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Functional and Compositional Responses of Periphyton Mats to Simulated Saltwater Intrusion in the Southern Everglades

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Abstract

Periphyton plays key ecological roles in karstic, freshwater wetlands and is extremely sensitive to environmental change making it a powerful tool to detect saltwater intrusion into these vulnerable and valuable ecosystems. We conducted field mesocosm experiments in the Florida Everglades, USA to test the effects of saltwater intrusion on periphyton metabolism, nutrient content, and diatom species composition, and how these responses differ between mats from a freshwater versus a brackish marsh. Pulsed saltwater intrusion was simulated by dosing treatment chambers monthly with a brine solution for 15 months; control chambers were simultaneously dosed with site water. Periphyton from the freshwater marsh responded to a 1-ppt increase in surface water salinity with reduced productivity and decreased concentrations of total carbon, nitrogen, and phosphorus. These functional responses were accompanied by significant shifts in periphytic diatom assemblages. Periphyton mats at the brackish marsh were more functionally resilient to the saltwater treatment (~ 2 ppt above ambient), but nonetheless experienced significant shifts in diatom composition. These findings suggest that freshwater periphyton is negatively affected by small, short-term increases in salinity and that periphytic diatom assemblages, particularly at the brackish marsh, are a better metric of salinity increases compared with periphyton functional metrics due to functional redundancy. This research provides new and valuable information regarding periphyton dynamics in response to changing water sources in the southern Everglades that will allow us to extend the use of periphyton, and their diatom assemblages, as tools for environmental assessments related to saltwater intrusion.

Introduction

Calcareous periphyton mats are an important and abundant component of the karst, freshwater wetlands found along the coastlines of the Caribbean Basin. In these landscapes, periphyton forms thick, dense mats several centimeters deep that blanket the substrate and marsh vegetation. These mats provide a unique microhabitat for a variety of autotrophic and heterotrophic organisms and play an important role in food web dynamics, organic and inorganic carbon cycling, nutrient cycling, and marl soil (calcium carbonate mud) formation (Rejmánková and Komárková 2000; Hagerthey et al. 2011; Gaiser et al. 2011; La Hée and Gaiser 2012; Schedlbauer et al. 2012; Troxler et al. 2013; Trexler et al. 2015). Furthermore, periphyton is an excellent bioindicator and is often used in ecological assessments of the changing environmental conditions in coastal wetlands, including water quality degradation and hydrologic modification (McCormick and Stevenson 1998; Gaiser 2009). Coastal, freshwater wetlands face the added pressure of saltwater intrusion as sea levels continue to rise and altered hydrologic regimes reduce freshwater inputs to downstream wetlands (White and Kaplan 2017). Even though periphyton communities can serve as excellent bioindicators of coastal change, little is known about their use in detecting novel rates of marine water intrusion into

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coastal, freshwater wetlands. In order to determine the utility of periphyton as an indicator of marine water intrusion, we need to understand how exposure to saltwater influences periphyton productivity, nutrient uptake, and species composition over short- and long-time scales.

Saltwater intrusion into coastal freshwater wetlands is largely driven by climate change and can be exacerbated by altered natural freshwater flows in ways that lessen inputs to coastal marshes and reduce groundwater and aquifer recharge (Briceño et al. 2014; Herbert et al. 2015). Reduced freshwater flow into downstream marshes combined with sea level rise allows saltwater to intrude further inland, particularly during the dry season (December–May), exposing freshwater wetlands to elevated salinity and altered ionic concentrations (Dessu et al. 2018). For example, Saha et al. (2012) reported increased mean surface water salinity from 2 psu at the end of the wet season (November) to 14 psu at the end of the dry season (May) at brackish site in the southwestern Everglades; at the same location, Saha et al. (2011) documented mean groundwater salinity increases from 2 psu in 2003 to 12 psu in 2009. The seasonal and long-term changes in natural salinity gradients caused by saltwater intrusion in the Everglades and other coastal, freshwater wetlands affects the ecological functioning and community structure of key ecosystem components, including vegetation, soil microbes, and periphyton (Ikenaga et al. 2010: Krauss et al. 2011: Troxler et al. 2014; Mazzei and Gaiser 2018).

Periphyton is exceptionally sensitive to environmental change and responds with shifts in species composition that in turn affect the physical structure and ecological roles of these mats (Gaiser 2009; Gaiser et al. 2011; Hagerthey et al. 2011). Calcareous periphyton mats are characterized as microbial assemblages of diatoms, cyanobacteria, chlorophytes, and other microorganisms surrounded by a matrix of mucilage and interstitial calcium carbonate secreted by certain resident algal species (Azim and Asaeda 2005; Hagerthey et al. 2011). In the Florida Everglades, periphyton has been shown to respond more quickly and predictably to changes in hydrology and water quality than other wetland plant communities because of the environmental specificity and short generation times of resident taxa (McCormick and Stevenson 1998; Gaiser et al. 2006; Gaiser 2009; Wachnicka et al. 2010; Gaiser et al. 2011; Lee et al. 2013). Diatoms are particularly sensitive to changing environmental conditions and are commonly used as bioindicators in water quality assessments (Stevenson et al. 1999; Potapova and Charles 2007; Desrosiers et al. 2013; Stevenson 2014). Diatoms are also relatively easy to identify compared with cyanobacteria and chlorophytes because of their species-specific cell wall ornamentation which can be discerned under light microscopy. Furthermore, many periphytic diatoms contribute to mat formation and cohesion through the production of extracellular mucilage and attachment structures (Johnson et al. 1997; Azim and Asaeda 2005;

Gaiser et al. 2010). Therefore, shifts in the composition and relative abundances of diatoms, and other mat-engineering species (e.g., cyanobacteria), will result in different periphyton mat types, such as non-calcareous biofilms or filamentous mats, that may not provide the same ecological services as the native calcareous mats found in karstic, freshwater wetlands (Vymazal and Richardson 1995; McCormick and O'Dell 1996; Pan et al. 2000; Rejmánková and Komárková 2005; Gaiser et al. 2006; Hagerthey et al. 2011; Gaiser et al. 2011).

The response of calcareous periphyton to changes in nutrient availability, particularly phosphorus, and modified hydroperiod has been well documented in the Florida Everglades (Iwaniec et al. 2006; Gaiser et al. 2006; Gaiser 2009; Sola et al. 2018); however, not much is known about how these mats respond to the increased salinity and changes in water chemistry caused by saltwater intrusion. Exposure of aquatic plants and algae to elevated salinity can lead to osmotic stress, ion toxicity, and nutrient uptake limitations (Alam 1999; Mendelssohn et al. 2006; Touchette 2007). Osmotic stress causes loss of turgor pressure and water deficits in plant and algal cells due to diffusion of water out of cells during hyperosmotic conditions that can eventually result in cell death (Hasegawa et al. 2000). Furthermore, exposure to saltwater disrupts cellular ion homeostasis. The accumulation of sodium ions has been shown to decrease chlorophyll content, photosynthetic electron transport activities, and the uptake of essential nutrients in non-salt-adapted autotrophs (Lu and Vonshak 2002; Sudhir and Murthy 2004; Hu and Schmidhalter 2005). Therefore, the physiological stressors imposed by saltwater intrusion on the periphytic algal communities native to freshwater, karstic wetlands have the potential to alter their productivity, nutrient uptake capacity, and species composition as freshwater populations decline in response to salt stress, and salt-tolerant species from adjacent coastal waters, that do not form calcareous, cohesive mats, gain a competitive advantage.

