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Short-Term Effects of Drying-Rewetting and Long-Term Effects of Nutrient Loading on Periphyton N:P Stoichiometry

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Abstract: Nitrogen (N) and phosphorus (P) concentrations and N:P ratios critically influence periphyton productivity and nutrient cycling in aquatic ecosystems. In coastal wetlands, variations in hydrology and water source (fresh or marine) influence nutrient availability, but short-term effects of drying and rewetting and long-term effects of nutrient exposure on periphyton nutrient retention are uncertain. An outdoor microcosm experiment simulated short-term exposure to variation in drying-rewetting frequency on periphyton mat nutrient retention. A 13-year dataset from freshwater marshes of the Florida Everglades was examined for the effect of long-term proximity to different N and P sources on mat-forming periphyton nutrient standing stocks and stoichiometry. Field sites were selected from one drainage with shorter hydroperiod and higher connectivity to freshwater anthropogenic nutrient supplies (Taylor Slough/Panhandle, TS/Ph) and another drainage with longer hydroperiod and higher connectivity to marine nutrient supplies (Shark River Slough, SRS). Total P, but not total N, increased in periphyton mats exposed to both low and high drying-rewetting frequency with respect to the control mats in our experimental microcosm. In SRS, N:P ratios slightly decreased downstream due to marine nutrient supplies, while TS/Ph increased. Mats exposed to short-term drying-rewetting had higher nutrient retention, similar to nutrient standing stocks from long-term field data. Periphyton mat microbial communities may undergo community shifts upon drying-rewetting and chronic exposure to nutrient loads. Additional work on microbial species composition may further explain how periphyton communities interact with drying-rewetting dynamics to influence nutrient cycling and retention in wetlands.

Keywords: Everglades; hydroperiod; nitrogen (N); periphyton mat; phosphorus (P); stoichiometric ratio (N:P); Shark River Slough (SRS); standing stock; Taylor Slough (TS/Ph); water availability

1. Introduction

Ecosystem structure and functions are partly regulated by nutrient loads and concentrations and stoichiometric ratios; the ratio of nitrogen (N) to phosphorus (P) in supplies relative to biological demand can control the cycling of other elements in aquatic ecosystems [1]. Historically high nutrient pollution due to fertilizer use can lead to eutrophication of P-sensitive freshwater and estuarine ecosystems [2–4], vegetation composition shifts [5], and altered food web dynamics [6,7]. Stoichiometric coupling of N and P in biomass describes how altering the concentrations of one nutrient will affect chemical cycles of the other [8]. Enrichment of both nutrients can create a stronger

synergistic response than enrichment of N or P in freshwater environments, suggesting both are of equal importance [9]. Immobilization of nutrients at water inputs can occur in P-rich systems [10]. Conversely, lower P and thus higher N:P ratio leads to greater algal biomass per unit of P [11–13]. Comprised of algae, cyanobacteria, eubacteria, and other microbes, periphyton communities (synonymous with benthic microbial communities or biofilms) are major contributors to primary productivity and can regulate biogeochemical cycling in aquatic systems [14–18]. The stoichiometry of periphyton communities influences the efficiency of trophic transfer and nutrient dynamics along the food web via secondary producers (i.e., insect larvae, small fish) [19–21]. The productivity and composition of periphyton provide an integrated measure of water depth, flow, and nutrient supply, and are therefore used as indicators of aquatic ecosystem function (e.g., [17,21–23]). However, the mechanisms through which depth and other drivers regulate nutrient dynamics of periphyton subject to drying and wetting in wetlands are poorly known. Thus, it is important to understand how and why stoichiometry of periphyton mat changes over spatio-temporal scales.

In shallow aquatic ecosystems, the productivity, structure and species composition of periphyton communities rapidly respond to hydrological changes through direct effects of water availability and movement as well as indirect effects through mediation of elemental availability and cycling [24,25]. Delivery of limiting nutrients by water flow affects rates of periphyton production and nutrient uptake [26], with cascading influences on ecosystem structure and function, resulting in gradients in resource availability from their source to downstream habitats [27,28]. Taxonomic composition changes in periphyton mats due to nutrient availability and distance from nutrient source, for example agricultural nutrient loads upstream [27]. In karstic (carbonate-rich) wetlands, such as the Everglades and other freshwater wetlands of the Caribbean, periphyton forms thick, calcareous mats [29]. These wetlands are prone to drying, so desiccation-resistant taxa tend to become more abundant in areas experiencing longer dry periods [30]. Specifically, green algae and diatoms tend to be more abundant in P-enriched and deeper-water areas, as compared to filamentous cyanobacteria that dominate in P-deprived and desiccation-prone mats [17,31]. Nutrient enrichment can cause microbial mats to disintegrate [32] and/or shift microbial production from the mat to the water column [33,34]. Conversely, a decrease in rainfall, extending dry periods, can result in increased concentrations of these nutrients into periphyton [35]. Nutrients trapped in desiccated mats are often quickly remineralize into the water column after rewetting [36]. Nutrient retention can therefore be controlled by abiotic influences of drying and inundation, but also through changes in algal community composition and biotic retention [30,36], especially when P supply is limited [37]. In periphyton mats, some species of, e.g., cyanobacteria, can absorb N by fixing N_2 from the atmosphere, introducing N into the water column [38,39]. These mechanisms have led to research into methods of manipulation of periphyton mat systems for water quality improvement [40].

Understanding the hydrologic drivers of nutrient uptake and release by periphyton communities is important for predicting the consequences of water flow and nutrient loading changes driven by hydrologic restoration and/or climate changes in wetlands. This study tested two questions: (1) how are periphyton mat nutrient standing stocks and ratios affected by short-term experimental manipulations of hydroperiod variability and drying? and (2) how is long-term variation in periphyton mat nutrient standing stocks and ratios explained by variation in water availability across spatio-temporal scales? The mechanisms underlying nutrient retention were investigated through an experimental manipulation of water availability; mats subject to less frequent drying were hypothesized to retain more N and P than mats subject to more frequent drying. The long-term variation in mat nutrient standing stocks was studied in a multi-year, seasonal study along nutrient and hydrologic gradients in the Florida Everglades, a globally important shallow wetland, to test the effects of hydroperiod changes and proximity to agricultural or marine supplies of nutrients and their ratios. Hydroperiod (flooding duration) was expected to affect intra-annual variability in periphyton mat nutrient concentrations [36,41]. Spatial variance in long-term datasets was expected to be controlled by connectivity to nutrient sources and the differential exposure of mats to drying

(desiccation) and rewetting (rehydration) [25,27,28]. Understanding these drivers and patterns of stoichiometric variance in periphyton mats can improve ecosystem change models under different scenarios of hydrologic and nutrient restoration in the Everglades and other wetlands.

