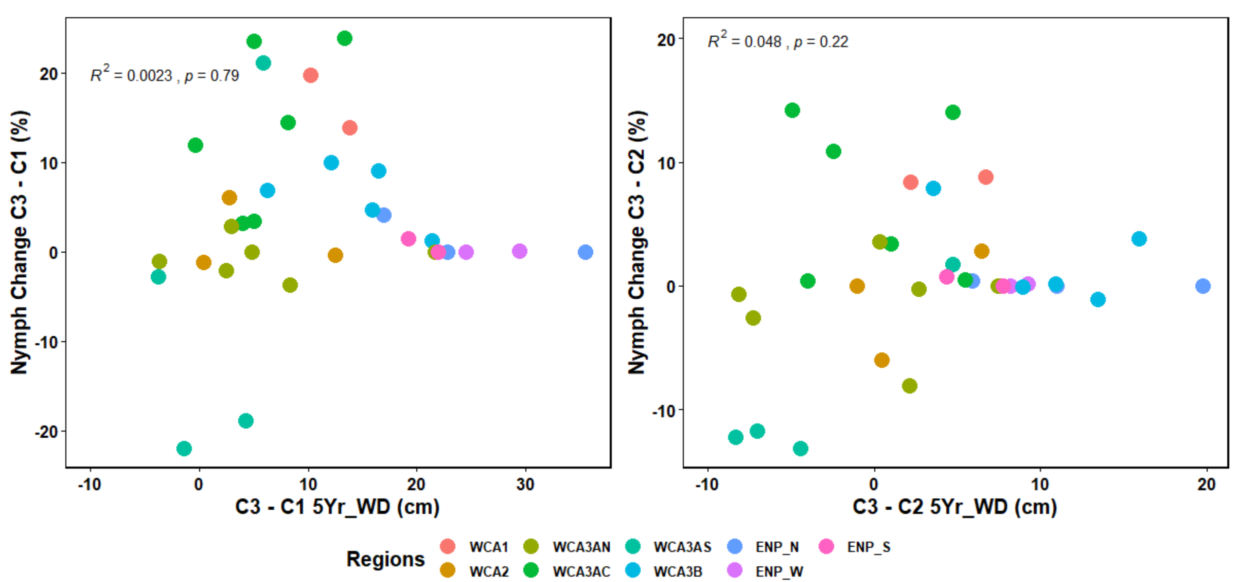

Figure 11: Change in 5-year average mean water level vs change in relative cover of lily plants in the 34 PSUs sampled over three years (Year 1-3; 2020-2023) of Cycle-3.

In non-metric multidimensional scaling (NMDS) ordination, sites were primarily arranged along hydrologic gradients (not shown), and species in the ordination space also followed the same pattern (Figure 12). Sawgrass (*C. jamaicense*), ferns (*Blechnum serrulatum*, *Osmunda regalis*), and other species common on ridges were clearly separated from slough species (water lily, *Nymphaea odorata;* banana lily, *Nymphoides aquatica*; bladderworts, *Utricularia* spp.) along Axis 1, while wet prairie species like spikerush (*E. cellulosa*), beakrush (*Rhytra* spp.) and others were intermediate along this axis, and somewhat differentiated along Axis 3.

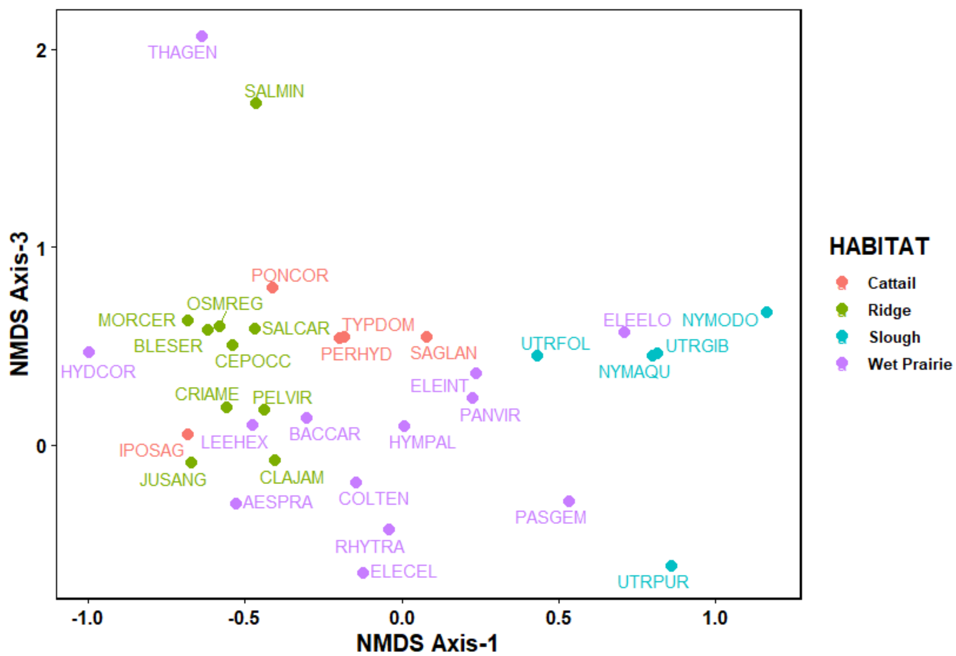

Figure 12: Distribution of major ridge-slough plant species in ordination space. It is noted that coherent clustering of species occurs by community type, which indicates relatively strong fidelity of species to their associated communities across the landscape. Species names are given in Appendix 2.

The global k-means clustering analysis for classifying the sites in two groups identified ridges dominated by sawgrass as one dominant cluster, and communities including both wet prairies and sloughs as a second dominant cluster. These groups were somewhat separated on the first ordination axis. Since both Cycle-1 and Cycle-2 data had shown that k-means clustering within individual PSUs mostly corresponded to the global k-means clustering (Ross et al. 2016; Sah et al. 2021), cluster distance within individual PSUs were used as a measure of community distinctness, which was the sum of squares distance between the two cluster centers (BSS) based on their Voronoi sets for each PSU. To maintain consistency for comparison of community distinctness among three cycles, species cover data for the PSUs sampled during the first three years of all the cycles (PSU 220 was sampled in Year-4 of Cycle-1 and 2, but is still included since it was sampled in Year-3 of Cylce-3), were used in the NMDS ordination, and community distinctness for each PSU for each cycle was calculated separately. In the first three years of Cycle-3, community distinctness ranged between 0.09 in PSU-9 (WCA2A) and 0.85 in PSU-23 (WCA3AC) (Table 4). Two thirds of the sampled PSUs had community distinctness values of <0.50, which represents various degree of degradation in R&S landscape. Those PSUs are mostly in WCA2A, WCA3AN, WCA3B and ENP areas (Figure 13).

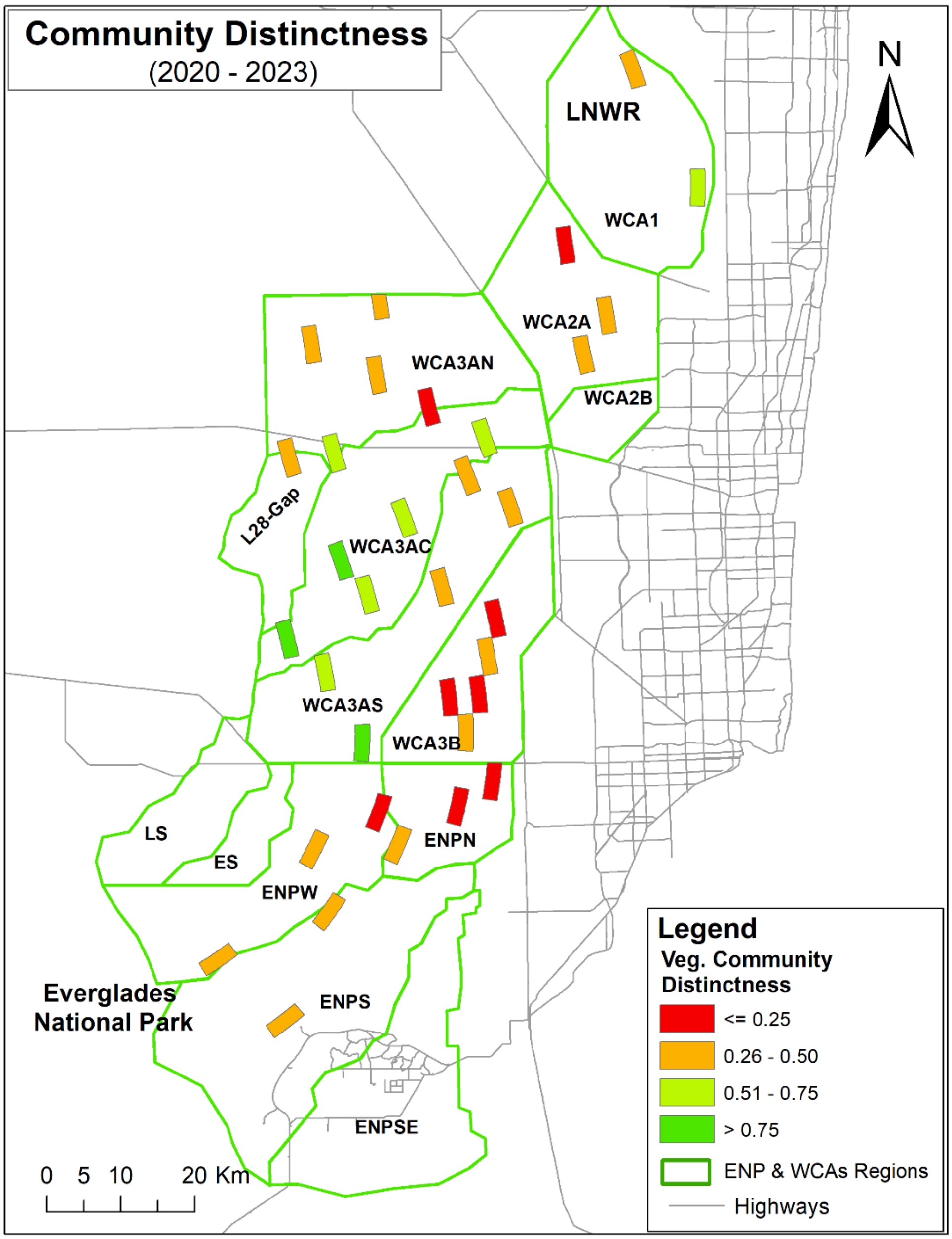

Figure 13: Spatial patterns of vegetation community distinctness measured as a distance between two clusters (k-means clustering) in 34 PSUs sampled over three years (Year 1-3; 2020-2023) of Cycle-3.