Diatom assemblage turnover along natural salinity gradients has been well documented in Caribbean karstic wetlands (Gaiser et al. 2005; Wachnicka et al. 2010; La Hée and Gaiser 2012; Nodine and Gaiser 2014; Mazzei and Gaiser 2018); however, the effect of elevated salinity on periphyton functional processes and species composition has not been explicitly tested. In this study, in situ mesocosm experiments were used to test the effects of exposure to pulses of saltwater on periphyton metabolism (net ecosystem productivity, ecosystem respiration, and gross primary productivity), nutrient content (total carbon, nitrogen, and phosphorus), and diatom species composition in a freshwater (FW) and brackish (BW) marsh. We hypothesized that exposure to artificial seawater would reduce periphyton productivity, total carbon content, and nutrient uptake due to the osmotic and ionic stresses caused by elevating salinity above ambient levels. We

expected that these changes would be accompanied by shifts in periphytic diatom assemblages that could be used to develop indicators of elevated salinity in fresh and brackish marshes of the southern Everglades. We also predicted that functional and compositional responses to simulated saltwater intrusion would be stronger in FW periphyton compared with BW mats, which are naturally exposed to fluctuating salinity because of their proximity to the coast.

Methods

An in situ salinity addition experiment simulating pulsed saltwater intrusion was conducted in a freshwater (25 26′ 6.11″ N, 80 46′ 50.78″ W) and brackish (25 13′ 13.38″ N, 80 50′ 36.66″ W) marsh in Everglades National Park, Florida, USA (Fig. 1). The FW site was located 32 km from the coast and was characterized by long hydroperiod (inundated year-round), the

presence of floating, epiphytic, and benthic calcareous periphyton mats, and dominated by *Eleocharis cellulosa* and *Cladium jamaiscense* macrophytes. Periphyton was absent in areas of high macrophyte density or during periods of extreme high-water levels. The BW site was located approximately 4.5 km from the coast and experienced seasonal dry-downs (~ 4 months/year) during which the benthos was exposed to the atmosphere. This site was characterized by non-calcareous benthic periphyton (i.e., biofilms and filamentous mats) and was dominated by *C. jamaicense* with some interspersed dwarf *Rhizophora mangle* and *Conocarpus erectus*. Periphyton was often absent or too desiccated to sample dry-down months at the BW marsh.

Boardwalks were constructed at both locations to avoid any disturbance caused by stepping on the soils during the installation and sampling of treatment chambers. At both locations, we inserted 12 clear, circular (1.4-m diameter, 0.6-m height) polycarbonate chambers 30 cm into the soil around the
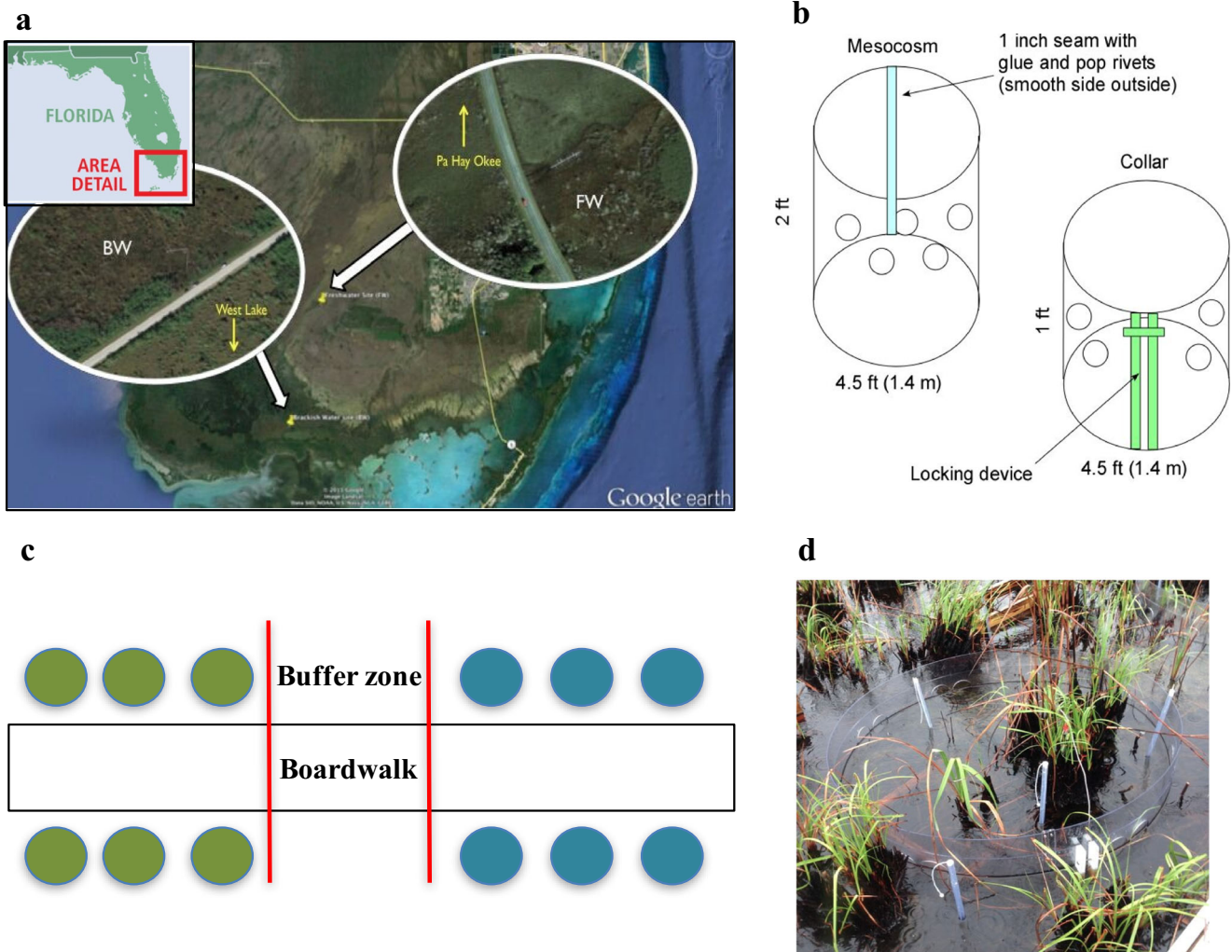
Fig. 1 Location of freshwater (FW) and brackish (BW) marsh sites within the park (**a**). Experimental mesocosm chamber design and dimensions (**b**). Diagram of the experimental design layout; blue circles represent control (+AMB) chambers and green circles represent the salinity treatment (+SAL) chambers (**c**). Image of an experimental chamber at the BW site (**d**)

boardwalks to enclose plots of marsh for the experimental treatments. The chambers had a series of 10-cm diameter holes around their perimeter and were equipped with a movable collar that allowed the chambers to be closed during treatment applications, to ensure the dosing water remained within the chamber, and opened the following day to allow natural exchange with the surrounding environment and minimize enclosure artifacts. Six of the twelve chambers were assigned the elevated salinity treatment while the remaining six were designated as controls; a buffer area was established between the control and salinity treatment chambers to avoid contamination of control plots. The experiment lasted 15 months from October 2014 to December 2015. During this time, salinity treatment (+SAL) chambers were dosed once a month with a known volume of brine prepared using source water and Instant Ocean© sea salt mix to attain surface and porewater salinities approximately double the ambient conditions at each marsh (Stachelek et al. 2018). The control (+AMB) chambers were dosed at the same time using site water alone to account for the effects elevating water levels in the treatment chambers when dosing. The dosing solution was applied by pumping dosing water from tubs (one with source water for the +AMB chambers and one with the salt-water mixture of the +SAL chambers) through a hose equipped with a gentle rain-style extension spray wand. The day after dosing, surface and porewater salinity was measured in each chamber (YSI Model 600 XL, Yellow Springs, OH) and water samples were collected for chemical analysis (see Wilson et al., in review for full water chemistries). Porewater salinity was measured from water samples extracted from three sampling wells (i.e., porewater sippers) placed randomly inside each chamber to a depth of 15 cm. Two porewater sippers were installed 0.5 m outside the edge of each chamber to monitor any potential leakage of saltwater.