To simulate the influence of wetting and drying on nutrient dynamics in periphyton mats in the Everglades drainages, a microcosm experiment was created to manipulate inundation frequency and measure nutrient standing stock, thus enabling inferences on retention and release trends. Mats subject to frequent drying and rewetting were expected to retain more N and P than those dried for a long period (Hypothesis 1) [30,41].

To test whether mat nutrient content was affected by source proximity, N, P and N:P were analyzed and compared at each site, while hydroperiod variation was compared between drainages and sites within drainages. These patterns were compared along the long hydroperiod Shark River Slough (SRS) and the short hydroperiod Taylor Slough/Panhandle (TS/Ph) (Figure 1). Periphyton mats at near-canal sites (SRS 1, TS/Ph 1) were hypothesized to have higher nutrient standing stocks due to their proximity to canals near agricultural N and P sources [25,27] (Hypothesis 2). In SRS, sites downstream were expected to have a lower ratio of N:P due to natural tidal and storm-derived P input from the Gulf of Mexico, while N:P is predicted to be higher downstream in TS/Ph as Florida Bay N inputs are higher (Hypothesis 3) [28]. Hydrology was hypothesized to control intra-annual variability in nutrient ratios due to rehydration effects on nutrient fluxes in the mats (Hypothesis 4) [36,41]. Sites in TS/Ph were hypothesized to show similar trends in N and P standing stocks to experimentally manipulated mats undergoing extended dry periods, while SRS sites were expected to have similar trends in N and P standing stocks to mats undergoing frequent drying and rewetting (Hypothesis 5) [36].

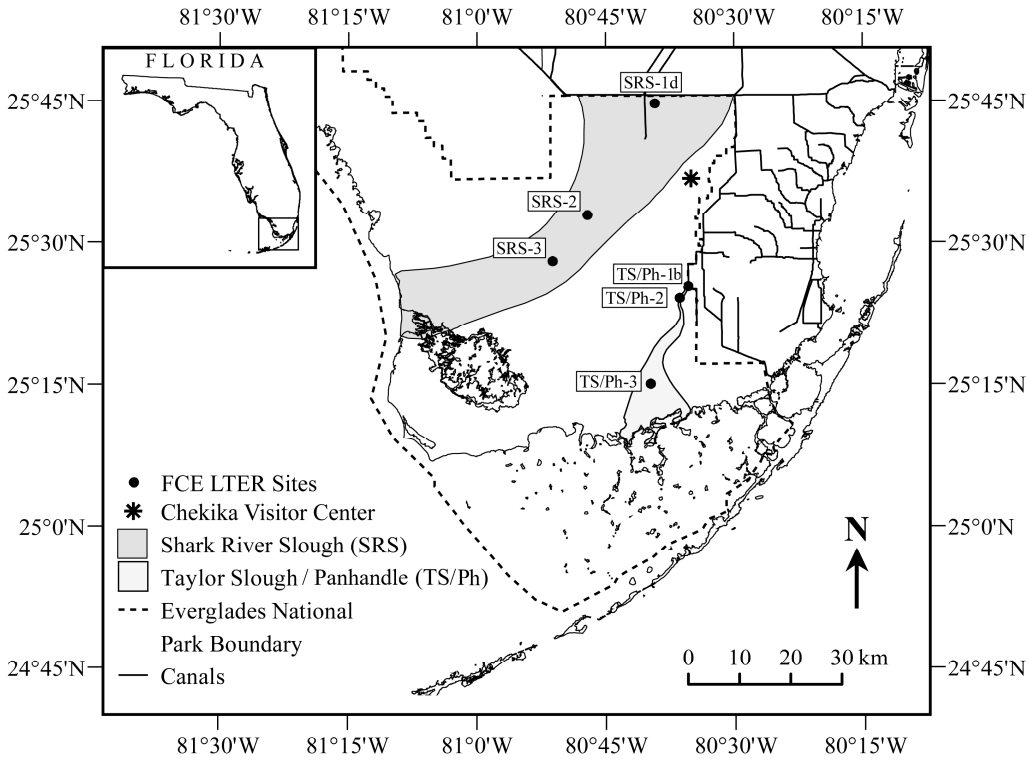

Figure 1. Map of the Florida Coastal Everglades Long Term Ecological Program sampling sites.

2. Materials and Methods

2.1. Study Region

Extremely P-limited wetlands such as the Florida Everglades are ideal ecosystems to test the effects of hydrological changes on periphyton mat stoichiometry. While SRS is an "upside-down"

estuary stretching from the Water Conservation Areas (WCA) north of Everglades National Park (ENP) to the Gulf of Mexico, TS/Ph comprises a series of relatively isolated basins extending east of SRS towards Florida Bay (Figure 1) [28]. Man-made canals and levees have largely disrupted the timing of natural water flow in the marshes [15], but vast restoration efforts are underway. The Comprehensive Everglades Restoration Plan (CERP) aims to address the major impacts of these hydrological diversions and impoundments and to ecologically restore this wetland by, e.g., increasing clean freshwater flow into ENP (evergladesrestoration.gov). To inform such restoration efforts, various authors have been studying the effects of drying frequency on mat primary production [42,43] and on nutrient fluxes in plant and algal communities [29,31,44,45].

2.2. Experiment on the Effects of Dry-Wet Cycles on Mat Nutrient Standing Stocks

2.2.1. Periphyton Mat Collection

About 75 mL of floating mats were collected from three neighboring 1 m^2 plots in the southeastern Everglades near the Chekika Visitor Center (25°36′49.2″ N 80°35′02.9″ W; Figure 1) and randomly assorted into 72 beakers (400 mL) that were kept in cool, dark conditions until the start of the experiment. The mats in this region are representative of the "short-hydroperiod" (6–9 months flooding duration and more than one wet-dry cycle per year) of the upstream areas of the TS/Ph and SRS drainages [25,32]. The area was flooded at the time of sampling. To measure the initial TP and TN in the three mats, six 1 cm^2 cores were removed from randomly selected mats. The cores were placed in pre-weighed, labeled bags, frozen, and analyzed for TP and TN content using the methods described in detail in 'Periphyton mat nutrient content' (the same used in the FCE LTER program). The mat nutrient content was not significantly different among the three plots ($p > 0.05$); thus, in the experiment, each mat from the three plots sampled was a replicate.