Spatially, community distinctness showed similar geographic patterns to those observed for microtopographic variability. As in Cycle-1 and Cycle-2, most of the PSUs with high community distinctness values were in WCA3AC, where the R&S landscape is relatively conserved. Four PSUs, including two within ENP, one in WCA2A and the other in WCA3B, had community distinctness values <= 0.25 (Figure 13), suggesting that those areas have almost uniform vegetation, an indicator of severely deteriorated condition of the R&S landscape.

Community distinctness was consistent across the three cycles ($r \Rightarrow 0.70$, $p < 0.001$), as there was no significant difference (Friedman ANOVA: Chi-square value $_{(n=332,\ df = 2)} = 4.26$, $p = 0.118$) in the mean community distinctness values among the cycles. However, the distinctness values in PSUs in Cycle-3 were closer to the values in Cycle-2 than those in Cycle-1; root mean square difference between Cycle-1 and Cycle-3 was 0.142, while the value between Cycle-2 and Cycle-3 was 0.133 (Figure 14). Almost three-fourth (73.5%) of the PSUs had a difference of <0.15 in distinctness values between Cycle-2 and Cycle-3. However, the PSU- 1 (WCA1) and PSU-3 (WCA3AN) had a difference of 0.3 in distinctness value. The PSU 3 is in the northern most part of WCA3AN and had the lowest 5- and 20-year mean water depth for both cycles.

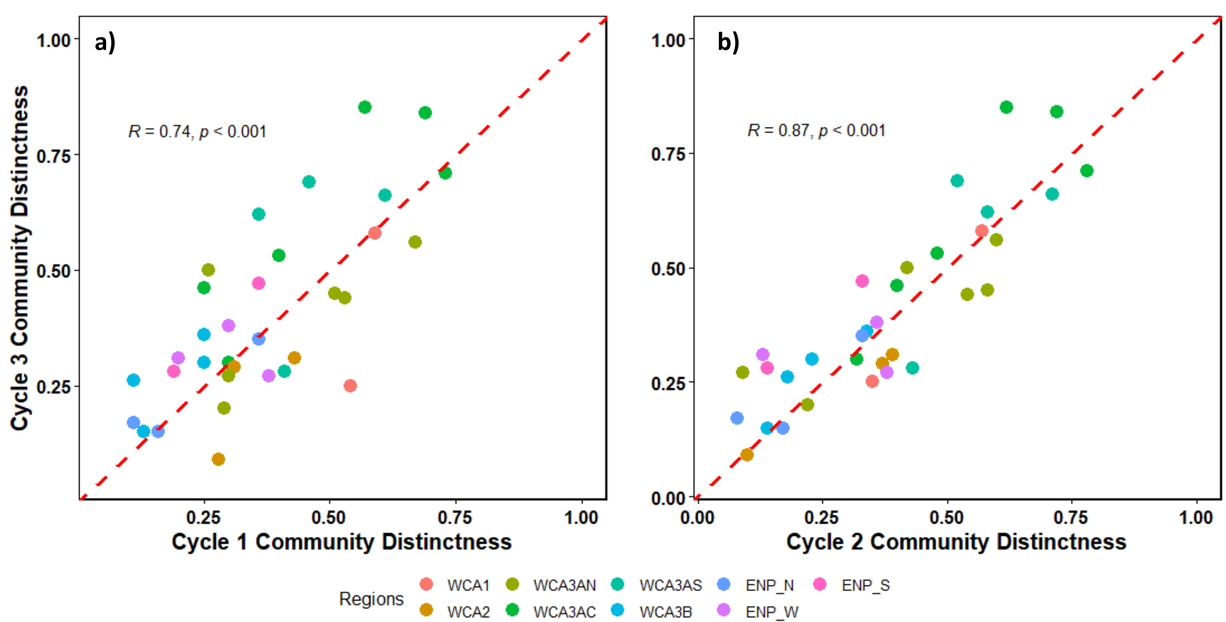

Figure 14: Cycle-3 PSU community distinctness in relation to that of Cycle-1 (n = 34) and Cycle-2 (n = 33). Only the PSUs that were sampled over three years (Year 1,2 & 3) of three cycles were considered. An exception was PSU-220, which was sampled in Year-4 of Cycle-1 and Cycle-2, but it was included in the analysis.

In the studied PSUs within the R&S landscape, community distinctness was not significantly correlated with either short- (5-year) or long-term (20-year) mean water depth (Figure 15a, c). Rather, with a few exceptions, maximal community distinctness (value >0.5) generally occurred within PSUs with LTMWD between 20 and 55 cm. Most of those PSUs are within WCA3AC. The community distinctness was positively and significantly correlated (r^2 = 0.27; p < 0.01) with heterogeneity in microtopographic variation, represented by 5-year as well as 20-year WD standard deviation (Figure 15b, d). The PSUs with high distinctiveness also had higher separation of those communities in water depth. In contrast, the PSUs in WCA2A, WCA3B and ENP areas had both low topographic variability and low community distinctness (Figure 15b, d). The exceptions were three PSUs: the PSU-21 and PSU-31 in WCA2A, and the PSU-36 in WCA3AS had high topographic variability but low (<0.35) community distinctness.

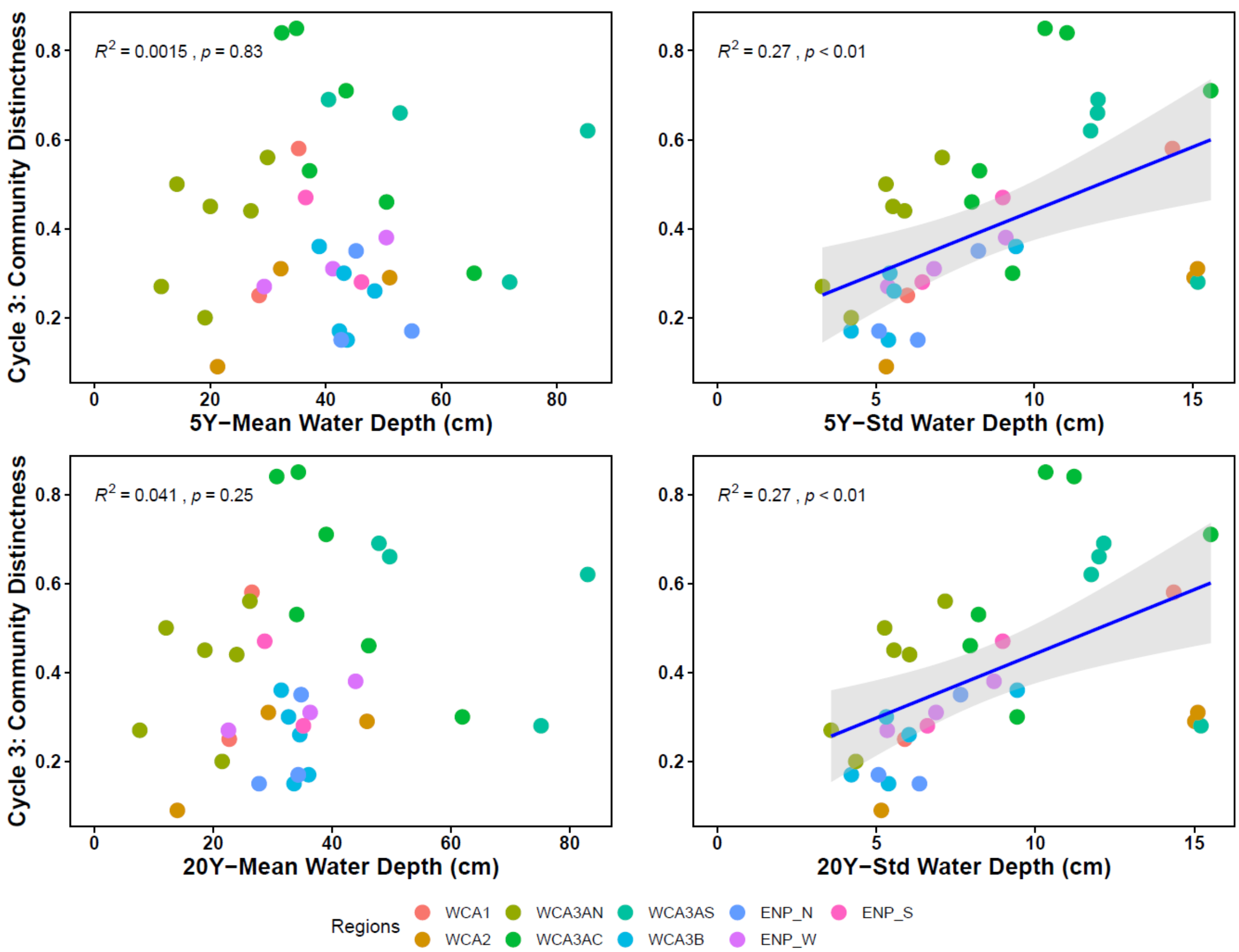

Figure 15: Relationship of community distinctness with 5-year and 20-year (LTMWD) mean water depth, and topographic relief, measured as standard deviation mean water depths.

In general, differences in elevation between two clusters within each PSU represent the status of R&S landscape within the area. Spatially, the distribution of the differences in elevation between two k-means clusters mirrored the distribution of community distinctness and topographic variability; PSUs with more than 10 cm difference in elevation between two clusters were mostly present in WCA3AC (Figure 16). The exceptions were four PSUs, one in each of WCA1, WCA2A, WCA3AS and ENP_S, which had low (<0.4) community distinctness, but high (>12 cm) elevation difference.