Periphyton samples were collected monthly for metabolism, nutrient content, and diatom species composition measurements except when mats were not present (e.g., BW dry-down events or extreme high-water levels at the FW site). When periphyton was present, two 10-mL periphyton samples were collected from each chamber at random for in situ biological oxygen demand (BOD) measurements using a 7.1-cm diameter plastic syringe. One 10-mL sample was placed in a 300-mL clear bottle to measure periphyton net ecosystem productivity (NEP), and the other was placed in a 300-mL dark bottle to measure periphyton ecosystem respiration (ER) as the change in dissolved oxygen (DO) over time using a YSI ProODO Dissolved Oxygen Meter (YSI, Yellow Springs, OH, USA). The paired clear and dark BOD bottles for each chamber were filled with site water and initial oxygen concentrations were measured before capping the bottles and submerging them in the marsh water where they incubated for ~2 h under ambient conditions. After the incubation period, final oxygen concentrations were measured and the periphyton

from the light bottles was transported back to the lab for further analyses. NEP and ER were standardized to the ash-free dry mass (AFDM) of the incubated sample. Oxygen production and consumption rates were converted to carbon production and consumption (mg C g^{-1} AFDM h^{-1}) by multiplying NEP and ER by the mass ratio of C:O_2 (0.357) and then dividing NEP by a photosynthetic quotient of 1.2. and ER by a respiratory quotient of 1 (Knapp et al. 2007). Gross primary production (GPP) was then calculated by adding the absolute value of the ER value to NEP. During months when periphyton biomass was too low for BOD incubations, we collected any periphyton material present in the mesocosm chambers for nutrient analyses and examination of diatom species composition.

In the lab, samples were placed in a 500-mL beaker and homogenized with deionized water (DI) water to create a periphyton slurry with a minimum volume of 200 mL that could be subsampled for various analyses. The beaker was placed on a stir plate to facilitate continuous mixing of the slurry which was subsampled for AFDM, total carbon (TC), total nitrogen (TN), total phosphorus (TP), and diatom species composition. The AFDM subsample was placed in a drying oven at 80 °C for ≥ 48 h to obtain a dry mass (g) measurement and then in a furnace where it was combusted at 500 °C for 1 h to obtain the ash (mineral) mass (g). We calculated AFDM by the loss-on-ignition method as the difference between the ash mass and the total dry mass. The organic content of the periphyton biomass was calculated as the % AFDM of the total dry mass and was used to later determine the organic and inorganic fractions of the TC. The TP subsample was dried at 80 °C and ground down to a fine powder with a mortar and pestle. Colorimetric analysis was used to estimate TP concentrations in the periphyton subsamples, expressed as micrograms per gram of AFDM, following the methods of Solorzano and Sharp (1980). Total C and TN subsamples were ground by mortar and pestle, dried at 60 °C, and analyzed for TC and TN concentrations using a Flash 1112 elemental analyzer (CE Elantech, Lakewood, NJ, USA) following standard procedures. The organic and inorganic fractions of TC were determined by calculating the % AFDM of the TC to get the organic fraction and then subtracting the organic fraction from the TC value to calculate the inorganic fraction.

The subsamples for diatom taxonomic analysis were cleaned of mineral debris and organic matter using the sulfuric acid oxidation methods described in Hasle and Fryxell (1970). We pipetted a known volume of cleaned diatom sample onto a glass coverslip, permanently mounted it on a microscope slide with Naphrax mounting medium (PhycoTech Inc., St. Joseph, MI, USA) and viewed the diatoms under a compound light microscope (Zeiss Axioskop 2) equipped with differential interference contrast and a Leica DFC425 digital camera. We counted at least 500 valves along random transects at ×600 magnification under oil immersion and identified them to the

species level. Raw diatom counts were converted to relative abundance by standardizing the number of valves counted for each species by the total number of valves counted.

Differences in surface and porewater salinity between +AMB and +SAL treatments and the effects of simulated pulses of saltwater intrusion on periphyton functional attributes were evaluated using analysis of variance (ANOVA) to test for treatment effects at the FW and BW sites separately. One-factor MANOVAs were used to evaluate treatment effects on periphyton metabolism (dependent variables: NEP, GPP, and ER) for each sampling date and across all sampling dates to test for mean effects over the entire study period. The same was done with TC, TN, TP, and chl-a as the dependent variables to test for saltwater exposure effects on periphyton nutrient content and algal biomass on each individual date and over the entire study period. Nutrient ratios (TC:TN, TC:TP, and TN:TP) were also analyzed in this manner. These analyses were conducted in SPSS (IBM SPSS Statistics V23.0).

The effect of elevated salinity on periphytic diatom assemblages was examined on months when data were available for both the FW and BW sites (Oct 2014, Nov 2014, Jun 2015, and Oct 2015). Species present in < 3 samples were removed from the dataset and relative abundances were arcsine-square root transformed. We used the PERMANOVA+ add-on package in PRIMER (v6, PRIMER-E Ltd.) to analyze the multivariate response of diatom species composition to elevated salinity using a 3-factor design (2 treatment levels (+AMB, +SAL); 2 site levels (FW, BW); and 4 month levels). Pairwise tests were conducted on the treatment × site × month interaction term to identify significant treatment effects during each of the four sampling months at each site. Nonmetric multidimensional scaling (NMDS) was used to illustrate Bray-Curtis compositional dissimilarity in ordination space. Using R (R Core Team 2017 version 3.3.3), we ran a one-way analysis of similarity (anosim{vegan}) on FW and BW species datasets separately to determine if significant compositional dissimilarity between +AMB and +SAL plots existed when the four sampling dates were considered together. Species driving compositional dissimilarity between +AMB and +SAL treatment chambers at the FW and BW sites were determined using the multilevel pattern analysis available in the indicspecies package (multipatt {indicspecies}) in R (De Cáceres and Legendre 2009).