2.2.2. Drying/Rewetting Manipulations

All samples were subjected to a 5-day preliminary drying phase to remove excess water, a conditioning phase wherein samples dried with different frequencies, and a final rewetting phase in which samples were kept wet at a total volume of 100 mL. Nutrient content in the deionized water (DIW) used for rewetting the samples was below detection limits. Three replicates were used for: (1) a low variance (LV) treatment, in which the mats were allowed to dry completely before being rewetted; (2) a high variance (HV) treatment, in which mats were dried and rewetted at 5-day intervals; (3) Control samples in which DIW was added every 2–3 days to maintain a total volume of 100 mL (Table 1). Low variance conditions simulate TS/Ph hydrology while HV conditions simulate SRS hydrology. Mat samples were randomly assorted inside a small Plexiglas greenhouse that provided protection from rainfall while allowing sunlight to enter and water vapor to escape. After the HV samples dried completely, all samples were brought to a total volume of 100 mL using DIW.

Table 1. Control, high variance and low variance samples were dried and rewetted as shown. Between 3–9 October, the experiment was suspended for one day, and the samples were moved indoors to protect them from the heavy rainfall and wind caused by Hurricane Matthew.

Treatment	Conditioning Phase							Final Rewetting
	26-Sept	3-Oct	9-Oct	14-Oct	19-Oct	25-Oct	31-Oct	1–25-Nov
Control								
High Variance								
Low Variance				Dry			Wet	

2.2.3. Periphyton Mat Sampling and Processing

During the final two-week rewetting period, six 1 cm^2 cores (subsamples) were collected into a plastic bag daily from each beaker using a coring device, the mass was recorded, and these subsamples

were kept frozen until analysis. The form and color of the mats were recorded for qualitative comparison. To extend the length of the sampling period, beakers were sampled on odd-numbered days. After collection, the subsamples were allowed to thaw in a dark container. In a 250-mL beaker, the subsamples were homogenized in DIW with a hand blender and distributed into a cup for nutrient analyses (120 mL) and an aluminum pan for ash-free dry weight analysis (30 mL).

2.2.4. Periphyton Mat Nutrient Standing Stocks

To measure ash-free dry weight, 40 mL subsamples were dried at 100 °C for three days and weighed before placing into a muffle furnace set at 500 °C for one hour to remove organic content via combustion; the subsamples were weighed and the ash-free dry weight calculated. Then, 120 mL subsamples were dried using the same procedure and then finely ground using a mortar and pestle; 17–20 mg was placed into vials for TP and TN analysis. To measure TP, 0.2 mL of 0.17 M $MgSO_4$ and 1 mL of H_2O were added to each vial, the subsamples were then re-dried in an oven at 70 °C overnight and then placed in a furnace at 500 °C for 4 h, and cooled overnight. To each vial, 5 mL of 0.2 N HCl was added and the subsamples were heated at 80 °C for 30 min; 10 mL of DIW were then added to each subsample, which were vortexed and left to settle overnight. From each subsample, 3 mL were extracted and dispensed into test tubes along with 7 mL of DIW. A standard curve using anhydrous dihydrogen P standard was prepared for 0 µM P, 0.5, 1.0, 10.0, 15.0 and 20.0 µM P concentrations. To each of the subsample, 1 mL of TP reagent (Appendix A) was added, the subsamples were vortexed and covered with aluminum foil for 20–30 min. Total P was then measured using a UV-2101PC Spectrophotometer at 885 nm [46]. To measure TN, 0.020 g of the ground subsamples were weighed into tin containers, sealed and compressed into a sphere and placed into a Thermo Fisher Flash Elemental Analyzer 1112 [47]. For both P and N analyses ground apple leaves, NIST SRM 1515, were used as standard reference samples containing certified N and P mass fraction values [48].

2.3. Long-Term Nutrient Standing Stock Dynamics

To examine the effects of upstream or downstream nutrient loads on periphyton nutrient standing stocks, spatio-temporal trends in the stoichiometry of Everglades periphyton mats, nutrient standing stock patterns were examined using data from the Florida Coastal Everglades Long-Term Ecological Research program (FCE LTER) (Figure 1). The FCE LTER program includes collections of floating or benthic periphyton mats from triplicate 1 m^2 sampling area three times per year in winter (January), spring (March–May), and autumn (September–November) at three sites in SRS and three sites in TS/Ph [49] (Figure 1). A total of 319 and 222 samples were collected from SRS (2003–2016) and TS/Ph (2007–2016), respectively. After removing plant and animal matter from the samples, these were divided into subsamples to measure dry mass by drying to constant mass in a 100 °C oven ($g \cdot m^{-2}$), mat total phosphorus (TP) ($\mu mol \cdot g^{-1}$ dry weight) by colorimetry after dry combustion and total nitrogen (TN) ($\mu mol \cdot g^{-1}$ dry weight) by elemental analysis [50]. Water depth was measured at the site and time of sampling using a metal ruler, as well as via continuous water level recordings from pressure transducers at each location to observe the daily trend at the sites [51,52].

2.4. Data Analyses

Nutrient standing stocks were calculated by multiplying the mat TN and TP concentration ($\mu g/g$) by subsample dry weight (g/m^2) and converting to mat nutrient stock (g/m^2) (Supplementary File S1: LTER). Experimental data was calculated as TP per core of incubated mat (μg/core) (Supplementary File S1: Exp). In the experiment, the dynamics of the nutrients relative to the starting point (no nutrients were added or removed) were of interest, so the expression per core enables standardization of the experimental unit. To describe spatial patterns of nutrient standing stock, N:P mass ratios, and water depth, box-plots, line and area graphs were created. Response ratios for HV and LV treatments were calculated at each day by subtracting TN and TP at the day of collection from each measurement, then dividing by mean TN and TP in the Control samples. Spearman correlation coefficients and

p-values between water, nutrient standing stocks, and ratios were calculated. Linear regressions were conducted using TN standing stock as a response variable and TP an explanatory variable to observe how mats respond to nutrient gradients along SRS and TS/Ph [53]. The mean, standard deviation, and range were calculated to compare nutrient standing stocks between drainages, sites within drainages, experimental treatments, and between treated mats and nutrient content upon mat collection. To identify significant differences in nutrient standing stocks, the One-way ANOVA test was used, followed by the univariate Tukey's honest significant difference (HSD) when variables were normally distributed and had homogeneous variances, or by the Games-Howell test when they were not. Line graphs, correlations, and linear regressions were conducted in Excel® (version 2013); box plots, One-way ANOVA and post-hoc tests were created / conducted in SPSS® (version 25).

3. Results

3.1. Short-Term Drying-Rewetting Microcosm

During and after the final rewetting, Control samples (left in Figure 2b–g) approximately maintained the same size, compared to the preliminary drying phase, but displayed evident discoloration, i.e., they became yellow-brown vs. the initial green-brown (Figure 2a). Samples experiencing frequent wetting and drying (HV) (center in Figure 2b–g) shrank and were yellow-brown to orange at the end of experiment, analogously to the Control samples (Figure 2), while samples undergoing extended drying (LV) lost all coloration and shrank in size (right in Figure 2b–g).