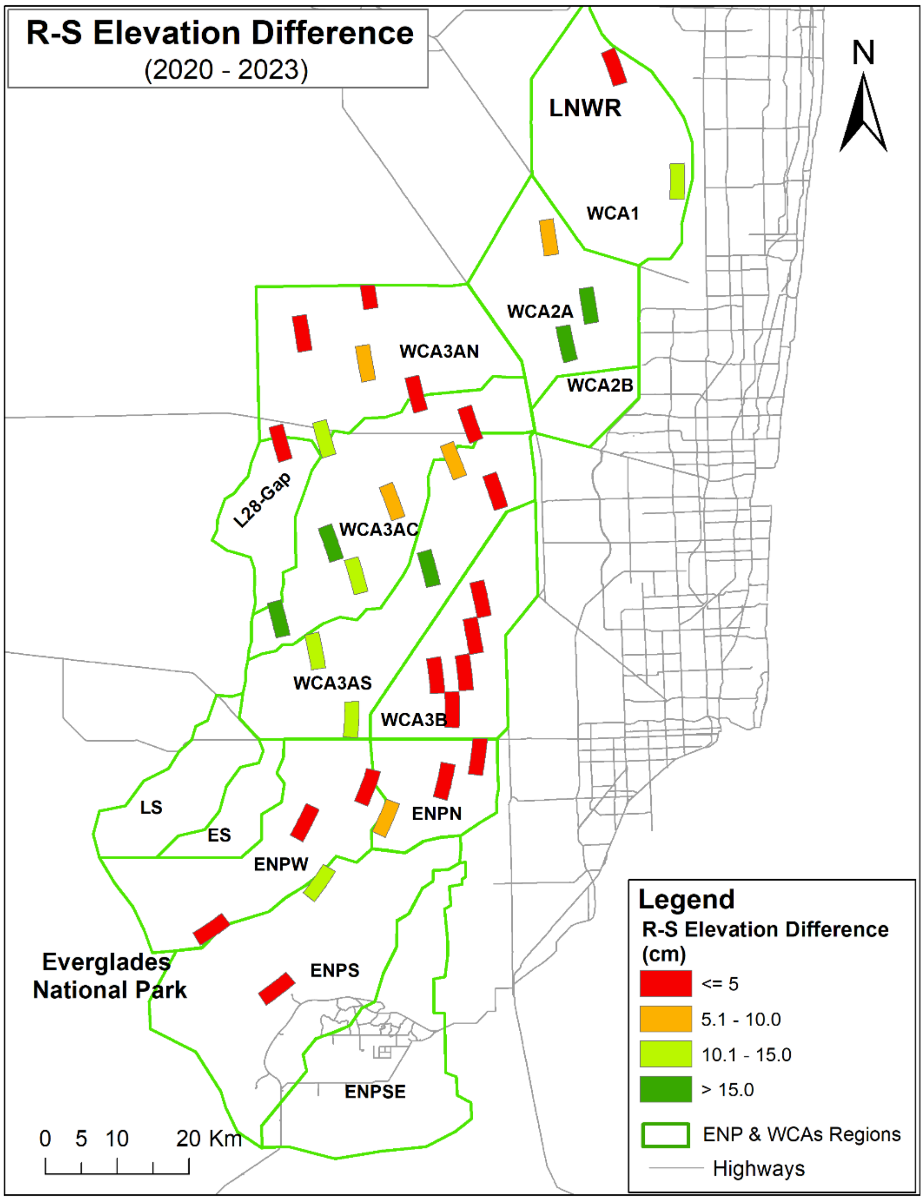

Figure 16: Spatial patterns of difference in long-term mean water level between two clusters (k-means clustering) in 34 PSUs sampled during the first three years (2020-2023) of Cycle-3.

The PSUs with high community distinctness also showed strong relationships ($r^2 = 0.43$) between local water depth and vegetation community composition (as measured by Mantel's r)

(Figure 17). The relationship between Mantel-r and long-term mean water depths was not significant. Most PSUs with high Mantel-r values had both 5-year average and long-term mean water depths between 25 and 55 cm (Figure 18a, c). Interestingly, three PSUs with both 5-year average and LTWD >60 cm had very low Mantel-r values. Two of those three PSUs were in WCA3AS, where the impoundment of water has been considered as degradation of ridge and slough landscape. Across all the surveyed PSUs (n = 34), the vegetation-environment association was significantly related (r^2 => 0.15) with microtopographic variation, represented by standard deviation of 5-year and long-term (20-year) water depths (Figure 18b, d).

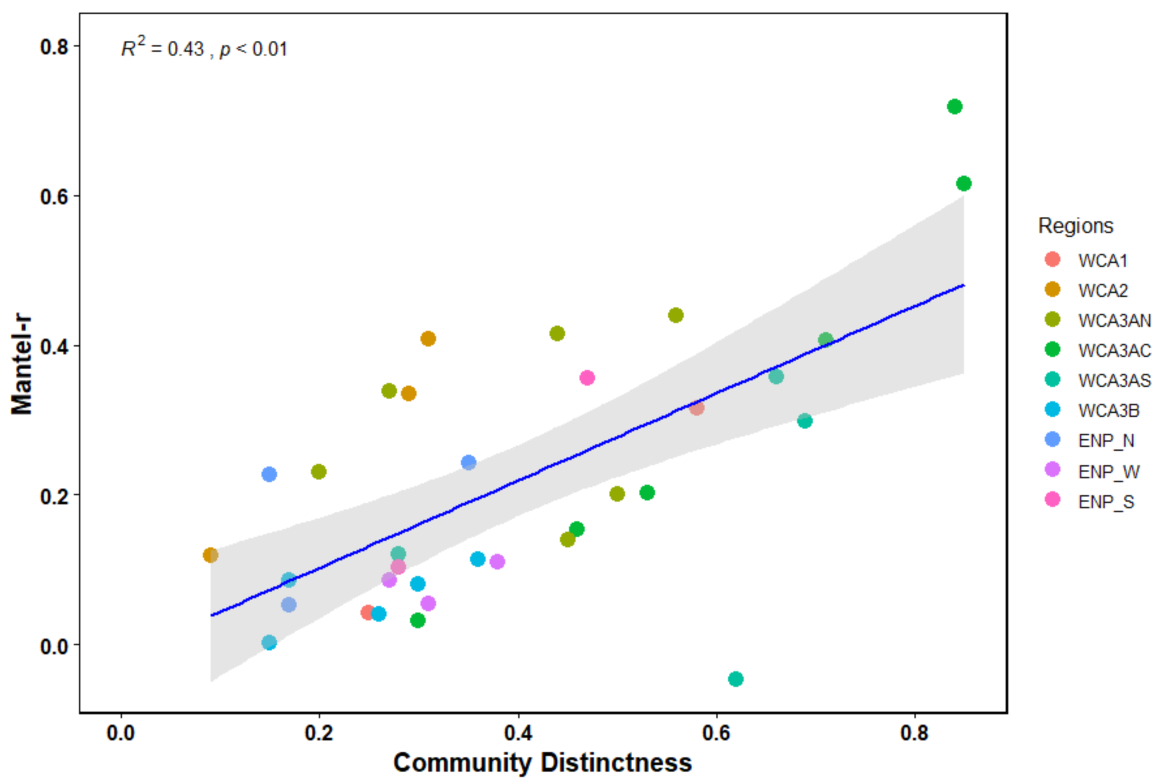

Figure 17: Relationship between community distinctness and mantel r (association between vegetation composition and water depth) across 34 PSUs sampled during the first three years (2020-2023) of Cycle-3.

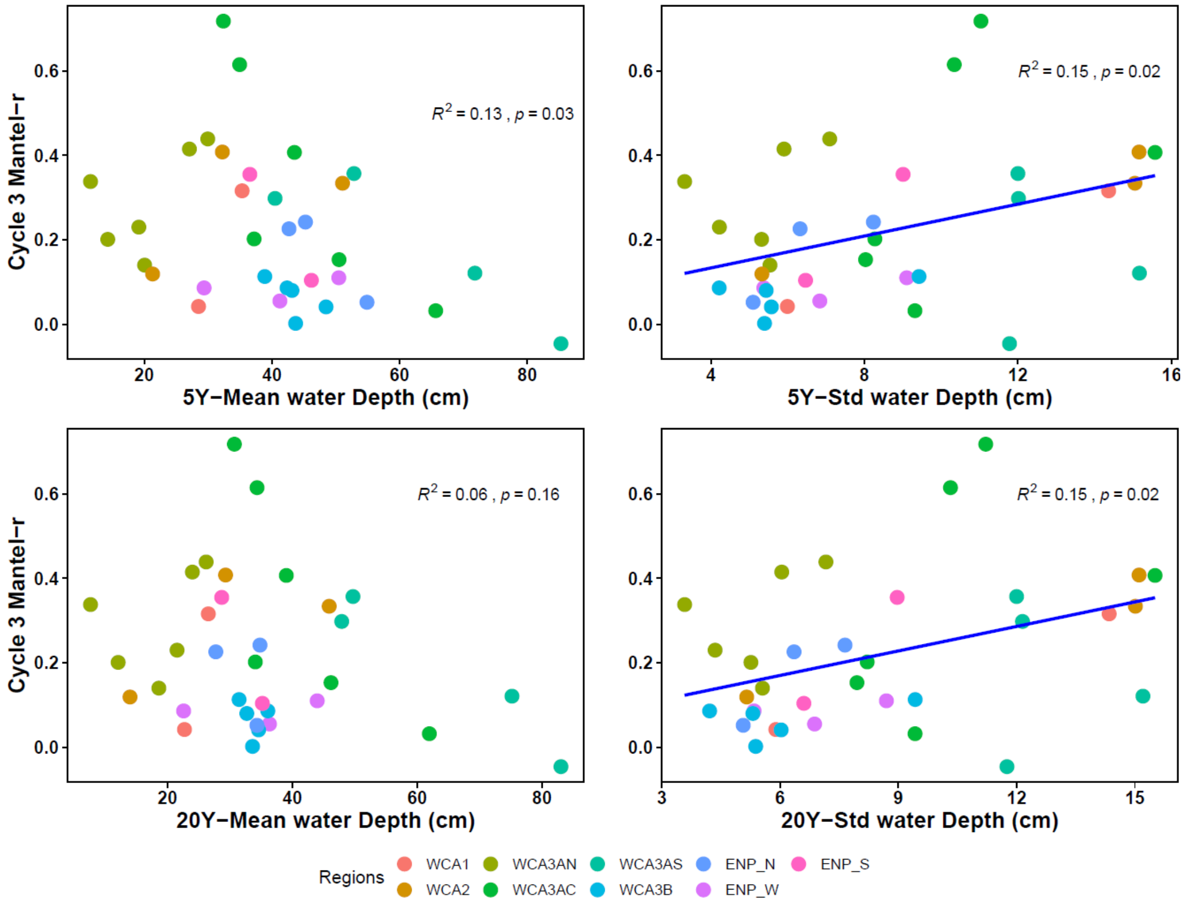

Figure 18: Relationship of Mantel-r with 5-year and 20-year (LTMWD) mean water depth, as well as topographic relief, measured as standard deviation of mean water depths.

The spatial distribution of the vegetation-elevation association followed similar patterns to those observed for microtopographic variability and vegetation community distinctness, as the vegetation-elevation correlation was stronger in PSUs within WCA3AC than in other regions (Figure 19). The vegetation-elevation correlation (Mantel r) is strongly correlated across cycles (Figure 20). Though, in general, Mantel r values during Cycle 3 were lower than the values during Cycle-2.