Results

Addition of the Instant Ocean© brine increased surface and porewater salinities in the treatment plots at both the FW and BW sites (Fig. 2). Mean surface water salinity at the FW site
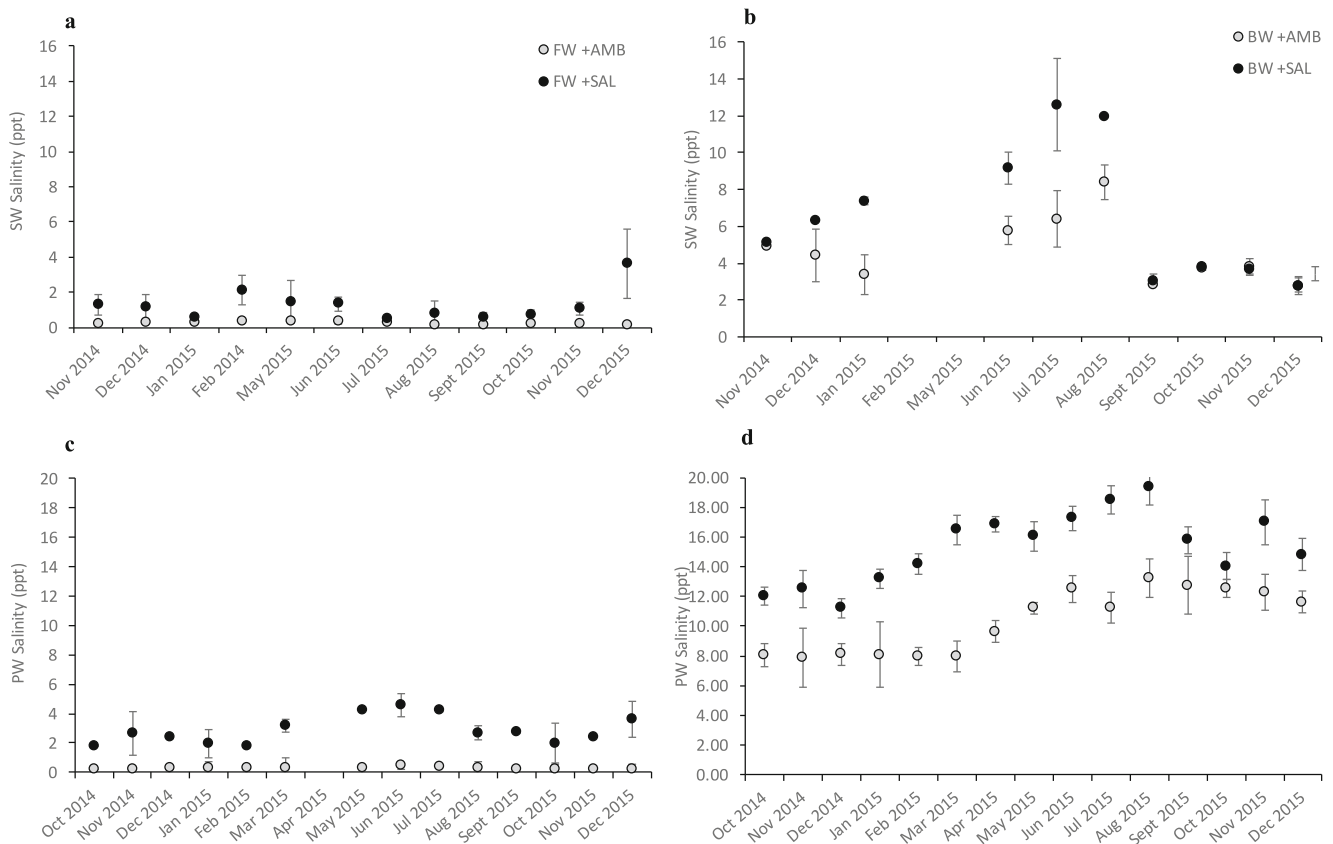

Fig. 2 Mean surface water and porewater salinity in control (+AMB) and treatment (+SAL) plots at the freshwater (a) and brackish water (b)

was significantly higher ($p < 0.05$) in +SAL treatment plots (1.3 ± 1.1 ppt) compared with ambient (+AMB plots) salinity (0.2 ± 0.1 ppt) when averaged over all sampling dates, as well as on all individual sampling dates. At the BW site, mean surface water salinity in the +SAL treatment plots (6.6 ± 3.6 ppt) was significantly ($p = 0.000$) elevated above ambient conditions (4.6 ± 1.9 ppt) when averaged over the duration of the experiment. However, salinity in +SAL plots was not significantly higher than +AMB plots on the last four sampling dates (Sept–Dec 2015). Mean porewater salinity in the +SAL treatment plots was elevated 3 ppt above ambient at the FW site and 5 ppt above ambient at the BW site. Average ambient porewater salinity at the FW site was 0.28 ppt (± 0.08) and was elevated to 2.9 ppt (± 1.2) in the +SAL plots; salinity was significantly higher in the +SAL plots compared with +AMB plots on all sampling dates ($p < 0.05$). Similarly, salinity in +SAL plots at the BW site was significantly greater than salinity in +AMB plots on all sampling dates ($p < 0.05$) with mean ambient porewater salinities of 10.3 ppt (± 2.2) and mean treatment salinities of 15.3 ppt (± 2.6).

Exposure of FW periphyton mats to an ~ 1-ppt elevation in surface water salinity and 3-ppt elevation in porewater salinity caused sufficient osmotic stress to induce physiological responses such as reduced productivity, C content, and nutrient concentrations. Periphyton NEP, GPP, TC, TN, and TP were all significantly lower ($p < 0.05$) in the +SAL plots compared with +AMB plots, but there was no effect on periphyton ER or chl-a concentrations (Table 1). Despite the significant overall treatment effects, we found temporal variability in periphyton functional responses to elevated salinity, particularly on the summer sampling dates (Fig. 3a). We found no treatment effects on periphyton TC or TP in June and August 2015 and no effect on TN in June 2015. Periphyton productivity was not measured in June, but no treatment effects on NEP or GPP were detected in the Aug or Sept 2015 samples.

BW periphyton mats were more resilient to the saltwater treatment compared with the FW periphyton. We found no significant treatment effect on periphyton NEP, GPP, ER, TC, TN, TP, or chl-a when averaged over all sampling dates (Table 1). However, temporal variability in treatment effects were detected (Fig. 3b). Periphyton NEP and GPP were suppressed with the salinity treatment immediately after the first saltwater dose but did not respond to the treatment in any of the later sampling dates where metabolism was measured. Periphyton collected after the first saltwater dosing had greater C content but no effect thereafter. TN and TP were unaffected with the first dosing and remained unaffected throughout the experiment, except for the Nov 2014 sample when TN was reduced and the Jun 2015 sample when both TN and TP were reduced in the treatment plots.

Periphyton mats from both the FW and BW marsh sites were net autotrophic during all sampling events regardless of treatment. At the FW site, mean NEP values ranged from 4.8 to 12.8 mg C g^{-1} AFDM h^{-1} in the +AMB plots and from 1.4 to 7.0 mg C g^{-1} AFDM h^{-1} in the +SAL plots. Mean NEP values at the BW sites ranged from 4.0 to 10.1 mg C g^{-1} AFDM h^{-1} in +AMB plots and from 0.3 to 12.0 mg C g^{-1} AFDM h^{-1} in +SAL plots. Periphyton ER rates were generally low at both the FW (− 0.9 and − 4.3 mg C g^{-1} AFDM h^{-1}) and BW (− 0.5 and − 4.0 mg C g^{-1} AFDM h^{-1}) sites regardless of the treatment group. Despite the reduction in periphyton TC, TN, and TP, particularly at FW site, mat C:P and N:P ratios

Table 1 Means and standard deviations (STDEV) for periphyton net ecosystem productivity (NEP), gross primary productivity (GPP), respiration (R), chlorophyll a (chl-a), total carbon (TC), total nitrogen (TN), and total phosphorus (TP) averaged across all sampling dates at the freshwater (FW) and brackish (BW) marsh

Site	Response	+AMB		+SAL		ANOVA	
		Mean	SD	Mean	SD	F statistic	p value
FW	NEP (mg C g^{-1} AFDM h^{-1})	9.0	4.3	5.0	3.1	14.04	0.001*
	GPP (mg C g^{-1} AFDM h^{-1})	11.7	5.1	6.9	4.2	2.49	0.001*
	ER (mg C g^{-1} AFDM h^{-1})	− 2.6	1.7	− 1.7	2.1	12.33	0.122
	Chl-a (mg g^{-1})	2.3	2.5	1.8	1.3	0.74	0.394
	TC (mg g^{-1})	293.7	37.0	248.7	16.1	46.65	0.000*
	TN (mg g^{-1})	14.8	4.7	10.7	2.3	22.05	0.000*
	TP (µg g^{-1})	194.0	68.0	153.0	73.0	5.26	0.025*
BW	NEP (mg C g^{-1} AFDM h^{-1})	5.1	4.6	5.4	5.6	0.05	0.826
	GPP (mg C g^{-1} AFDM h^{-1})	6.7	5.5	7.5	6.5	1.61	0.211
	ER (mg C g^{-1} AFDM h^{-1})	− 1.4	1.4	− 1.9	1.5	0.26	0.611
	Chl-a (mg g^{-1})	2.8	3.9	1.6	0.8	2.51	0.119
	TC (mg g^{-1})	353.8	27.6	360.8	37.9	0.75	0.390
	TN (mg g^{-1})	25.0	3.4	24.1	5.0	0.75	0.391
	TP (µg g^{-1})	525.4	111.4	500.7	150.6	1.69	0.199