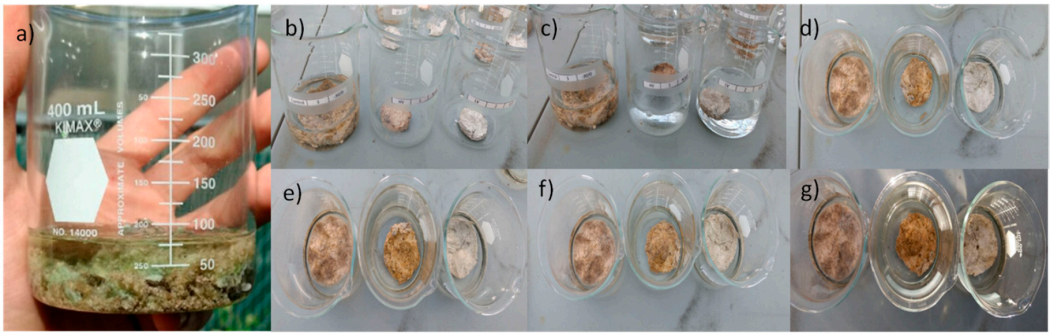

Figure 2. Floating mats in each image, (**b**–**g**), from left: Control, high-variance (HV) drying-rewetting, low-variance (LV) drying-rewetting were subjected to a final 2-week rewetting period following the conditioning phase (Table 1). (**a**) Mat sample, pre-treatment; (**b**) Day 0, immediately before the final inundation period; (**c**) Day 0, immediately after wetting; (**d**) Day 1, approximately 24 h after rewetting, (**e**) Day 5; (**f**) Day 9; (**g**) Day 13.

Total N and TP in treated samples were at least three times greater than pre-treatment mats (Table 2). Among the treated mats, LV samples had the highest mean and range TP and HV had the largest TN (Figure 3a,c; Table 2), whereas Control samples had the lowest mean TN and TP, variability, and range and the largest mean N:P (Figure 3a,c,e; Table 2). Total P did not vary significantly between LV and HV, but was significantly higher in LV and HV than Control samples (Figure 3a). Total N did not show significant variations (Figure 3c). Nitrogen:P in Control samples was significantly higher than in the treated mats (Figure 3e). All treatments showed a strong positive linear relationship between TP and TN (Table 2; Figure 4a–c), with similar slopes between Control and HV (Figure 4a,b) and smaller for LV (Figure 4c). Response ratios of TP for both treatments decreased immediately after rewetting and slightly increased after one week of continuous submersion, with HV maintaining higher TP (Figure 5a). Response ratios for TN decreased more abruptly than that of TP after rewetting and slightly increased after one week with smaller differences between treatments than for TP (Figure 5b). Total N and TP response ratios did not vary significantly between HV and LV, as per One-way ANOVA and Tukey-HSD tests.

Table 2. Means and ranges of mat total phosphorus (TP), total nitrogen (TN), and N:P mass ratio. N:P correlations compared pre-treatment mats to experimentally treated mats after the final inundation period.

Treatment	TP (µg/Core)				TN (µg/Core)				N:P	N:P Correlation	
	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	*r*	*p*
Pre-treatment	29.3	8.3	19.3	42.2	4345	1025	3289	6538	155	0.58	0.10
Control	73.4	29.4	29.7	139.2	11,642	4966	4824	22,034	160	0.85	1.40×10^{-7}
High Variance	109.4	44.8	39.3	251.5	14,073	7173	7958	33,177	129	0.89	7.37×10^{-9}
Low Variance	112.5	56.7	43.7	301.8	12,183	5428	6167	30,983	116	0.84	2.52×10^{-7}

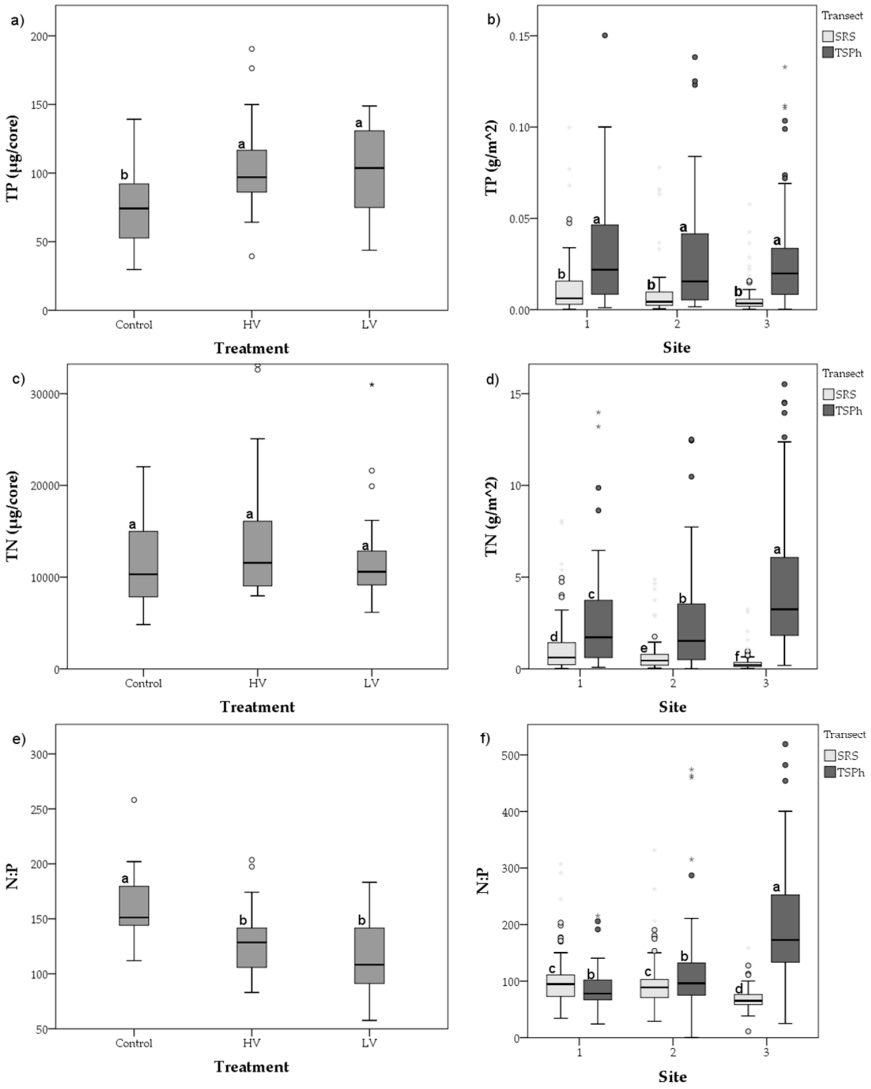

Figure 3. Spatial trends of: (**a**) mean mat total phosphorus (TP, g/m^2) in SRS and TS/Ph; (**b**) mean TP (µg/core) in the experimentally treated mats; (**c**) mean mat total nitrogen (TN, g/m^2) in SRS and TS/Ph; (**d**) mean TN (µg/core) per treatment; (**e**) mean N:P in SRS and TS/Ph; (**f**) mean N:P molar ratio per treatment. Significant differences (Long-term: Games-Howell; Experiment: Tukey's HSD; $p < 0.05$) are indicated by different letters. Site 1 refers to SRS 1d and TS/Ph 1b. Note the different axis units. Site number increases downstream. Site name abbreviations as in Figure 1.