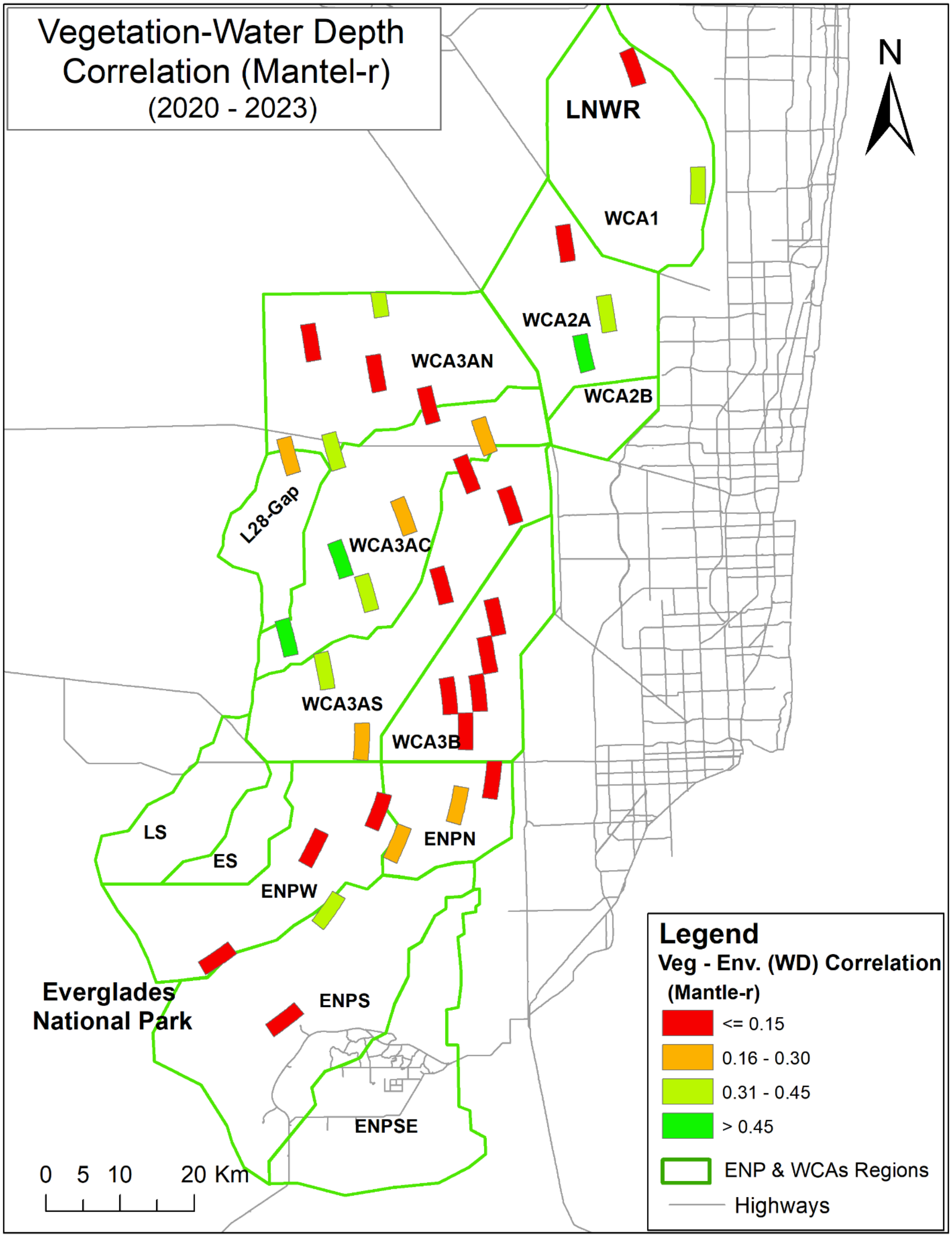

Figure 19: Spatial patterns of elevation-vegetation associations (as measured by Mantel's correlation coefficient [r]) in 34 PSUs sampled during the first three years (2020-2023) of Cycle-3.

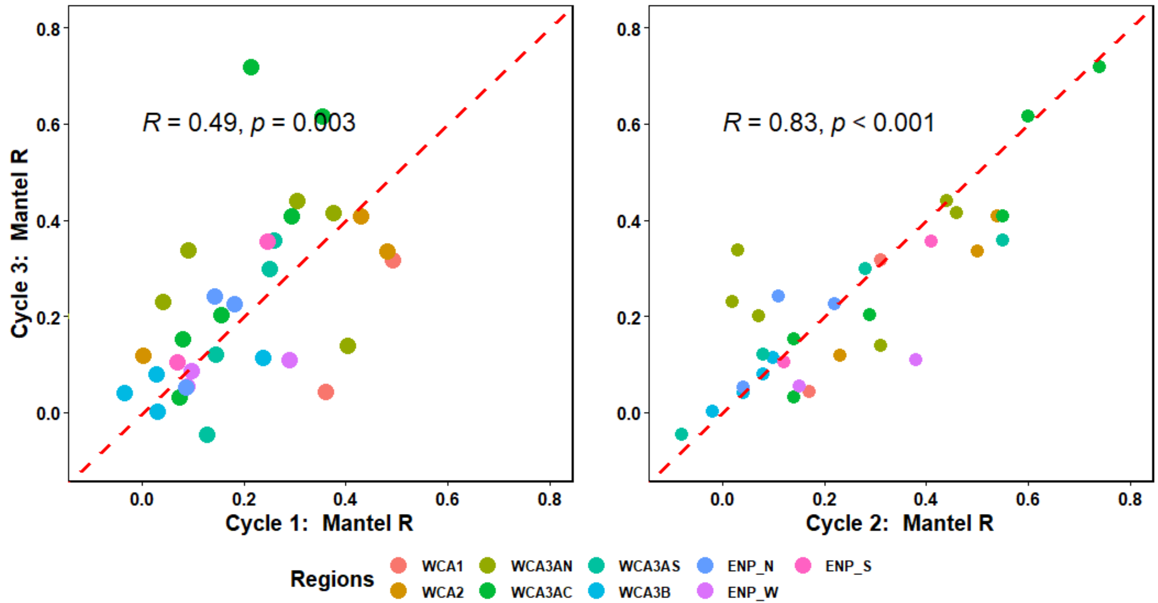

Figure 20: Cycle-3 PSU long-term mean water depth-vegetation associations (as measured by Mantel's correlation coefficient (r) in relation to that of Cycle-1 (n =34) and Cycle-2 (n=33). Only the PSUs that were sampled over three years (Year 1, 2 and 3) of at least two cycles were considered. Exception was PSU-220, which was sampled in Year-4 of Cycle-1 and Cycle-2, but it was included in the analysis.

3.3.2 *Species richness and evenness*

The total number of species recorded within the PSUs during the first three years (Year 1, 2 and 3; 2020-2023) of Cycle-3 survey thus far has been 89, ranging between 5 species in PSU-9 and 50 species in PSU-35 (Appendix 3). Within each PSU, the average species richness, number of species per 1 m^2 plot (defined here as alpha diversity, α), showed a range of 1.6 to 4.6 species/plot. Across the 34 PSUs sampled, the number of species in a plot ranged from 1 to 11. The alpha diversity (α) varied greatly across all ranges of LTMWD, and a maximal number of species per plot occurred in the areas with LTMWD ranging between 15 and 50 cm, except some of relatively dry PSUs with mean LTWD of <10 cm (Figure 21). The plots with mean water depth >55 cm tend to have low (<6 species) species richness. Generalized Linear Model results revealed that LTMWD had a significant effect on plot-level species richness (Appendix 4). The effect of time since the last fire (TSLF) was also significant ($p = 0.002$), and the interaction between LTMWD and FF Index was marginally insignificant, suggesting that water depth could modify the effects of fire.

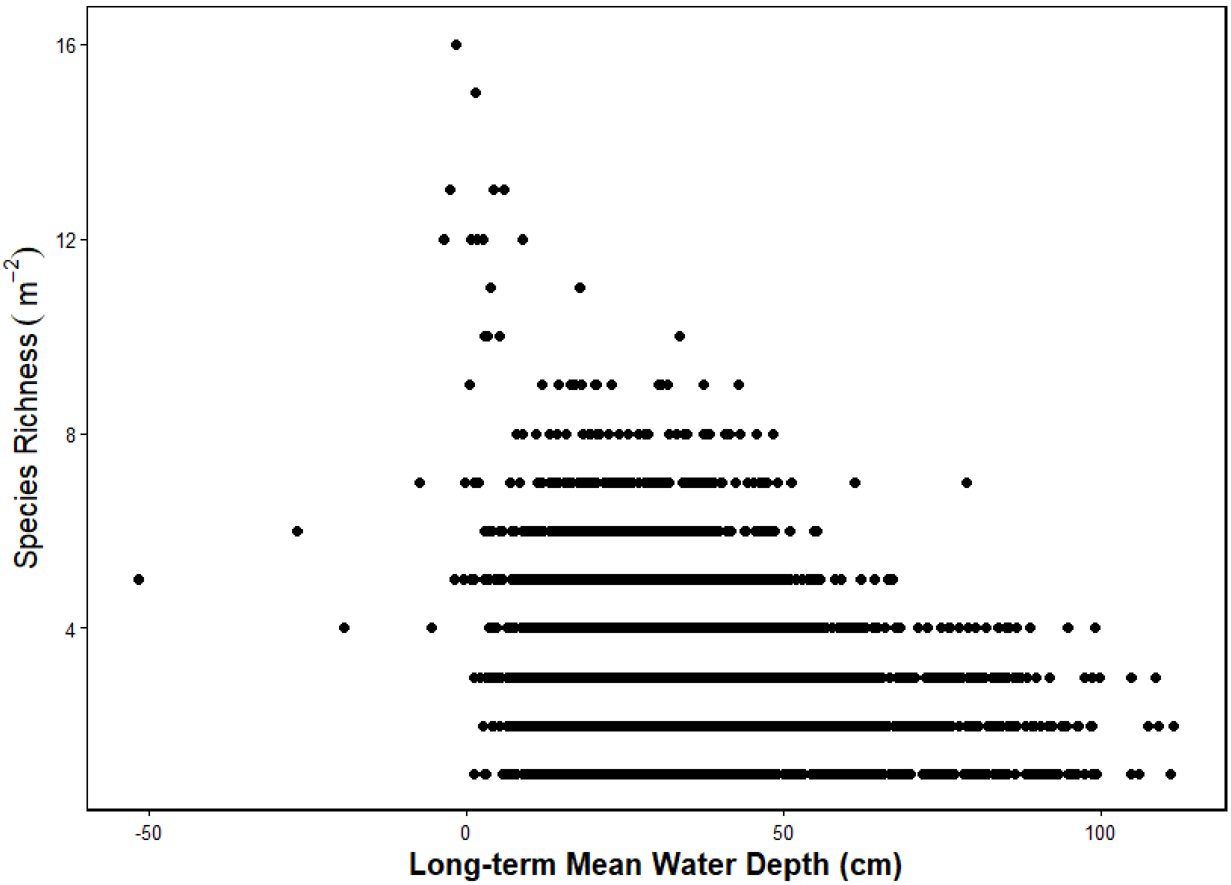

Figure 21: Long-term mean water depth (cm) vs species richness (# of species m^{-2}) in 34 PSUs surveyed during the first three years (2020-2023) of Cycle-3.