*$p < 0.05$—ANOVA p values indicating the probability that each variable is significantly different between control (+AMB) and treatment (+SAL) plots
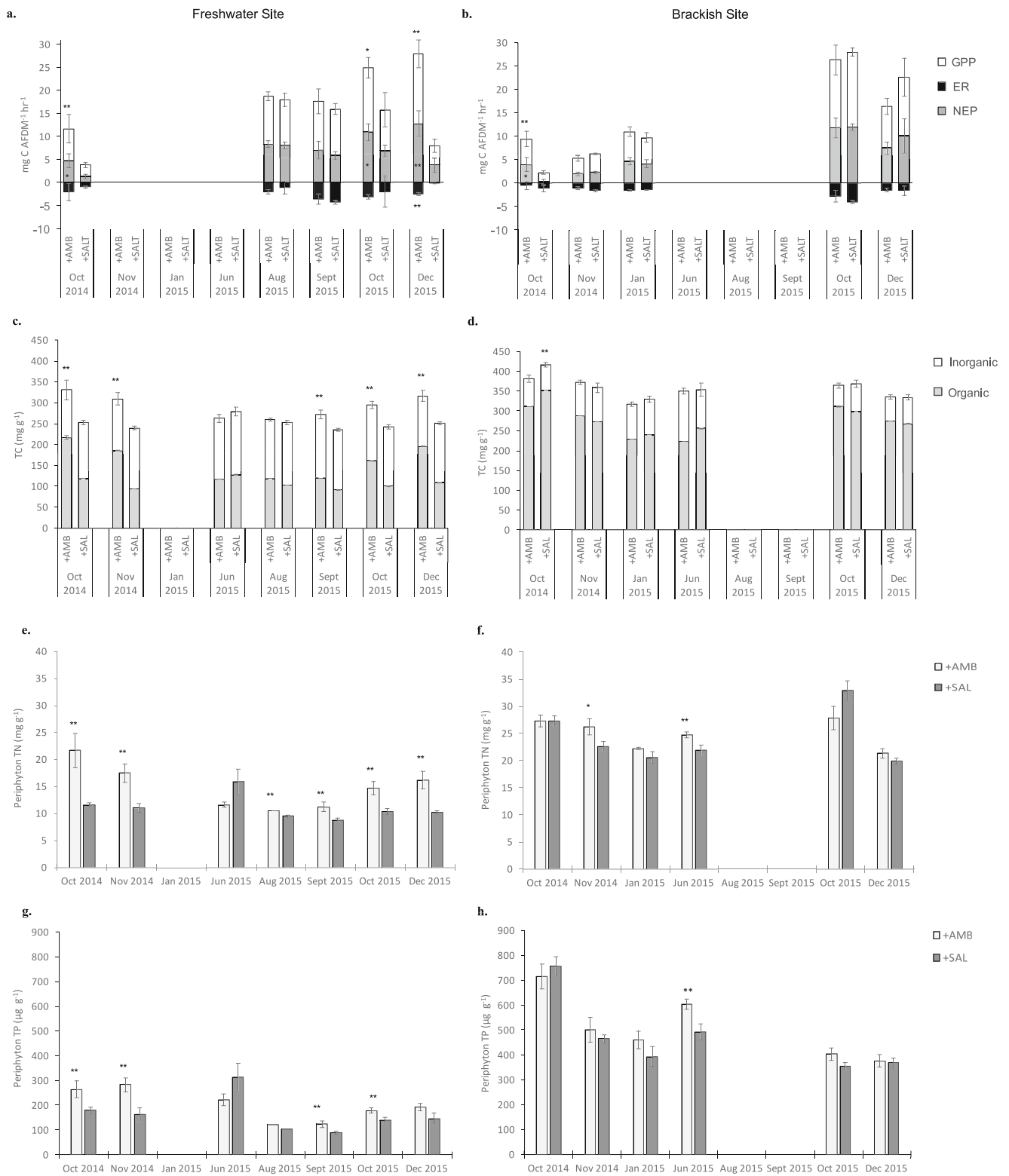
Fig. 3 Periphyton metabolism (net ecosystem productivity (NEP), ecosystem respiration (ER), and gross primary productivity (GPP), periphyton carbon content (total carbon (TC), organic carbon (OC), and inorganic carbon (IC)), periphyton total nitrogen (TN), and periphyton total phosphorous (TP) in control (+AMB) and treatment (+SAL) plots at the freshwater (left) and brackish water (right) sites in order from top to bottom. Asterisk represent significant treatment effects within each sampling date

were not significantly affected by the elevated salinity treatment at either the fresh ($F = 1.90$, $p = 0.173$; $F = 0.05$, $p = 0.823$) or brackish ($F = 0.379$, $p = 0.541$; $F = 0.953$, $p = 0.333$) marsh when averaged over all sampling dates.

However, periphyton C:N was significantly higher in the +SAL compared with +AMB plots at both the fresh ($F = 7.893$, $p = 0.007$) and brackish ($F = 6.624$, $p = 0.012$) sites.

The PERMANOVA analysis detected significant dissimilarity in diatom species composition in response to all three main effects (treatment, site, and month) and for all interaction terms (Table 2). Based on these results, we conducted post hoc pairwise contrasts on the three-way interaction term (treatment × site × month) to search for seasonal patterns in treatment (+AMB and +SAL) effects at the FW and BW sites separately. We found significant compositional dissimilarity between treatment and control plots at the FW site on all four sampling months and the ordination illustrated that the separation between +AMB and +SAL groups increased over time (Table 3; Fig. 4). Compositional dissimilarity between treatments at the BW site was significant only after the first monthly dosing event (Oct 2014) and in June 2015 (Table 3; Fig. 4). The ordinations confirm that divergence between treatment groups was greatest after the first saltwater dosing and that the significance of the dissimilarity between treatments in the June samples may have been driven by an outlier in the +SAL group.

We also observed seasonal shifts in species composition within each treatment group at both sites (Fig. 4a). At the FW site, diatom assemblages within the +AMB group remained relatively similar during the first three sampling dates but deviated considerably on the last date (Oct 2015). Within the +SAL group, the first two sampling dates clustered closer together, while the June 2015 and Oct 2015 samples each formed separate clusters. We observed similar within-treatment group variance trends at the BW site. The +AMB samples from Oct and Nov 2014 grouped closely together, while the June and Oct 2015 samples formed two separate clusters. This same within-treatment seasonal pattern was observed for the +SAL group.

Species dissimilarity between +AMB and +SAL treatments was examined across the four sampling dates to look for overall treatment effects at each site without considering seasonal variability and found that treatment groups had significantly different diatom assemblages at both the FW

(ANOSIM $R = 0.530$, $p = 0.001$) and the BW (ANOSIM $R = 0.748$, p = 0.001) site (Fig. 4b). This was evident from the boxplots of ranked species dissimilarity which showed that between treatment group dissimilarity was greater than within group dissimilarity. In general, periphytic diatoms in FW and BW marshes formed two distinct assemblages and exhibited similar statistical dispersion in the dissimilarity metric (ANOSIM $R = 1$, $p = 0.001$) (Fig. 4b). Interestingly, dispersion within the +SAL treatment group was much greater than within +AMB group for both the FW and BW marsh indicating that saltwater exposure causes higher variance in diatom assemblages (Fig. 4b). Diatom indicators selected by the multilevel pattern analysis (multipatt{indecspecies}) for +AMB and +SAL treatment groups at the FW and BW sites are listed in Table 4 (see Fig. 5).