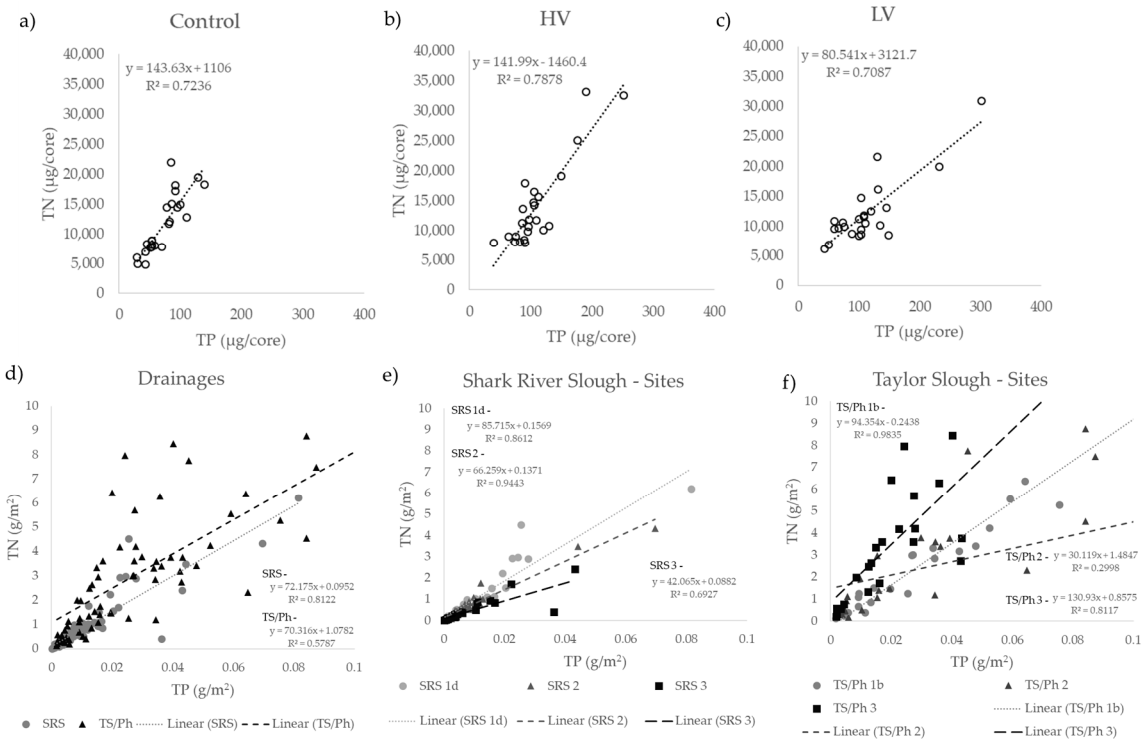

Figure 4. (**a**) Linear regressions of TN vs. TP of long-term data in drainages; linear regressions of TN vs. TP measured in per site ((**b**) SRS; (**c**) TS/Ph); linear regressions of TN vs. TP measured from the experiment ((**d**) Control; (**e**) high variance (HV) drying-rewetting; (**f**) low-variance (LV) drying-rewetting. Abbreviations as in previous figures.

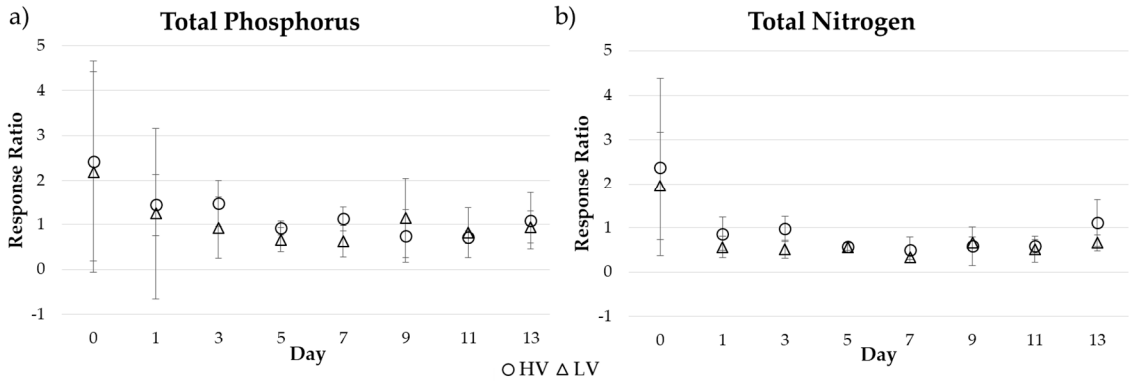

Figure 5. Response ratios for (**a**) TP and (**b**) TN of HV and LV treatments relative to Control mats, using the original nutrient concentrations from the field as a baseline. Ratios were calculated by subtracting concentrations by original field data (pre-treatment), then dividing by the relative daily average in the Control samples. Bars indicate standard deviations (SD). HV and LV did not differ significantly in TP or TN (Tukey HSD; $p < 0.05$). Abbreviations as in previous figures.