Total species richness in each PSU, here defined as 'gamma diversity, γ', was significantly related to both LTWD_SD, LTWD_SD^2 and FF Index^2 (Generalized Linear Model; p <0.010, 0.017 and 0.004). While PSU-level species richness showed a negative relationship with LTWD (GLM: Estimate = -0.145) (Figure 22a), its relationship with FF Index exhibited an inverted hump-shaped curve, showing that species richness was higher in both unburned and most frequently burned areas (Figure 22c; Appendix 4). Total number of species recorded in individual PSUs were much lower in areas with LTWD > 60cm. As expected, species richness at PSU-level was significantly affected by microtopographic variation, expressed as the standard deviation of LTMWD (Appendix 4).

Beta diversity (β), expressed as γ/α for each PSU (Whittaker 1960; Tuomisto 2010), was not much affected by long-term mean water level (General Linear Model (GLM), p >0.05) and by water depth variation (General Linear Model (GLM), p >0.05) (Figure 22d, e; Appendix 4). However, beta diversity had a hump-shaped relationship with mean water level, showing that it tended to be higher at intermediate water levels. Beta diversity did not respond to fire frequency either (Appendix 4). The results of General Linear Model (GLM) also revealed that the effect of LTMWD on evenness was significant (p = 0.015), while the interaction between LTMWD and FF Index had no significant (GLM, p = 0.855) effects on species evenness (Appendix 4).

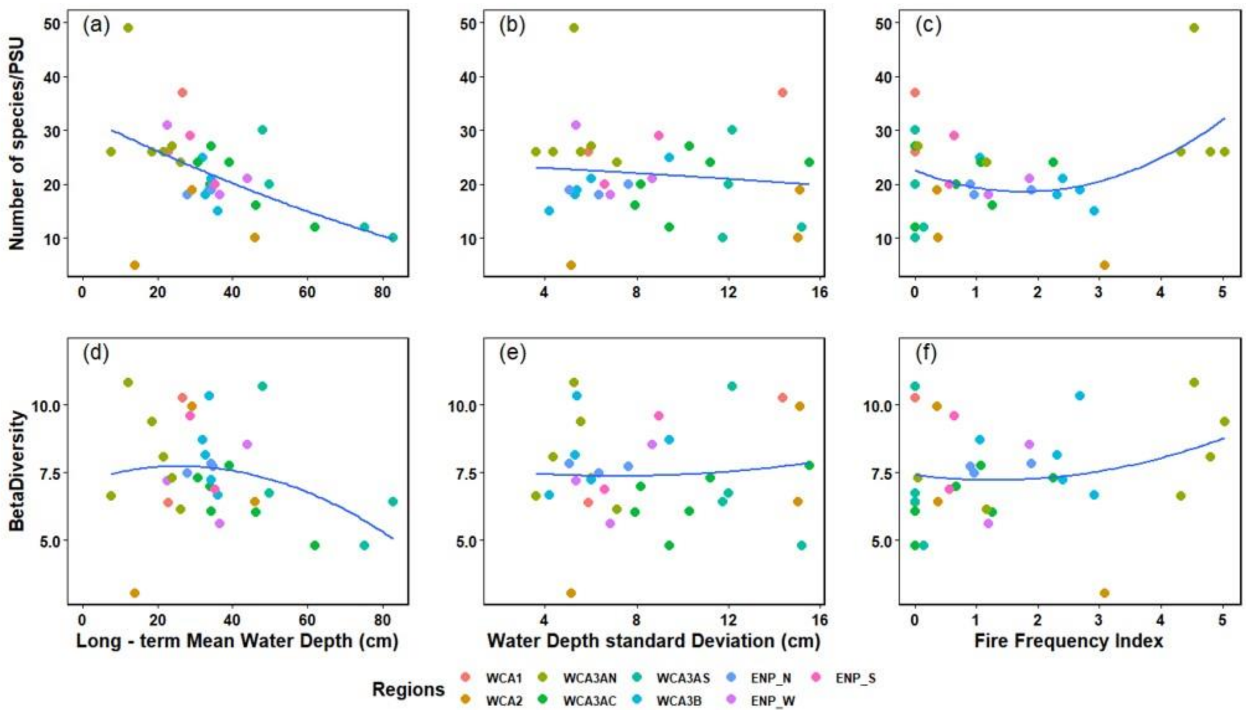

Figure 22: Relationships of species richness (# of species/PSU) and beta diversity (β) with long-term mean water depth (LTMWD) and fire frequency index (FF Index) across 34 PSUs surveyed during the first three years (2020-2023) of Cycle-3.

4. Discussion

In the Ridge and Slough landscape, microtopography is one critical component of historic landscape structure, characterized by dense sawgrass ridges >30 cm higher than the adjacent sloughs (McVoy et al. 2011). However, human modification of the hydrologic regime that began in the early 20th century has resulted in significant reduction in topographic variation, particularly, a loss of elevation differences between ridge height and slough depths throughout the historical R&S landscape (Ogden 2005, Bernhardt and Willard 2009, Larsen et al. 2011, Harvey et al. 2017). Such a flattening of microtopography, together with a loss of distinct ridge and slough vegetation, has been a focus of concern for maintaining Everglades ecosystems (Ogden et al. 2005). Thus, the maintenance and re-establishment of distinct modes of soil elevation (associated with sawgrass ridges and open water sloughs, respectively) is a central goal of Everglades conservation and restoration (USACE and SFWMD 1999).

Previous studies of landscapes throughout the historic R&S landscape have established that bimodality of soil elevations is the key measure of microtopography within this landscape (Watts et al. 2010, Ross et al. 2016). During two surveys, conducted between 2009 and 2015, and then again between 2015 and 2020, the presence of bimodal soil elevations was found to be largely restricted to PSUs within the central WCA3A (Ross et al. 2016; Sah et al. 2021). In these most conserved landscapes, the elevation difference between the high and low elevation modes was generally between 10 and 25 cm, and occurred in PSUs with long-term mean water depths between 25 and 50 cm. The study done over the first three years (2000-2023) of the current 5-year cycle (2020-2025) reiterates that R&S landscape condition varies among different regions. Relatively conserved R&S with distinct bimodality in soil elevations and vegetation communities is mostly confined within central WCA3A, while PSUs in WCA2A, WCA3AN, WCA3B and most of ENP have unimodal soil elevation distributions and are in varied degrees of degradation. As in the previous two surveys, during this study the statistical analysis of bimodality of elevation distributions involved comparing the goodness-of-fit of a single normal distribution with the fit of two normal distributions, which might have equal or unequal variances and equal or unequal weighting. PSUs in which modes had extremely unequal weights (i.e., 75% or more points fall within the higher weighted mode) were not considered to have conserved microtopography, both because such uneven modes are more likely to arise as statistical artifacts, and because the historic ridge-slough landscape was composed of somewhere between 1:1 and 1:3 proportions of ridge and sloughs. (McVoy et al. 2011).

When the bimodality results for the PSUs sampled during the first three years of all three cycles were compared, the number of PSUs showing the bimodal elevations was less during Cycle-3 than during Cycle-1 and Cycle-2 (Table 3). However, four PSUs that had shown bimodality during the first survey did not show bimodality during the next two survey periods. The PSUs in which a shift from detection of bimodal soil elevations in Cycle-1 to their non-detection during Cycle-2 and Cycle-3 were mostly in areas that have experienced dry conditions in recent decades, including WCA3AN and ENP. Since the interval between the successive sampling events is short (5 years), this shift may not necessarily indicate ongoing degradation of remnant patterns in WCA3AN and ENP, although this possibility should be a cause for concern. In many PSUs, fewer points were sampled during Cycle-2 and Cycle-3 than in Cycle-1, owing to logistical and budgetary constraints. Detection of bimodality requires substantial statistical power. While ~135 points in a PSU in Cycle-2 and 3 are also a considerable number typically adequate for distribution modeling, among five PSUs that showed bimodality in Cycle-1 but non-bimodality in Cycle-2 and/or Cycle-3, three PSUs (PSU-3, 18 and 19) had fewer than 135 sampling points. PSUs 3 and 19 are in WCA3AN, experiencing relatively dry conditions, and PSU-18 in ENP is encompassing the areas between SRS loop road, thus experience high variability in water depths. However, such a reduction in sampling intensity between samplings as well as high variation in water depths might have impacted the power to detect subtle bimodality. However, the shift from statistically significant to non-significant bimodality does not necessarily indicate a substantial loss of microtopographic relief. For example, PSU 2, which had a 2-mode model in all three cycles, had elevation modes with unequal weights in both Cycle-1 and Cyle-3 (i.e., one mode >75%), and thus were deemed to have unimodal distributions in Table 3.

Throughout the R&S landscape, some regions, especially WCA3B, ENP_N and ENP_S, that experienced relatively dry conditions for several decades were wetter during Cycle-2 (2015-2020) and Cycle-3 (2020-present) than Cycle-1, due mainly to higher than average annual rainfall in four of eight years since WY2016 (Abtew and Ciuca 2017, Abtew et al. 2019; Cortez et al. 2022; Cortez 2024) and an increase in water delivery resulting from activities associated with DECOMP Physical Model (DPM) and Combined Operational Plan (COP) (Saunders et al. 2018; USACE 2022). Thus, an improvement in R&S conditions in those regions can be expected. In fact, two PSUs, PSU-6 in southern ENP and PSU-15 in the northeastern corner of WCA3AC, did not show bimodality during the Cycle-1 and 2 surveys, but did only during Cycle-3. These PSUs currently have 5-year average water depth of 46 cm and 65 cm, i.e., within the range of optimum water depths or slightly higher for R&S landscape, in comparison to 22 cm and 30 cm during Cycle-1 and Cycle-2. Moreover, six PSUs, three in WCA3B and three in ENP_N, had shown unimodal distribution of soil elevations during the Cycle-1 sampling, but four of them showed bimodal distributions, although the modes in four of them still had unequal weights (i.e., 75% or more points fall within the higher weighted mode). Thus, only the subsequent monitoring will show if the improvement in landscape in this region is happening or not.

In the ridge and slough landscape, the distinct zonation of plant communities is shaped by abrupt differences in elevation between ridges and sloughs (Ogden 2005, McVoy et al. 2011). In this study, the distinctness between ridge and slough communities was represented by a test statistic "community distinctness," which was measured using dissimilarity between R&S vegetation community composition, defined as the distance (in multivariate space) between two forcefully imposed vegetation clusters (Isherwood 2013, Ross et al. 2016, Sah et al. 2021) that to some extent represent ridge and slough vegetation communities. Our approach to measuring community distinctness is a novel measure based on measurements of distances between two clusters of plant communities in ordination space (Isherwood 2013, Ross et al. 2016). During the first three years of Cycle-3, high community distinctness values representing highly distinct sawgrass-dominated ridges and *Nymphaea*- and *Utricularia*-dominated sloughs observed in conserved landscapes of WCA3AC are consistent with the findings during Cycle-1 and Cycle-2 of this ongoing monitoring study (Ross et al. 2016, Sah et al. 2021) and in other studies (Watts et al. 2010; Nungesser 2011). Likewise, in areas subject to increased or decreased water levels due to water management or altered infrastructure, this distinctness is reduced. For instance, the degraded ridge and slough community pattern observed in WCA2A, WCA3AN, WCA3B and ENP during all three cycles was consistent with loss of characteristic microtopography variability in those areas, suggesting that this metric is appropriate to assess the system-wide status of the ridge and slough landscape.