Discussion

Periphyton is considered a primary ecosystem engineer in karstic, freshwater wetlands. However, because these landscapes are typically located along coastlines, saltwater intrusion has the potential to alter the performance, and thus the ecological roles, of these dominant primary producers. In this study, we demonstrated that simulated monthly pulses of saltwater intrusion into naturally freshwater marshes suppressed periphyton primary productivity and reduced the TC, TN, and TP concentrations of the mats. These functional responses to pulsed salinity increases of only 1 ppt above ambient surface water levels were accompanied by significant shifts in periphytic diatom assemblages, underscoring the effectiveness of diatoms as fast and sensitive indicators of environmental variability. Brackish water periphyton was fundamentally different in physical structure and species composition than the cohesive, calcareous mats found in freshwater marshes and was more resilient to the monthly pulses of elevated salinity. With a 2-ppt increase in surface water salinity, these mats exhibited suppressed productivity immediately after the first salinity dosing but were unaffected thereafter. Likewise, the salinity pulses did not significantly affect the overall TC, TN,

Table 2 PERMANOVA output table showing significant main effects of treatment, site, and month, and a significant three-way interaction effect, on diatom species dissimilarity

Source	df	SS	MS	Pseudo-F	P (perm)
Treatment	1	1344.9	1344.9	5.0866	0.001
Site	1	61,280	61,280	231.76	0.001
Month	3	6015.4	2005.1	7.5834	0.001
Treatment × site	1	1611	1611	6.0929	0.001
Treatment × month	3	1863.4	621.13	2.3491	0.001
Site × month	3	12,138	4045.9	15.302	0.001
Treatment × site × month	3	1847.6	615.88	2.3293	0.001
Res	32	8461.1	264.41		
Total	47	94,561			

Table 3 Significance of post hoc comparisons on the PERMANOVA three-way interaction term for treatment factor levels (+AMB and +SAL) at each site during each sampling date

Time	FW	BW
Oct 2014	0.001**	0.001**
Nov 2014	0.001**	0.575
Jun 2015	0.001**	0.001**
Oct 2015	0.001**	0.111

** $p < 0.005$

or TP content of the mats at the brackish site. Despite the metabolic and nutritional resilience of brackish water periphyton mats to the experimentally elevated salinity, the periphytic-diatom assemblages responded to the saltwater pulses with altered species composition.

The movement of marine water into the freshwater marshes of the coastal Everglades subjects stenohaline organisms to stressors that can have detrimental effects on their physiological functions. We show that freshwater periphyton communities respond to relatively low-level salinity increases with
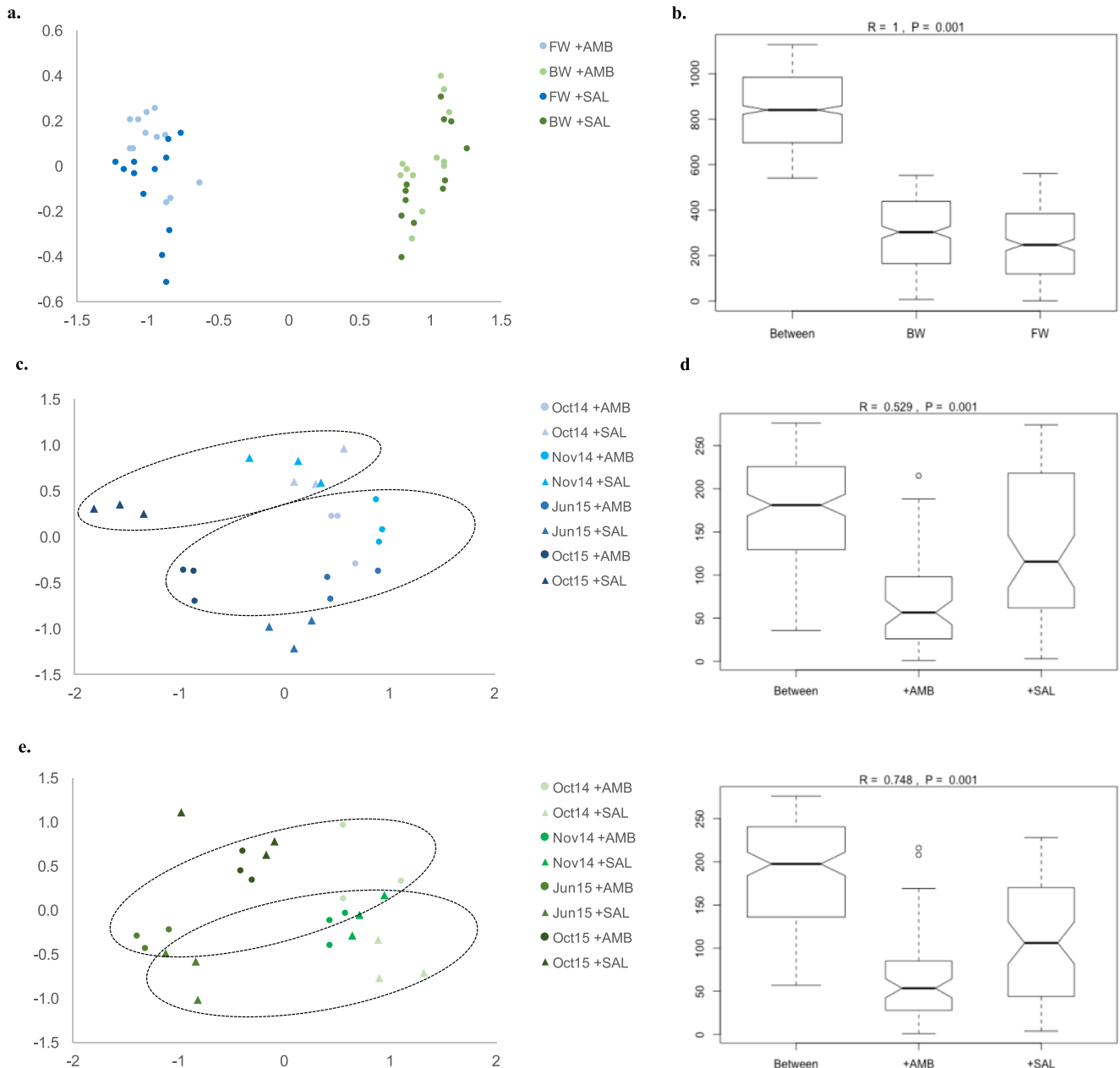

Fig. 4 Nonmetric multidimensional scaling (NMDS) ordinations (left) and analysis of similarity (ANOSIM) boxplots showing ranked species dissimilarity within and between groups (right). Freshwater (FW) and brackish (BW) diatom assemblages were highly dissimilar throughout the experiment (top). Diatom assemblages were significantly different between control (+AMB) and treatment (+SAL) plots at the freshwater (center) and brackish water (bottom) site