3.2. Long-Term Nutrient Standing Stock Dynamics

The mean and range of the standing stocks of TP and TN were higher in TS/Ph than in SRS (Table 3) mainly due to a higher biomass of periphyton in TS/Ph [29]. Mean TP decreased downstream in SRS and TS/Ph (Figure 3b). Total P was not significantly different across sites in each drainage; however, sites near to anthropogenic sources (SRS 1d and TS/Ph 1b) varied significantly from sites near marine sources (SRS 3 and TS/Ph 3) (Figure 3b,d). Total N across all sites were significantly different (Figure 3d). Mean TN decreased downstream in SRS sites and increased towards Florida

Bay in TS/Ph and had high variance (Figure 3d). Nutrient standing stocks were more variable within sites in TS/Ph than in SRS (Figure 3b,d). In SRS, N:P decreased towards the Gulf of Mexico, whereas, in TS/Ph, the ratio increased and became more variable at downstream sites closer to Florida Bay (Figure 3f). Nitrogen:P ratios were not significantly different between upstream sites (SRS 1d,2 and TS/Ph 1b,2), but SRS 3 and TS/Ph 3 had lower and higher N:P, respectively (Figure 3f). In SRS, TP and TN mean, standard deviation, and range were highest in SRS 1 (Table 3). In TS/Ph, site 3 had the highest mean TN and lowest mean TP, while TS/Ph 1b had the highest mean TP; TS/Ph 2 had the lowest mean TN (Table 3). Mean water depth at sample collection dates was highest in SRS 2 and lowest in SRS 3, whereas TS/Ph 2 and 3 were on average deeper than TS/Ph 1b (Figure A1). TN and TP standing stocks were mostly strongly positively correlated at all sites (Table 3) in both drainages, and thus TN was strongly predicted by TP, as per regression analysis (Figure 4d). The slopes of N:P were similar in SRS and TS/Ph, though, with very low TP, TS/Ph mats contained higher TN than SRS (Figure 4d). In SRS, the slope of the N:P linear relationship decreased downstream (Figure 4e); TS/Ph 1b had a higher N:P slope than TS/Ph 2, but this increased dramatically in TS/Ph 3 (Figure 4f).

Water depth seasonally fluctuated in SRS sites, infrequently drying completely, whereas TS/Ph sites completely dried in most years (e.g., Figure 6, most years in TS/Ph 1, 2009 and 2011–2012 in TS/Ph 2). Total P and TN synchronously changed with water depth in SRS in a few cases (Figure 6a–c), while nutrient standing stocks were inconsistently high or low in relation to varying water depth in TS/Ph (Figure 6d–f). For example, in SRS 2 and SRS 3 from 2005–2007, TP and TN peaked as water levels peaked at the end of the wet season (June–November), then stabilized at lower values with the onset of the dry season in spring (Figure 6b,c). Mean seasonal water depth (at collection sites) and nutrient ratios were not significantly correlated (Table 4).

Table 3. Means, standard deviations (SD), and ranges of mat TP and TN in g/m^2, and the mean mass ratio and correlation coefficient (r) and its significance (p) of their standing stocks (N:P) collectively as a drainage and at each site in Shark River Slough (SRS) and Taylor Slough (TS/Ph) from 2003–2016. Abbreviations as in Table 2.

Drainage/Site	TP (g/m^2)			TN (g/m^2)			N:P			N:P Correlation	
	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	r	p
SRS	0.009	0.014	0.000–0.100	0.78	1.18	0.01–8.08	90.7	42.0	11.1–331.7	0.901	1.23×10^{-36}
1d	0.012	0.016	0.000–0.100	1.20	1.61	0.01–8.08	103.1	46.9	34.3–306.9	0.928	5.79×10^{-14}
2	0.008	0.013	0.000–0.078	0.68	0.89	0.04–4.88	94.4	42.8	28.9–331.7	0.972	3.66×10^{-24}
3	0.006	0.010	0.000–0.058	0.40	0.59	0.02–3.24	68.9	20.6	11.2–158.7	0.832	2.17×10^{-08}
TS/Ph	0.033	0.050	0.000–0.496	3.39	4.14	0.00–40.07	145.3	147.0	0.0–1731.7	0.761	5.78×10^{-15}
1b	0.036	0.051	0.001–0.343	3.14	5.32	0.08–40.07	87.5	34.7	24.1–215.7	0.992	3.31×10^{-20}
2	0.035	0.063	0.002–0.496	2.56	2.74	0.00–12.50	118.2	89.7	0.0–474.2	0.548	4.61×10^{-3}
3	0.027	0.029	0.000–0.133	4.44	3.89	0.19–15.52	226.2	210.4	24.9–1731.7	0.901	8.30×10^{-10}

Table 4. Results of correlation analysis of periphyton mat standing stock mass N:P and water depth (cm) in SRS and TS/Ph within seasons—autumn (September–November), winter (December–February), and spring (March–May)—and within hydrological seasons—dry (December–May) and wet (June–November)—from 2003–2016. Significance calculated at or above weak correlation ($r \geq 0.30$ or $r \leq -0.30$). Abbreviations as in previous tables.

Drainage/Site	Season						Hydrological Season			
	Autumn		Winter		Spring		Dry		Wet	
	r	p	r	p	r	p	r	p	r	p
SRS										
1d	0.13		−0.30	0.47	−0.71	0.07	−0.32	0.36	−0.17	
2	0.25		−0.60	0.12	−0.71	0.05	−0.35	0.29	−0.35	0.50
3	0.19		0.38	0.45	N/A		0.03		0.03	
TS/Ph										
1b	−0.29		N/A		N/A		N/A		−0.16	
2	−0.20		−0.69	0.09	N/A		−0.69	0.09	−0.69	0.53
3	0.49	0.18	−0.07		N/A		−0.07		−0.07	