While community distinctness was consistent across three cycles (RMSE <= 0.15), several PSUs had reduced distinctness in Cycle-3 compared to Cycle-1 and 2. However, the reduction in community distinctness between Cycle-2 and Cycle-3 was much less than the reduction in distinctness between Cycle-1 and Cycle-3. The reduction in community distinctness was observed in PSUs, mostly within WCA1, WCA2A, WCA3AN (Figure 14), where ridge and sloughs have long disintegrated and topographic variation is very patchy. Three PSUs, one within each of WCA1, WCA3AC and WCA3AS regions had a reduction in community distinctness of >0.20 between Cycle-1 and Cycle-3, and those PSUs also had a reduction of >0.02 between Cycle-2 and Cycle-3. In contrast, PSU-11 in WCA3AC and PSU-34 in WCA3AS showed an increase in community distinctness by >0.17 during the Cycle-3 survey in comparison to the previous two surveys. Interestingly, almost all surveyed PSUs within WCA3B and ENP, the areas which have become wetter in recent years, have shown an increase in community distinctness values, suggesting an improvement in R&S conditions in those areas.

Several studies have documented rapid shifts (within 3-5 years) in prairie and marsh plant community composition in response to changing hydrologic regimes (Armentano et al. 2006; Zweig and Kitchens 2008; Sah et al. 2014). Hence, the difference in community distinctness might have resulted from a shift in species composition at a local scale. In general, hydrologic conditions during Cycle-3 were slightly wetter than in the previous two cycles, and in some PSUs, the difference in mean water depth was greater than 10 cm, which might have extended the hydroperiod as well. During Cycle-1 (2009-2015), relatively high distinctness values were observed in PSUs that had mean water levels between 20 and 50 cm (Ross et al. 2016). A shift in hydrologic conditions within this range, especially in some portions of WCA3B and northeastern and southern ENP might have helped to realize an increase in distinctness. Other studies also have found that a decrease in sawgrass in SRS and an increase in abundance of hydrophilic species in Northeast Shark River Slough (NESRS) have occurred since 2015, primarily in response to an increase in mean annual water depth due to increased water delivery to the Park (Sah et al. 2024; Nocentini et al. 2024).

Several other factors might have contributed to the observed changes in microtopography and community distinctness. Among them, fire, an integral component of Everglades ecosystem (Gunderson and Snyder 1994; Osborne et al. 2013), is also believed to have an important role in R&S landscape dynamics. An analysis of fire frequency over 23 years (1997-2019) suggests that the northern WCA3A and some parts of WCA3B, which have experienced dry conditions in recent decades, have burned more frequently than other areas (Sah et al. 2021). Since fire severity data were not available, we were unable to assess whether those fires consumed peat and affected topography or not. However, it is logical to assume that if a relatively dry area burns frequently, especially during the dry seasons when there is no standing water, the fires are likely to consume peat materials and affect topography, thereby impacting water regions and vegetation communities in the area (Gunderson 1994; Ogden 2005). Likewise, the discrepancy in burn season in different regions, for instance PSUs in WCAs mostly burned in dry season while PSUs in NESRS burned in wet seasons, might have affected vegetation communities differently. Between Cycle-1 and 2, four of nine burned PSUs in WCAs decreased in community distinctness by >0.2, whereas in the burned PSUs within ENP, a change in distinctness between the two surveys was much less, usually >0.1 (Sah et al. 2021).

Environmental heterogeneity (EH) is usually positively correlated with species diversity (Stein et al. 2014). In this study, microtopographic heterogeneity within each PSU was represented by the standard deviation of long-term water depth, which exhibited a significant relationship with plant species richness across the 34 sampled PSUs (Appendix 4). Microtopography in PSUs is affected by hydrologic conditions and variation in fire regimes. In this study, both plot-level and PSU-level species richness (α and γ diversity, respectively) tended to be higher in mid-range of water depth (20-55 cm; Figures 21, 22a), which is prevalent in conserved PSUs with relatively distinct ridge and slough features. In contrast, PSU-level species richness had an inverted hump-shaped relationship with fire frequency index. This is plausible, since relatively high fire frequency tends to burn the peat on the high ground (here, 'ridge') and reduce the microtopographic variation in the area, which can have negative effects on species richness. Across the surveyed PSUs (n = 34), beta diversity (β) was not affected by LTMWD or microtopographic variation (Appendix 4). However, as expected, the relationship of beta diversity with water depth tended to be negative, while it showed positive relationship with microtopography (LTMWD_SD) and FF_Index though the relationships were not statistically significant. We have defined beta diversity simply as γ/α and explored its relationship with environmental variables at the PSU-scale. In fact, there is a whole family of beta diversities, defined in different ways and at different scales (Tuomisto 2010). Moreover, the relationship between beta diversity and environmental heterogeneity and its drivers depends on the scale of study and several other factors (Stein et al. 2014 and others). Hence, more detailed analysis is planned by the end of this monitoring cycle to understand the true nature of spatio-temporal variation in beta diversity and its relationship with environmental drivers in the R&S landscape throughout the system.

5. Summary

Metrics of both microtopography and plant community distinctness in 34 PSUs revealed a spatial pattern of R&S conditions consistent with system-wide findings based on much large number of PSUs sampled in the previous two cycles (2009-2015; 2015-2020), suggesting that both metrics are robust measures of R&S condition in the Everglades. Some PSUs have experienced shifts in microtopographic variability, changing from bimodality to unimodality, and are experiencing a reduction in community distinctness (especially in WCA1, WCA2, WCA3AN) since previous surveys. Extreme drought conditions in two of five years during the first survey (2009-2015), which possibly had adverse effects on peat soils and microtopography, followed high water levels during subsequent surveys and might have a role in such changes in microtopography and community distinctness. In contrast, PSUs in southern WCA3B and throughout ENP have shown an increase in community distinctness. Since these are the areas which are currently experiencing increased water level, resulting from ongoing restoration efforts, an increase in community distinctness could be a positive sign. Several other factors, including fires, might also have contributed to the observed changes in microtopographic variability and community characteristics.

Assessment of R&S stability by examining temporal changes in landscape indices may require vegetation mapping showing distinct ridge and slough features at regular intervals. In fact, in the original design of R&S study using PSUs, vegetation mapping was also a component, and was done during the first three years of Cycle-1 (2009-2015) of the monitoring project (Heffernan et al. 2013, Ross et al. 2015), but it was then dropped due to budgetary limitation. Another round of vegetation mapping would help to assess long term system changes in landscape indices. Likewise, finer scale responses of ridge and slough features that may reveal the mechanisms underlying change may require a sampling design that also incorporates measurement of ground elevations and vegetation composition at short intervals along multiple transects that encompass ridge, slough, and transient communities.

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Appendix

Appendix 1: Soil depth (cm) in 34 PSUs surveyed during Year 1, 2 and 3 (2020-2023) of Cycle-3 (2020-2025).

PSU-Cycle	Cycle-Year	PSU	PSU_ID	Regions	Soil Depth (cm)			
					Mean	SD	Minimum	Maximum
C3	1	0	P000	ENP_W	81.1	30.4	6	183
C3	1	1	P001	WCA1	318.9	55.1	214	379
C3	1	2	P002	WCA3AS	145.9	37.3	51	255
C3	1	3	P003	WCA3AN	65.1	31.3	34	236
C3	1	4	P004	WCA3AC	118	16.9	28	168
C3	1	6	P006	ENP_S	61.2	31.9	2	296
C3	1	7	P007	WCA3AN	70.9	25.7	15	184
C3	1	9	P009	WCA2	203.2	26.8	159	264
C3	1	11	P011	WCA3AC	111.7	45.4	54	264
C3	1	15	P015	WCA3AC	106.4	31.3	54	220
C3	1	108	P108	WCA3B	240.8	43.6	156	382
C3	2	17	P017	WCA1	28.8	21.1	1	135
C3	2	18	P018	ENP_W	50.5	26.7	17	138
C3	2	19	P019	WCA3AN	189.9	35	127	386
C3	2	20	P020	WCA3B	129.7	45.5	65	264
C3	2	21	P021	WCA2	53.9	23.6	10	168
C3	2	22	P022	ENP_S	92.2	19.9	48	187
C3	2	23	P023	WCA3AC	40.6	21.4	0	171
C3	2	24	P024	ENP_N	89.6	37.8	37	259
C3	2	26	P026	WCA3AC	91.2	12.7	58	123
C3	2	28	P028	WCA3B	74.2	26.6	19	149
C3	2	30	P030	ENP_S	123.6	18.1	79	189
C3	2	31	P031	WCA3AC	179.7	29.2	91	260
C3	3	32	P032	ENP_N	61.2	38.8	6	264
C3	3	34	P034	WCA3AS	107.9	34.4	30	189
C3	3	35	P035	WCA3AN	44.5	28.0	6	127
C3	3	36	P036	WCA3AS	129.4	28.9	28	221
C3	3	37	P037	WCA2A	143.5	33.3	66	263
C3	3	39	P039	WCA3AN	48.1	24.8	7	145
C3	3	43	P043	WCA3AN	63.2	22.9	14	159
C3	3	44	P044	WCA3B	135.6	24.7	87	207
C3	3	45	P045	WCA3AS	79.5	42.2	15	259
C3	3	220	P220	WCA3B	107.1	25.9	67	217
C3	3	513	P513	ENP_N	79.7	25.5	30	173

Appendix 2: Mean species cover (%) in PSU sampled during Years 1, 2, and 3 (2020-2023). The number of 1x1 m plots sampled in each PSU is given in Table 1.