Table 4 Indicator diatom taxa classified by multilevel pattern analysis (multipatt{indicspecies}) for control (+AMB) and treatment (+SAL) conditions at the freshwater (FW) and brackish water (BW) marsh sites with mean relative abundance of each indicator species in each of the four groups

| Taxa no.	Taxa	Authority	FW + AMB	FW + SAL	BW + AMB	BW + SAL
FW + AMB						
1	*Achnanthes minutissima*	Kützing 1833	0.077	0.044	0.000	0.000
2	*Brachysira microcephela*	(Grunow) Compère, 1986	0.402	0.348	0.183	0.153
3	*Encyonema silesiacum elegans*	Krammer, 1997	0.122	0.073	0.007	0.000
4	*Encyonopsis microcephela*	(Grunow) Krammer, 1997	0.703	0.637	0.017	0.000
5	*Eunotia flexuosa*	(Brébisson ex Kützing) Kützing,1849	0.052	0.014	0.000	0.004
6	*Eunotia naegeli*	Migula 1905	0.082	0.012	0.000	0.000
7	*Gomphonema intricatum vibrio*	(Ehrenberg) Cleve, 1894	0.195	0.103	0.077	0.089
8	*Navicula cryptotenella*	Lange-Bertalot, 1985	0.290	0.281	0.000	0.010
9	*Ulnaria delicatissima*	(W.Smith) Aboal & Silva 2004	0.086	0.014	0.000	0.005
FW + SAL						
10	*Encyonema evergladianum*	Krammer, 1997	0.157	0.314	0.009	0.009
11	*Encyonema mesianum*	(Cholnoky) Mann, 1990	0.208	0.244	0.000	0.000
12	*Kobayasiella subtilissima*	Lange-Bertalot, 1999	0.051	0.053	0.000	0.000
BW + AMB						
13	*Amphora sulcata*	Brébisson, 1854	0.004	0.000	0.104	0.090
14	*Caponea caribbea*	Podzorski, 1984	0.000	0.000	0.069	0.065
15	*Diploneis oblongella*	(Nägeli ex Kützing) Cleve-Euler, 1922	0.005	0.024	0.213	0.194
16	*Envekadea metezeltinii*	Lee et al., 2013	0.000	0.000	0.080	0.061
17	*Envekadea vanlandinghamii*	Graeff, 2013	0.000	0.009	0.154	0.117
18	*Fragilaria ftsp16*		0.010	0.004	0.297	0.252
19	*Fragilaria minuscula*	(Grunow) Williams,1987	0.005	0.005	0.119	0.073
20	*Navicula bulnheimii*	Grunow, 1863	0.000	0.000	0.032	0.015
21	*Navicula* cf. *vmsp2*		0.000	0.000	0.077	0.078
22	*Nitzschia acicularis*	(Kützing) W.Smith, 1853	0.000	0.000	0.047	0.041
23	*Nitzschia ftsp16*		0.015	0.000	0.199	0.078
24	*Nitzschia microcephela*	Grunow, 1880	0.000	0.000	0.026	0.010
25	*Nitzschia nana*	Grunow in Van Heurck, 1881	0.031	0.031	0.099	0.105
26	*Planothidium rostratum*	(Østrup) Lange-Bertalot 1999	0.000	0.000	0.026	0.010
27	*Seminavis pusilla*	(Grunow) Cox & Reid, 2004	0.005	0.015	0.299	0.233
28	*Stephanocyclus menghiniana*	Kützing,1844	0.016	0.030	0.101	0.090
29	*Synedra filiformis exilis*	Cleve, 1939	0.007	0.020	0.111	0.087
BW + SAL						
30	*Amphora acutiuscula*	Kützing, 1844	0.000	0.007	0.103	0.111
31	*Amphora coffeaeformis aponina*	Archibald & Schoeman, 1984	0.006	0.000	0.106	0.143
32	*Amphora montana*	Krasske 1932	0.000	0.004	0.026	0.048
33	*Amphora punctata*	Stepanek and Kociolek 2013	0.004	0.000	0.200	0.225
34	*Amphora veneta*	Kützing, 1844	0.000	0.000	0.064	0.120
35	*Caloneis bacillum*	(Grunow) Cleve 1894	0.004	0.000	0.016	0.065
36	*Fragilaria synegrotesca*	Lange-Bertalot, 1993	0.223	0.120	0.247	0.288
37	*Mastogloia pseduosmithii*	Lee et al., 2014	0.000	0.000	0.039	0.071
38	*Mastogloia braunii*	Grunow, 1863	0.000	0.000	0.070	0.095
39	*Mastogloia lanceolata*	Thwaites ex W.Smith 1856	0.011	0.011	0.317	0.319
40	*Navicula salinicola*	Hustedt, 1939	0.004	0.007	0.143	0.180
41	*Pleurosigma salinarum*	(Grunow) Grunow, 1880	0.000	0.000	0.013	0.023
42	*Rhopalodia pacifica*	Lange-Bertalot & Krammer, 1987	0.000	0.004	0.059	0.161
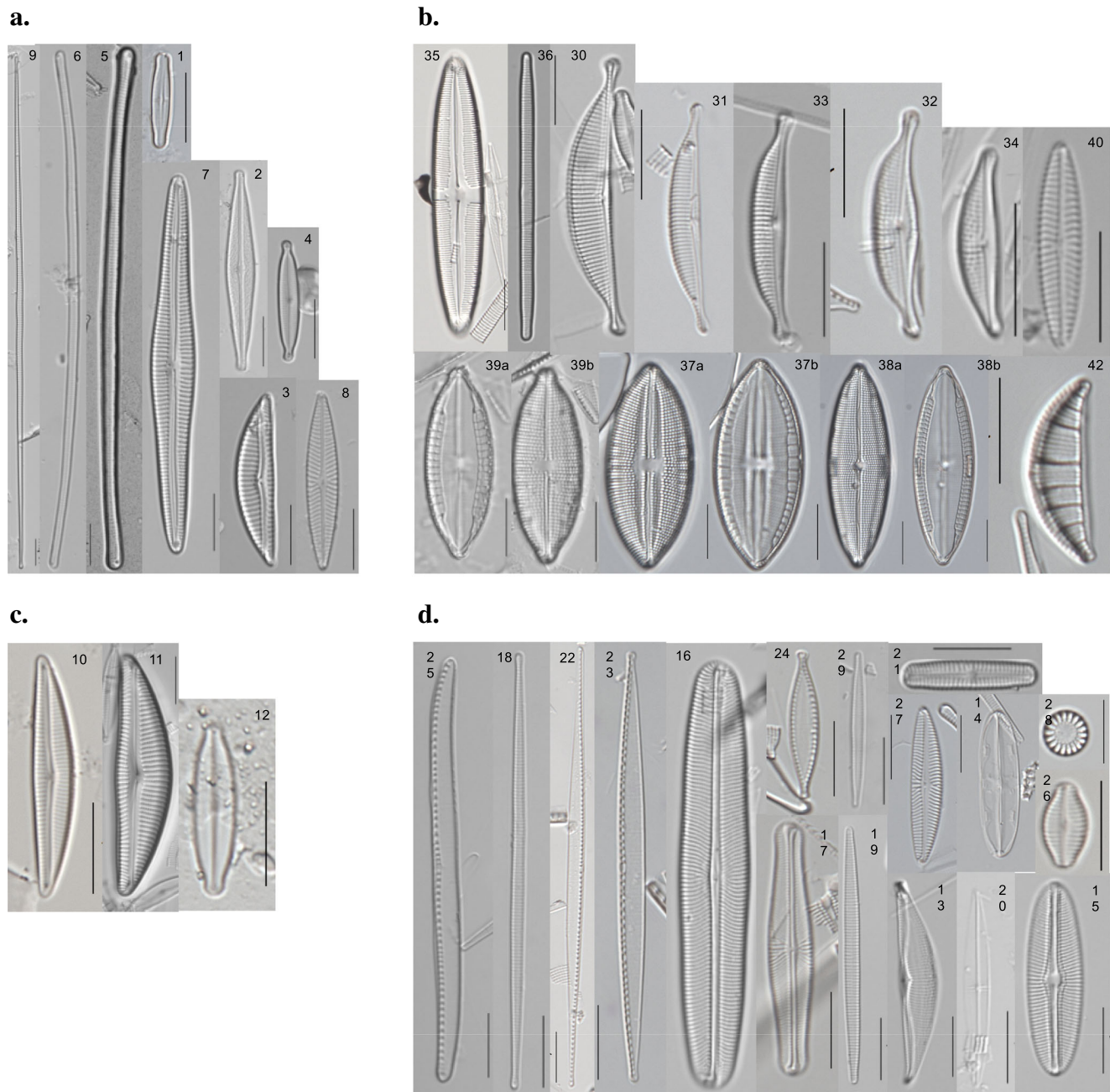
Fig. 5 Indicator taxa plates for freshwater +AMB (**a**), brackish water +AMB (**b**), freshwater +SAL (**c**), and brackish water +SAL (**d**) conditions. Image numbers correspond to taxa number in Table 4