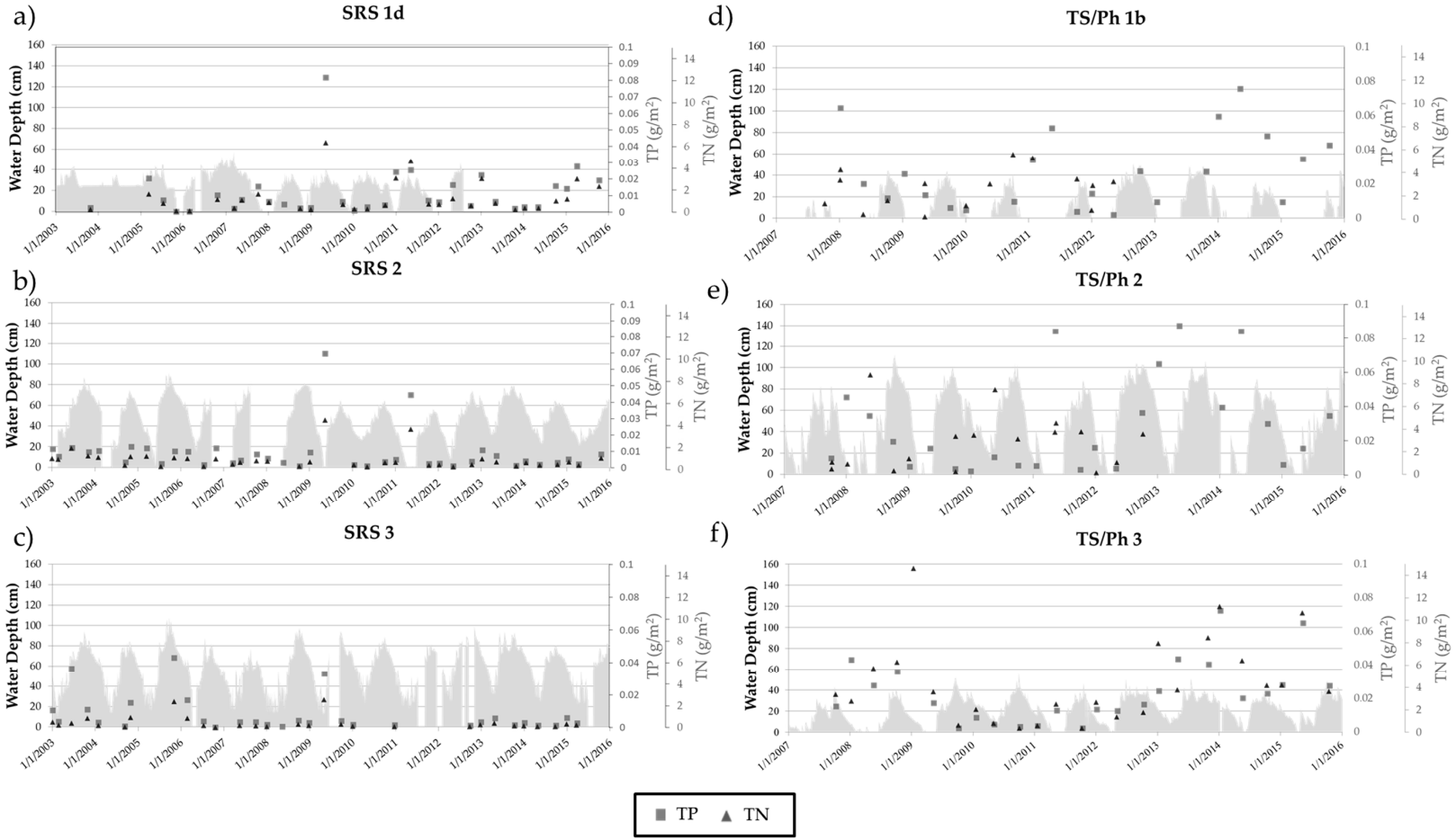

Figure 6. (**a**–**f**) Temporal trends in daily water depth measured from dataloggers (shaded region in figures) and periphyton mat TP and TN standing stock (three times per year) in SRS (2003–2016) and TS/Ph (2007–2016). Abrupt changes in water depth to 0 cm indicate missing data. Nutrients show synchronous change with one another, but show few patterns with water depth. Abbreviations as in previous figures.

4. Discussion

4.1. Short-Term Drying-Rewetting Effects on Periphyton Nutrient Dynamics

Nutrient content in Control samples was about three times greater than the original mats upon collection (pre-treatment; Table 2), likely due to preliminary drying to eliminate excess water. The wider range and increase in TN in HV and LV mats as compared pre-treatment mats may suggest N fixation from the atmosphere may be substantial in drought-stressed mats becoming wet, while Liao et al. [39] suggests rates are highest during early wet season.

The color of the mats during the conditioning phase showed a loss in green pigment of all mats possibly due to dormant or dead algal cells [41], chlorophyll degradation from high temperatures [54] or increased acidity [55] as well as to species changes in the mats, for example increased abundances of cyanobacteria that produce the yellow-brown pigment scytonemin [31,56,57] (Figure 2). Low variance samples were completely desiccated and became white, and mats shrank; HV samples also showed compaction, but in contrast retained a yellow-brown pigment at the end of the experiment. Control samples were unchanged in size, but also became yellow-brown. Mats exposed to frequent drying and rewetting (HV) were hypothesized to retain more nutrients than constantly dry (LV) mats (Hypothesis 1). However, this hypothesis is rejected as mean TN was not statistically significantly different between HV, LV and Control samples and TP was significantly higher and more variable in LV and HV than Control samples, but treatments were not different between them (Figure 3a; Table 2). Nutrient content was higher in treated mats, possibly a result of shrinkage of the mat, compaction of nutrient molecules, and a shift in composition that provided resistance to these conditions. Dried mats likely have a larger abundance of cyanobacteria than those that retained more moisture [17,31], which can be separately assessed with samples that were preserved for such potential microscope analysis. Response ratios showed how mats subject to different drying and rewetting frequencies initially lost N and P and then regained some of it, perhaps due algal and bacteria species composition changes with more abrupt N loss. The averages of response ratios in treated mats were not significantly different. Nutrient standing stocks tended to first decrease and then increase after approximately one week (Figure 5a,b) similar to the experimental results of Thomas et al. [41]. The linear regression slopes show that HV samples were more similar in N-P coupling, here as the rate and strength of the regression in which P predicts N, to Control than LV, which absorbed less TN per TP (Figure 4a–c). This suggests that, though constantly dry mats were able to withstand desiccation, possibly reflecting a shift in taxonomic composition (to cyanobacteria), they retain less TN per TP than mats exposed to dry and wet periods (Figure 4b,c), and strong N:P correlations (Table 2) are consistent in nutrient uptake and release for both, as was observed, for example, in an experiment in the Danube River [36]. As N-P coupling differed for both treatments, but was similar in average N:P, it can be suggested that the alternating dry/wet mats adapted to their condition, which may cause an increase in absorption/desorption due to lack of consistent nutrient cycling, but overall stabilizes to an average N:P. Average TP and TN response ratios were not significantly different between treated samples, though HV mats consistently had slightly higher TP response.

4.2. Long-Term Periphyton Nutrient Dynamics across Space

Periphyton standing stocks near canals (SRS 1d and TS/Ph 1b; Figure 1) were expected to have higher nutrient standing stocks due to proximity to anthropogenic inputs (Hypothesis 2). The data did show that mean TP and median TP and TN decreased from SRS 1 to SRS 3 (Figure 3a,c; Table 3), comparable to Rudnick et al. [58] and Gaiser et al. [29] observing a TP increase and a TN decrease downstream in SRS. In more upstream areas of the Everglades, McCormick et al. [27] and Gaiser et al. [29] also found TP to decrease downstream of canals. According to Wozniak et al. [59], N should be rather stable in the interior marshes and decrease towards the estuaries via denitrification; in this study, mean TN was shown to decrease downstream in SRS and become more stable (Figure 3d). In upstream sites, nutrient standing stocks were higher (Figure 3b,d), as expected, considering the