SPPCODE	Species Name	Year-1										
		0	**1**	**2**	**3**	**4**	**6**	**7**	**9**	**11**	**15**	**108**
AESPRA	*Aeschynomene pratensis* var. *pratensis*	0.20						0.07				0.02
ANDVIR	*Andropogon virginicus*		0.04			0.07						
BACCAR	*Bacopa caroliniana*	0.13	0.60	0.34	2.64	0.67	0.80	3.64				0.58
BLESER	*Blechnum serrulatum*	0.04	0.14	0.19	0.13	0.27						0.01
CEPOCC	*Cephalanthus occidentalis*		0.19	0.35		0.22		0.47		0.01		0.04
CHRICA	*Chrysobalanus icaco*						0.23					
CLAJAM	*Cladium jamaicense*	23.75	7.09	15.70	43.86	29.32	17.21	33.18	61.45	18.72	11.59	36.59
COLTEN	*Coleataenia tenera*				0.66							
CRIAME	*Crinum americanum*	0.23		0.21		0.21	0.12	0.75				0.08
DICDIC	*Dichanthelium dichotomum*				0.98							
ELECEL	*Eleocharis cellulosa*	6.36	12.90	0.93		1.44	9.29	6.62	0.48	0.02	0.17	1.64
ELEELO	*Eleocharis elongata*				0.06	0.15		0.11		0.19		
ELEINT	*Eleocharis interstincta*		0.17									0.04
ERAELL	*Eragrostis elliottii*				0.06							
FUIBRE	*Fuirena breviseta*				0.12							
HYMPAL	*Hymenocallis palmeri*	0.01			0.31	0.11	0.03	0.84				0.04
IPOSAG	*Ipomoea sagittata*	0.01		0.04		0.10	0.01	0.07				0.02
JUSANG	*Justicia angusta*	0.04		0.11	0.18	0.19	0.02	0.07				0.17
LEEHEX	*Leersia hexandra*	0.10	0.03	0.02		0.17	0.01	0.12		0.63	0.05	0.05
LUDMIC	*Ludwigia microcarpa*				0.02							
LUDREP	*Ludwigia repens*				0.28							
LYGMIC	*Lygodium microphyllum*		0.07									
MELQUI	*Melaleuca quinquenervia*		0.25									
MIKSCA	*Mikania scandens*				0.61							
MORCER	*Morella cerifera*		0.13		4.10		0.15					
NUPADV	*Nuphar advena*									0.59		
NYMAQU	*Nymphoides aquatica*	0.27	0.11	1.86		0.99	0.01	1.18			0.03	0.64
NYMODO	*Nymphaea odorata*	0.04	11.70	32.03		27.46		10.13	4.13	27.73	22.47	6.58
PANHEM	*Panicum hemitomon*	0.19	1.50	0.15		0.13	0.04			0.19	0.05	0.01
PANVIR	*Panicum virgatum L.*		0.02		0.40	0.37		1.11		0.57	0.16	0.05
PASGEM	*Paspalidium geminatum*	0.10	0.93	0.08		0.07	0.06	0.29		0.05	0.27	0.07
PELVIR	*Peltandra virginica*	0.10	2.48	1.31	0.07	0.29	0.18	0.09				0.66
PERHYD	*Persicaria hydropiperoides*									0.15		
PLUBAC	*Pluchea baccharis*				1.29			0.01				
PONCOR	*Pontederia cordata*	0.01	0.28	0.39	0.83	0.81	0.52	0.29		0.96	0.05	1.02
PROPAL	*Proserpinaca palustris*				0.48							
RHYINU	*Rhynchospora inundata*	0.01	0.06	0.02		0.01		0.08				0.09

SPPCODE	Species Name	Year-1										
		0	1	2	3	4	6	7	9	11	15	108
RHYMIC	*Rhynchospora microcarpa*		0.01									
RHYTRA	*Rhynchospora tracyi*		1.13		0.21		0.13	0.75				
SACGIG	*Saccharum giganteum*		0.04		0.29							
SAGLAN	*Sagittaria lancifolia* ssp. *lancifolia*	0.02	0.08	0.04	3.89		0.07	0.94		0.10		0.39
SALCAR	*Salix caroliniana*					0.30						0.50
SCHTER	*Schinus terebinthifolia*		0.03									
SYMDUM	*Symphyotrichum dumosum*				0.71							
TYPDOM	*Typha domingensis*	1.04		0.43	0.11	0.47	0.11	2.48	2.33	7.18	0.93	2.12
UNKC3Y11	*Unknown C3Y1_1*		0.12									
UTRFOL	*Utricularia foliosa*	1.76	0.46	4.85	1.72	3.79	0.93	3.21	1.10	7.88	2.49	0.81
UTRPUR	*Utricularia purpurea*	7.88	11.63	14.81	1.17	7.90	12.42	2.37		0.24	14.33	2.98

Appendix 2: Contd.

SPPCODE	Species Name	Year-2											
		17	18	19	20	21	22	23	24	26	28	30	31
AESPRA	*Aeschynomene pratensis* var. *pratensis*		0.44				0.55		0.27			0.09	
ANNGLA	*Annona glabra*	0.33										0.62	
BACCAR	*Bacopa caroliniana*	0.09	1.54	0.70	0.81	1.50	8.28	2.04	0.64	1.74	0.39	0.98	1.02
BACMON	*Bacopa monnieri*			0.07									
BLESER	*Blechnum serrulatum*	1.57						0.21		0.47		0.04	
CASFIL	*Cassytha filiformis*						0.04						
CEPOCC	*Cephalanthus occidentalis*	1.06		0.06	0.19			0.25	0.04	1.33	0.04	0.03	0.26
CHRICA	*Chrysobalanus icaco*	0.22	0.14										
CLAJAM	*Cladium jamaicense*	28.67	13.44	17.63	58.89	23.10	13.14	33.40	21.82	35.60	53.53	53.88	29.74
COLTEN	*Coleataenia tenera*	0.04			0.03		0.17		0.04				
CRIAME	*Crinum americanum*			0.49	0.46		1.64	0.77	0.29	1.76	0.39	0.29	0.57
CYPHAS	*Cyperus haspan*						0.04						
DICSPP	*Dichanthelium* sp.	0.04											
ELECEL	*Eleocharis cellulosa*	1.42	19.67	3.21	2.27	3.09	21.55	2.00	3.66	1.83	2.48	6.03	0.96
ELEELO	*Eleocharis elongata*	1.92			0.20	1.25		0.92		1.35		0.62	3.10
ELEINT	*Eleocharis interstincta*	0.33		0.54									
FUIBRE	*Fuirena breviseta*									0.07		0.02	
HYDCOR	*Hydrolea corymbosa*			0.01			0.20			0.31		0.02	
HYDUMB	*Hydrocotyle umbellata*			0.02									
HYMPAL	*Hymenocallis palmeri*			0.01	0.06		0.05	0.73	0.06	0.42	0.49	0.08	0.38
IPOSAG	*Ipomoea sagittata*	0.01			0.01	0.01		0.15		0.01		0.02	
IVAMIC	*Iva microcephala*						0.01					0.01	
JUSANG	*Justicia angusta*	0.01	0.12	0.36	0.01		0.17	0.07	0.04	0.90	0.00	0.29	
LEEHEX	*Leersia hexandra*	0.03		0.19			0.27	0.08		0.19		0.01	0.16
LUDALA	*Ludwigia alata*			0.07									
LUDMIC	*Ludwigia microcarpa*	0.02											
LYGMIC	*Lygodium microphyllum*	0.48											
MIKSCA	*Mikania scandens*			0.28									
MORCER	*Morella cerifera*	3.10											

SPPCODE	Species Name	Year-2											
		17	18	19	20	21	22	23	24	26	28	30	31
NYMAQU	*Nymphoides aquatica*	0.17	0.33		0.12		0.79	1.14	0.07	2.17	0.50	0.86	0.60
NYMODO	*Nymphaea odorata*	21.30		0.07	1.89		0.15	19.88		21.07	8.86	1.74	29.89
OSMREG	*Osmunda regalis* var. *spectabilis*	1.07						0.52					
OXYFIL	*Oxypolis filiformis*						0.04						
PANHEM	*Panicum hemitomon*	0.58	0.39	0.02	0.07		1.13	0.47	0.34	0.57	0.35	0.64	0.21
PASGEM	*Paspalidium geminatum*	0.01	0.09		0.02	0.07	0.21	0.21	0.12	0.18	0.09	0.38	0.06
PELVIR	*Peltandra virginica*	1.62	0.29	0.04	0.36		0.38	0.11		1.46	0.07	0.55	0.39
PERHYD	*Persicaria hydropiperoides*	0.02		1.09			0.01						
PERSET	*Persicaria setaceum*	0.04											
PISSTR	*Pistia stratiotes*	0.01											
PLUBAC	*Pluchea baccharis*						0.02					0.01	
PONCOR	*Pontederia cordata*	2.72		0.60	0.11		2.78	0.40	0.26	0.78		0.25	0.41
POTILL	*Potamogeton illinoensis*										0.04		
PROPAL	*Proserpinaca palustris*			0.37									
RHYINU	*Rhynchospora inundata*		0.36				0.56	0.63		0.43		0.12	0.36
RHYMIC	*Rhynchospora microcarpa*	0.06											
RHYTRA	*Rhynchospora tracyi*	0.18	2.02	0.04			1.79	1.82	0.01	0.13	0.01	0.14	1.05
SACGIG	*Saccharum giganteum*	0.07											
SAGLAN	*Sagittaria lancifolia* ssp. *lancifolia*	0.04	0.08	4.28	0.36	0.04	1.14	0.33	0.19	0.44	0.27	0.61	0.39
SALCAR	*Salix caroliniana*	0.22	0.10	1.22			0.30			0.37			
SALMIN	*Salvinia minima*	1.36											
SCHTER	*Schinus terebinthifolia*	0.14											
TAXDIS	*Taxodium distichum*									0.15			
THAGEN	*Thalia geniculata*						2.07						
TYPDOM	*Typha domingensis*	0.86	0.10	25.04	0.01	1.89	4.76	0.27	1.24	0.96	2.63	0.04	1.56
UNKC3Y21	*Unknown C3Y2_1*	0.09		0.03			0.09			0.06			
UNKC3Y22	*Unknown C3Y2_2*	0.02											
UTRFOL	*Utricularia foliosa*	2.17	1.93	1.00		0.13	2.86	0.06	1.26	2.01	0.59	3.74	1.58
UTRGIB	*Utricularia gibba*	0.73	0.01				0.44						
UTRPUR	*Utricularia purpurea*	4.08	9.79	0.59	0.65	0.33	7.84	5.54	9.11	9.61	2.13	9.47	2.05

Appendix 2: Contd.