depressed NEP while periphyton from the brackish marsh were less sensitive to elevated salinity, despite an initial reduction in NEP. This was not surprising given that periphyton in brackish marshes are naturally subjected to fluctuating salinity given their proximity to the coast and that species comprising these mats are adapted to these conditions. However, we were only able to achieve salinities of 2 ppt above ambient in the surface water and 5 ppt above ambient in the porewater; exposure to higher salinities are expected to illicit stronger functional responses in BW periphyton. Periphyton ER rates were unaffected by the salinity treatment in both the FW and BW

marsh sites suggesting that the decline in photosynthetic carbon sequestration (NEP) was largely driven by lowered periphyton GPP. One explanation for the reduction in GPP is the osmotic stress and resulting intracellular water deficits caused by elevated salinity (Hasegawa et al. 2000). Water is essential to photosynthesis because it acts as the electron donor in photosystem II replacing the reaction center electron that is mobilized from the chlorophyll molecule into the electron transport chain when excited by light energy (Falkowski and Raven 2013). Therefore, plasmolysis, and many other cellular disturbances caused by exposure to saltwater, can

limit photosynthetic carbon fixation and eventually result in cell death. Affenzeller et al. (2009) experimentally tested the effect of elevated salinity on cultured samples of a freshwater green alga and found significant declines in GPP which they attributed to changes in photosynthetic enzyme activity and disruption of transport across the chloroplast envelop membrane. Although several studies have tested the effect of salinity on intertidal or marine algae (Macler 1988; Satoh et al. 1983), our study is one of the few to experimentally test the effects of elevated salinity on freshwater algae (Affenzeller et al. 2009), and particularly freshwater periphyton mats. Rejmánková and Komárková (2005) conducted a factorial mesocosm experiment to assess the response of periphyton from karstic wetlands in Belize to combinations of low, medium, and high salinity, P, and N but found that, despite trends of lower primary productivity at elevated salinity, the overall effect of salinity was not significant. Similarly, Ewe et al. (2006) examined periphyton GPP along a natural salinity gradient in the Florida Everglades and found that, although productivity values were higher in the freshwater marshes of Taylor Slough compared with downstream in Florida Bay, these results were highly variable. These results suggest that the mechanisms driving periphyton responses to saltwater exposure may be less clear than those driving organism-level responses because these mats are essentially microecosystems inhabited by a suite of auto- and heterotroph organisms.

Salinity-induced declines in freshwater periphyton GPP and NEP were reflected by the lower TC content of salt-treated mats. We hypothesize that reduced photosynthetic carbon fixation leaves less organic carbon available for biomass production and that the potential loss of calcium carbonate precipitating cyanobacteria in response to greater salinity reduces the amount of inorganic carbon stored in the mats. Periphyton TN and TP concentrations were also reduced in salt-treated mats suggesting nutrient uptake suppression caused by ionic stress. The accumulation of Na^+ and Cl^- ions may create ionic competition that inhibits assimilation of essential nutrients, such as NH^+_4, NO^-_3, PO^-_4, K^+, and Ca^{2+} (Alam 1999; Sudhir and Murthy 2004; Hu and Schmidhalter 2005). The reduction in N and P uptake is another possible explanation for the decline in NEP and GPP, given the essential roles these nutrients play in synthesizing chlorophyll a (N) and ATP and NADPH (P). Studies looking at the effects of salinity on plant nutrient uptake support our findings that salt stress reduces uptake of essential nutrients (Kozlowski 1997; Grattan and Grieve 1998). For example, Brown et al. (2006) reported reduced N and P uptakes by Spartina alterniflora exposed to experimentally elevated salinity levels, and Choi et al. (2010) showed that nitrate and phosphate uptakes were reduced in the estuarine macroalgal Ulva pertusa when salinity was above or below its 15–20 psu optimum. Lower TP concentrations in periphyton mats with added saltwater may also be caused by abiotic mechanisms. Saltwater has been

shown to cause P desorption from $CaCO_3$ bedrock through anion competition in the Everglades (Price et al. 2006; Flower et al. 2017), and a similar mechanism may be at play within the calcareous periphyton mats that causes $CaCO_3$-bound P to be released into the surrounding water.

Changes in freshwater periphyton functionality were accompanied by significant shifts in diatom composition. Compositional shifts were evident after one pulse of seawater dosing and continued to diverge further from the ambient community with monthly pulsed additions over the year study period. The growing separation between +AMB and +SAL treatment groups over time suggests that divergence in diatom assemblages in response to monthly pulses of saltwater exposure may be additive. However, because samples were only collected the day after dosing, we cannot be certain that the diatom assemblage (and periphyton functions) did not recover before the following monthly dosing and therefore we cannot draw conclusions on the resiliency of FW periphyton and diatom assemblages to pulsed saltwater intrusion. Exposure to monthly pulses of saltwater, as opposed to continuously elevated salinity, may allow freshwater communities to recover or impede the establishment of a mostly salt-adapted assemblage before the following dosing. This may explain why we did not see functional redundancy in freshwater periphyton which would be expected if a salt-adapted community had become established (Rosenfeld 2002). Functional redundancy was evident at the BW site, where periphyton functionality was largely unaffected by the monthly pulses of elevated salinity while the diatom assemblage exhibited significant shifts in response to the saltwater treatments. Although the periphytic-diatom assemblage at the brackish marsh responded to a 2-ppt salinity elevation with altered species composition, albeit less so than FW periphytic diatoms, other BW periphyton communities (e.g., cyanobacteria and green algae) may be less sensitive to small, discontinuous salinity increases explaining why we did not observe significant functional responses in BW periphyton. These findings highlight the sensitivity of diatoms to small scale changes in salinity compared with other periphytic communities and supports the idea that diatoms are stronger indicators of small, short-term changes in salinity compared with periphyton functional metrics.

Saltwater intrusion not only elicits negative physiological responses in freshwater communities, but also stimulates changes in community structure as coastal transgression modifies environmental conditions to favor salt-tolerant communities (Ross et al. 2000; Davis et al. 2005; Troxler et al. 2014). This study represents a first step in understanding how freshwater, mat-forming algal communities respond to elevated salinity, an important area of research given the threat of saltwater intrusion on coastal, freshwater wetlands and the importance of algal communities in these systems. In general, much of the literature on the effects of elevated salinity on primary

productivity in freshwater coastal wetlands, whether experimental or field based, is focused either on macrophyte or whole ecosystem productivity (Webb and Mendelssohn 1996; Childers et al. 2006a; Neubauer 2013; Troxler et al. 2014; Wilson et al., in review). While this information is imperative to our understanding of saltwater intrusion impacts on the ecosystem, we need to assess impacts to other key ecosystem components, such as periphyton, to achieve a comprehensive understanding of the potential consequences of saltwater intrusion. Although salinity is a dominant driver of plant and algal community composition and metabolic functioning, saltwater intrusion into coastal freshwater marshes not only elevates salinity but alters the elemental composition of site water as well. Among other water chemistry changes, saltwater intrusion is expected to provide a phosphorus subsidy to the naturally P-limited marshes of the coastal Everglades (Childers et al. 2006b; Price et al. 2006). Therefore, it is important to understand how periphyton, and other key ecosystem components, respond to the individual and synergistic effects of increased salinity and nutrient (e.g., P) availability. Studies addressing these interactive effects will allow us to expand the use of periphyton and their diatom assemblages as tools for environmental assessments related to sea-level rise and saltwater intrusion in the Everglades and other karstic, freshwater wetlands.

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