historically high P loading in the WCAs [37] and the fact that canalization reduced water flow [28] and depth in SRS ([60], unpublished) (Hypothesis 2). In TS/Ph, mean TP and TN were significantly higher than SRS. In upstream sites of TS/Ph, TP and TN were higher than downstream, likely due to anthropogenic nutrient loads from upstream, as per Hypothesis 2. However, TN increased downstream in TS/Ph (Figure 3b) with consequent increase in N:P (Figure 3f; Table 3), as expected (Hypothesis 3), consistent with findings by Iwaniec et al. [25], but in contrast with results in Rudnick et al. [58]. This trend may be due to the more limited marine P supply from Florida Bay and/or higher N supplies, as Figure 3d suggests, perhaps from agricultural activities, into the lower part of TS/Ph [28,61]. In SRS, mean TP and TN decreased downstream, decreasing N:P, as expected due to TN decreasing more than TP (Figure 3b,d,f; Table 3) (Hypothesis 3). Nitrogen-P coupling was evident in the field data; mat TN was significantly correlated with and predicted by mat TP in both drainages (Table 3; Figure 4d–f). In SRS and TS/Ph, TN increased with TP at a similar rate (Figure 4d). At low nutrient standing stocks, TS/Ph had higher TN, but the slope of the N:P regression was slightly higher in SRS. In SRS, the N:P slope decreased downstream (Figure 4e), suggesting increasing N uptake from the substrates towards anthropogenic sites. In TS/Ph 3 the N:P slope dramatically increased, as compared to upstream sites (Figure 4f). The Homestead agricultural sources and isolated basins of TS/Ph likely cause this higher N uptake from the landscape [28].

4.3. Long-Term Periphyton Nutrient Dynamics across Time

Contrary to what was expected (Hypothesis 4), there was no significant correlation between N:P and water depth in either drainage (Table 4). Temporal trends rarely showed the mats taking up nutrients during the wet period in either drainage (Figure 6). Nutrient standing stocks in SRS showed very small fluctuations with a few synchronous changes, such as increasing and stabilizing over the wet periods of 2004–2006, while water depth and nutrient standing stocks showed peaks in both low and high-water phases in TS/Ph (Figure 6a–c). Historically, samples were not collected immediately before or after the lowest depth (SRS) or total dry-down (TS/Ph). Therefore, it is recommended that samples for future analyses be collected before total dry-down and after the end of the dry season to better test the effects of drying and rewetting on nutrient standing stocks and ratios.

4.4. Short- and Long-Term Drivers of Periphyton Nutrient Retention

Regression analysis showed that the slopes of the linear relationships show a greater change in TN per TP in mats simulating SRS (HV) and Control mats than in mats simulating TS/Ph (LV). Control samples and pre-treatment mats were similar in N:P, while LV and HV both had lower N:P; experimental dry conditions (LV and HV) have led to an increased nutrient retention, especially of P (Figure 3a; Table 2). Low variance samples were hypothesized to have similar N:P uptake as mats in TS/Ph, while mats exposed to drying and rewetting (HV) would have a different rate of TN movement to TP than SRS. Because the slopes of the linear regressions by drainage were similar and that of the experimental treatments were not, Hypothesis 5 must be rejected. The nature of the experiment being a closed system is limiting in substituting natural factors that affect biogeochemical activity, such as water flow and nutrient input and output. Therefore, future experimental efforts need to refine ways of mimicking natural mechanisms that affect mats within the laboratory. The periphyton mats may demonstrate sensitivity to these drying scenarios, emblematic of climate change, and might change in nutrient dynamics to respond to hydrological variances [35].

5. Conclusions

The spatial and temporal patterns and drivers of nutrient standing stocks and retention in periphyton mats were tested in a globally important subtropical wetland. A larger input of natural P from marine sources enters SRS than anthropogenic sources according to periphyton mat nutrient stocks, suggesting progress is being made for efforts to reduce agricultural pollution. Increases in water discharge in long-hydroperiod marshes may lead to greater retention on mat TP via P loading and

overall decreases in mat N:P, showing that increased wetting in wetlands may lead to consequences in nutrient dynamics downstream. Mats used in the experiment maintained a similarly strong N-P coupling despite different hydrological conditions, showing predictability remains the same for different drying durations. These findings can be used to inform further experimental and modeling efforts to understand and predict the effects of water availability on nutrient fluxes in periphyton mats in this and other ecosystems. Ongoing restoration activities aimed to increase freshwater flow to both drainages may result in larger intra-annual mat nutrient load changes and stronger N-P coupling, with likely consequences for other biota depending on algae and other organisms constituting periphyton mats.

Supplementary Materials: Data from FCE LTER sites are available online at http://fcelter.fiu.edu/data/. Resulting nutrient standing stocks for field and experimental data are included as a supplementary file S1: LTER and S1: Exp, respectively). The following materials are available online at http://www.mdpi.com/2073-4441/10/2/105/s1

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Author Contributions: E.E.G., A.D.S., L.M., and J.S.K. conceived and designed the experiment; A.D.S. and M.M.F. collected and analyzed the samples and the data; A.D.S. and L.M. wrote the manuscript and M.M.F. contributed on Materials and Methods; E.E.G., and J.S.K. contributed on the interpretation of the results and with suggestions in all the project phases.

Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Anhydrous phosphorus dihydrogen standard: A sulfuric acid solution was prepared by mixing 62 mL of concentrated H_2SO_4 in 400 mL of DI water in a 1 L glass bottle. In a separate bottle 136.1 mg anhydrous potassium dihydrogen phosphate, 1000 mL DI water, and 0.2 mL of sulfuric acid solution were mixed and cooled. *TP Reagent*: The TP reagent was made by mixing 37.5 mL of 0.0243 M ammonium molybdate solution, 93.0 mL of 2.85 M sulfuric acid solution, 37.5 mL of 0.307 M ascorbic acid solution, and 18.75 mL of 0.00407 M potassium antimonyl tartrate solution in a 250 mL beaker.

Appendix B

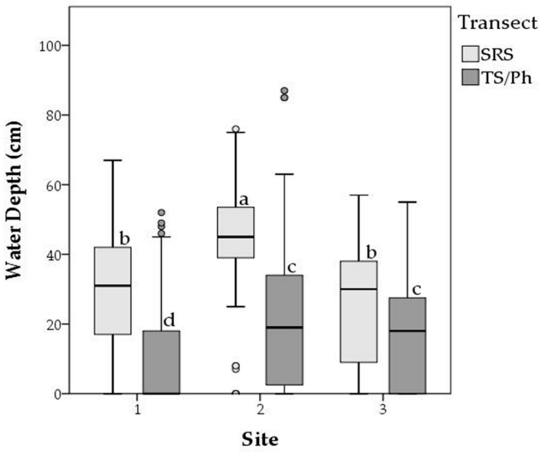

Figure A1. Water depth (cm) along SRS and TS/Ph measured when collecting samples. Significant differences (Games-Howell; $p < 0.05$) are indicated by different letters.

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