SPPCODE	Species Name	Year 3										
		32	34	35	36	37	39	43	44	45	220	513
AESPRA	*Aeschynomene pratensis* var. *pratensis*	0.02					0.02	0.01				0.04
ANDSPP	*Andropogon sp.*			0.07								
ANNGLA	*Annona glabra*		0.00	0.12								
ASTSPP	*Aster sp.*			0.05								
BACCAR	*Bacopa caroliniana*	0.27	0.39	5.41			1.41	1.53	0.90		0.77	0.42
BACMON	*Bacopa monnieri*			0.04		0.04						
BLESER	*Blechnum serrulatum*	0.00	0.58	0.17		0.04						
BOECYL	*Boehmeria cylindrica*			0.01								

SPPCODE	Species Name	Year 3										
		32	34	35	36	37	39	43	44	45	220	513
CENASI	*Centella asiatica*			0.25								
CEPOCC	*Cephalanthus occidentalis*	0.02	0.98	0.03		0.02	0.74	0.51	0.07			0.10
CHARA	*Chara sp.*	1.15		0.18	0.07	5.56	0.19		0.41	14.78	1.24	0.21
CLAJAM	*Cladium jamaicense*	38.36	35.27	16.85	8.09	31.50	19.69	46.33	40.78	4.28	25.96	28.40
COLTEN	*Coleataenia tenera*						0.03					
CRIAME	*Crinum americanum*	0.12	1.26	0.20		0.01	1.25	4.05	0.35		0.16	0.18
CYPODO	*Cyperus odoratus*		0.01							0.01		
DICDIC	*Dichanthelium* sp.			0.06								
ELEBAL	*Eleocharis baldwinii*									0.01		
ELECEL	*Eleocharis cellulosa*	2.79	1.36	20.34	0.33	1.19	5.06	4.67	4.90	1.79	0.76	4.13
ELEELO	*Eleocharis elongata*	0.23	3.13		0.19	0.04			0.68		0.26	
ELEGEN	*Eleocharis geniculata*			0.02		0.04						
ELEINT	*Eleocharis interstincta*											0.22
ELESPP	*Eleocharis sp.*			0.04								
EUPCAP	*Eupatorium capillifolium*		0.01									
FUIBRE	*Fuirena breviseta*		0.01	0.27								
FUISCI	*Fuirena scirpoidea*						0.05					
HYDCOR	*Hydrolea corymbosa*		0.01	0.02								
HYDUMB	*Hydrocotyle umbellata*					0.04						
HYMPAL	*Hymenocallis palmeri*	0.12	0.07	0.51			0.02	0.32	0.34		0.17	0.02
HYPALA	*Hyptis alata*			0.12								
IPOSAG	*Ipomoea sagittata*		0.05				0.17		0.01			0.02
IVAMIK	*Iva microcephala*					0.07						
JUSANG	*Justicia angusta*	0.03	0.07	0.08			0.11	0.03	0.09		0.04	0.13
LEEHEX	*Leersia hexandra*	0.02	0.09	0.81			0.03	0.05	0.03		0.04	0.10
LUDALA	*Ludwigia alata*			0.07				0.04				
LUDMIC	*Ludwigia microcarpa*		0.01									
LUDOCT	*Ludwigia octovalvis*									0.01		
LUDPER	*Ludwigia peruviana*		0.01									
LUDREP	*Ludwigia repens*					0.01		0.01				
MIKSCA	*Mikania scandens*			0.06								
MORCER	*Morella cerifera*	0.00		0.63								
NYMAQU	*Nymphoides aquatica*	1.18	0.30		0.01	0.04	0.09	0.07	0.27		0.01	
NYMODO	*Nymphaea odorata*	3.17	17.93		26.07	1.89	0.26	0.24	5.19	0.01	6.93	
OXYFIL	*Oxypolis filiformis*			0.12			0.04		0.02			
PANHEM	*Panicum hemitomon*	0.17	0.24	2.60	0.02		1.15	0.79	0.10		0.01	0.03
PANRIG	*Panicum rigidula*			0.07								
PANVIR	*Panicum virgatum*			0.09								
PASGEM	*Paspalidium geminatum*	0.14	0.23	0.35	0.36		0.12	0.11	0.18	0.33	0.04	
PASMON	*Paspalum monostachyum*											0.01

SPPCODE	Species Name	Year 3										
		32	**34**	**35**	**36**	**37**	**39**	**43**	**44**	**45**	**220**	**513**
PELVIR	*Peltandra virginica*	0.05	0.67	0.09					0.04		0.36	0.04
PERHYD	*Persicaria hydropiperoides*		0.05	0.01		0.10	0.01	0.04				0.01
PHYNOD	*Phyla nodiflora*						0.01					
PISSTR	*Pistia stratiotes*	0.17										
PLUBAC	*Pluchea baccharis*			0.49				0.02				
PONCOR	*Pontederia cordata*	0.73	1.79	0.05	0.19			0.04				0.19
POTILL	*Potamogeton illinoensis*	0.05		0.02								
PROPAL	*Proserpinaca palustris*			0.14				0.04				
RHYCOL	*Rhynchospora colorata*			0.14								
RHYDIV	*Rhynchospora divergens*			0.04								
RHYINT	*Rhynchospora intermedia*			0.04								
RHYINU	*Rhynchospora inundata*		1.16	0.07			0.76	0.10				
RHYMIC	*Rhynchospora microcarpa*		0.07	0.58			0.02					
RHYTRA	*Rhynchospora tracyi*		0.15	2.06			1.21	2.44	0.01			0.04
SACGIG	*Saccharum giganteum*			0.01								
SAGLAN	*Sagittaria lancifolia* ssp. *lancifolia*	0.01	0.23	3.86	0.01	0.94	0.12	3.62	0.33		0.07	
SALCAR	*Salix caroliniana*	0.00	0.33	0.02		0.83		0.86				0.07
SPABAK	*Spartina bakeri*						0.08					
TYPDOM	*Typha domingensis*	0.60	3.64	3.11	1.36	15.10	0.33	1.13	0.52	4.24	0.76	0.88
UNK6	*Unknown C3Y3_6*							0.01				
UTRCOR	*Utricularia cornuta*									0.01		
UTRFOL	*Utricularia foliosa*	2.21	0.26	0.33	1.37	1.67	0.19	0.08	0.41		0.93	0.54
UTRGIB	*Utricularia gibba*		0.59	0.07		0.06						0.19
UTRPUR	*Utricularia purpurea*	6.83	1.96	0.17	24.04	0.04	2.56		5.48	0.01	5.37	4.43
VICACU	Vicia acutifolia			0.02								
XYRSPP	Xyris sp.		0.04									

Appendix 3: Plant species richness, evenness, and diversity indices in 34 PSUs surveyed during Cycle 3 Years 1, 2 & 3 (2021-2023).

Cycle-3 Year	PSU	PSU_ID	Region	Number of plots (1 m^2)	Species Richness /m^2 (α)	Species Richness/ PSU (γ)	Evenness	Shannon`s diversity	Beta Diversity (γ/α)
1	0	P000	ENP_W	135	2.5	21	0.81	2.48	8.54
1	1	P001	WCA1	117	4.1	26	0.92	2.99	6.38
1	2	P002	WCA3AS	132	3.0	20	0.81	2.42	6.74
1	3	P003	WCA3AN	89	3.9	26	0.83	2.71	6.63
1	4	P004	WCA3AC	132	3.1	24	0.82	2.60	7.76
1	6	P006	ENP_S	130	2.9	20	0.83	2.50	6.86
1	7	P007	WCA3AN	135	3.9	24	0.87	2.76	6.15
1	9	P009	WCA2A	120	1.6	5	0.75	1.20	3.05
1	11	P011	WCA3AC	135	2.7	16	0.78	2.17	6.03
1	15	P015	WCA3AC	134	2.5	12	0.89	2.21	4.79
1	108	P108	WCA3B	132	2.9	25	0.83	2.67	8.71
2	17	P017	WCA1	131	3.6	37	0.89	3.21	10.23
2	18	P018	ENP_W	99	3.2	18	0.90	2.59	5.60
2	19	P019	WCA3AN	114	2.8	26	0.87	2.83	9.36
2	20	P020	WCA3B	135	1.8	19	0.77	2.27	10.31
2	21	P021	WCA2A	135	1.6	10	0.82	1.89	6.42
2	22	P022	ENP_W	135	4.3	31	0.87	2.99	7.19
2	23	P023	WCA3AC	135	3.3	24	0.84	2.66	7.28
2	24	P024	ENP_N	133	2.4	18	0.90	2.61	7.48
2	26	P026	WCA3AC	135	4.5	27	0.86	2.82	6.05
2	28	P028	WCA3B	132	2.2	18	0.83	2.40	8.15
2	30	P030	ENP_S	121	3.0	29	0.82	2.75	9.59
2	31	P031	WCA3AC	132	2.9	20	0.89	2.65	6.99
3	32	P032	ENP_N	135	2.6	21	0.84	2.54	7.97
3	34	P033	WCA3AS	138	2.8	30	0.85	2.89	10.68
3	35	P034	WCA3AN	135	4.6	50	0.88	3.45	10.89
3	36	P036	WCA3AS	135	2.5	14	0.80	2.11	5.56
3	37	P037	WCA2	129	2.0	19	0.89	2.62	9.35
3	39	P039	WCA3AN	135	3.8	28	0.87	2.89	7.46
3	43	P043	WCA3AN	135	3.2	26	0.82	2.67	8.05
3	44	P044	WCA3B	135	3.0	22	0.83	2.55	7.35
3	45	P045	WCA3AS	135	1.7	11	0.64	1.53	6.58
3	220	P220	WCA3B	135	2.4	16	0.88	2.44	6.79
3	513	P513	ENP_N	135	2.4	20	0.87	2.60	8.17

Appendix 4: Results of Generalized Linear Model for Species Richness (species/plot, α diversity, or species/PSU, γ diversity) and General Linear Model for Beta diversity (β) and Evenness showing the effects of long-term (20 years) mean water depth (LTMWD, cm), standard deviation of long-term water depth (LTMWD_SD, cm), fire frequency (FF, fires/decade), PSU-level fire frequency index (FF Index), and time since last fire (TSLF, years).

Generalized Linear Model			
	Estimate	**Std. Error**	**p-value**
Plot-level Species Richness (n= 4303)			
(Intercept)	1.1936	0.0421	**<0.001**
LTMWD	-0.0051	0.0007	**<0.001**
FF	-0.0164	0.2965	0.956
TSLF	0.0040	0.0018	**0.022**
LTMWD:FF	-0.0186	0.0110	**0.091**
PSU-level Species Richness (n=34)			
(Intercept)	2.5850	0.5180	**<0.001**
LTMWD	-0.0145	0.0220	0.510
LTMWD^2	-0.0001	0.0002	0.793
LTMWD_SD	0.2346	0.0912	**0.010**
LTMWD_SD^2	-0.0103	0.0043	**0.017**
FF_Index	-0.2871	0.1786	0.108
(FF_Index)^2	0.0631	0.0221	**0.004**
LTMWD*FF_Index	0.0017	0.0043	0.701
General Linear Model			
Beta Diversity (β) n=34			
(Intercept)	6.8946	3.5605	**0.064**
LTMWD	-0.0266	0.1573	0.867
LTMWD^2	-0.0004	0.0015	0.797
LTMWD_SD	0.2338	0.1585	0.153
FF_Index	-1.6333	1.7732	0.366
FF_Index^2	0.3006	0.2084	0.162
LTMWD:FF_Index	0.0394	0.0347	0.267
LTMWD_SD:FF_Index	-0.0382	0.1269	0.766
PSU-level Species Evenness (n=34)			
(Intercept)	0.9211	0.0347	**<0.001**
LTMWD	-0.0017	0.0006	**0.015**
LTMWD_SD	0.0000	0.0035	0.996
FF_Index	-0.0101	0.0139	0.475
LTMWD:FF_Index	0.0001	0.0005	0.835
LTMWD_SD:FF_Index	-0.0011	0.0028	0.696