Wetlands (2019) 39:337–347
https://doi.org/10.1007/s13157-018-1100-z

GENERAL WETLAND SCIENCE

Effects of Nutrient-Limitation on Disturbance Recovery in Experimental Mangrove Wetlands

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Received: 31 May 2018 / Accepted: 22 October 2018 / Published online: 10 November 2018

Abstract

Coastal wetlands are exposed to high-energy storms that influence plant and soil structure. To understand how nutrient availability interacts with storm-induced plant stress, we tested how defoliation interacts with nutrient enrichment to affect carbon (C) and nutrient (nitrogen, N; phosphorus, P) cycling and storage within soils and plants. In outdoor experimental mesocosms, we defoliated red mangrove saplings (*Rhizophora mangle*), added 30 g of inorganic P to peat soils, and quantified plant [elemental stoichiometry (C:N, C:P, N:P), leaf count, and above- and below- ground biomass] and soil responses [C:N, C:P, N:P, litter breakdown rate (k), soil CO_2 efflux] during a 42-d recovery period. Mangroves rapidly regrew all removed leaves and recovered nearly 30% of leaf biomass. Mangrove biomass %P increased by 50% with added P; however, soil stoichiometry remained unchanged. Defoliation reduced Soil CO_2 efflux by 40% and root litter k by 30%. Phosphorus was quickly incorporated into mangrove biomass and stimulated nighttime soil CO2 efflux. This work highlights the importance of testing interactions of nutrient availability and plant stress on plant and soil biogeochemical cycling and suggests that plants quickly incorporate available nutrients into biomass and defoliation can lead to reduced soil C losses.

Keywords Peat · Nutrients · Coastal storms · Climate change · Hurricanes

Introduction

Coastal wetlands are exposed to multiple types of disturbances that influence attributes of plant and soil structure that can ultimately affect recovery of ecosystem function. Resilience is defined as an ecosystem's ability to recover to a persistent state following a disturbance (Holling 1973; Boesch 1974), and ecosystems that recover functions more quickly have higher resilience (White and Jentsch 2001; Johnstone et al. 2016). Changes in the patterns of disturbance drivers and increases in acute stressors like nutrient pollution can influence ecosystem recovery following disturbances (Odum et al. 1995).

High-energy tropical storms and hurricanes are intermittent disturbance events that can affect the structure and function of coastal environments (Michener et al. 1997; Lugo 2000, 2008) through exposure to wind damage, storm surge, and sediment deposition (Smith et al. 1994; Doyle et al. 1995; Deng et al. 2010; Barr et al. 2012). Mangrove forests are considered particularly vulnerable to damage caused by high-energy storms because of their position at the coast (Sherman et al. 2001; Piou et al. 2006). Despite high exposure, mangrove forests can recover quickly following storm disturbance because of key life history traits, such as translocation of above- and below-ground nutrient stores, rapid nutrient recycling rates, and quick leaf regrowth that allow mangroves to respond to stressors associated with storms (Alongi 2008; Barr et al. 2012).

Mangroves in the Florida Coastal Everglades experience a high recurrence of tropical storms and hurricanes (Duever et al. 1994; Smith et al. 1994; Krauss et al. 2005; Deng et al. 2010). Since the twentieth Century, the Florida Coastal Everglades was in the path of several devastating storms;

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13157-018-1100-z) contains supplementary material, which is available to authorized users.

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Labor Day Storm (1935), Hurricane Donna (1960), and Hurricane Andrew (1992). High-energy storms contribute both stresses (wind damage, tree mortality) and subsidies (marine nutrients, allocthonous energy inputs) to coastal ecosystems (Castañeda-Moya et al. 2010). Karst-based, Caribbean coastal wetlands, like the Florida Coastal Everglades, are P-limited (Fourqurean et al. 1993; Boyer et al. 1999; Noe et al. 2001) and depend on marine-derived P to support coastal productivity (Chen and Twilley 1999a, 1999b; Childers et al. 2006; Barr et al. 2012). Hurricane Wilma (2005) defoliated large areas of coastal mangrove forest and deposited P-rich sediment, altering biogeochemistry by increasing total P concentrations to 0.19 mg cm^{-3}, which is double the average soil nutrient total P (Castañeda-Moya et al. 2010). As observed from Hurricane Wilma, storms defoliate mangroves while simultaneously subsidizing wetland soils with P-rich marine sediments (Smith et al. 2009). Although mangroves regenerated leaves following Hurricane Wilma, there is evidence that some of the mangroves in the Everglades failed to recover fully (Barr et al. 2012; Danielson et al. 2017). It is not clear how nutrient deposits, associated with the storm contributed to mangrove regeneration. Therefore, it is critical for us to better understand mechanistic differences in plant and soil responses to changes in storm-derived subsidies and stresses.

To test how nutrient limitation affects recovery from disturbance we investigated plant- and soil-mediated subsidy and stress responses. We manipulated disturbance and nutrient subsidies in outdoor wetland mesocosms containing red mangrove (*Rhizophora mangle*) saplings. We used mangrove saplings because the effects of hurricane and storm surge on mangrove saplings have not been explicitly tested, this information is critical as mangroves continue to expand into continental interiors and latitudinally with climate change (Comeaux et al. 2012; Bianchi et al. 2013). We defoliated mangroves, added inorganic P to soils, and measured changes in plant and soil C storage and P uptake as indicators of recovery. We addressed the following questions: 1) How does simultaneous exposure to a subsidy (P addition) affect mangrove plants and soils exposed to stress (defoliation)? 2) How do plant defoliation and added P interactively modify short-term mangrove plant and soil C storage and P uptake? and 3) How do defoliation and P addition differentially affect above-and belowground plant and soil responses? We predicted that addition of P would increase mangrove leaf and root biomass and P content, soil P content, litter breakdown rates, and soil CO_2 efflux. We predicted net gains in plant and soil P and net losses in soil C with the addition of P. We also predicted that defoliation would increase soil CO_2 efflux because of increased soil microbial and root respiration. In contrast, we predicted defoliation would lead to decrease litter breakdown rates caused by reduced levels of plant exudates known to stimulate the degradation of recalcitrant organic compounds by soil microbes. We anticipated that the most significant losses in plant C and leaf count would be attributed to defoliation while the majority of the losses in soil C would be attributed to P addition (Howarth and Fischer 1976; Robinson and Gessner 2000). Finally, we predicted that plants with added P would recover more quickly and completely after defoliation than those without added P and that C storage would be positive with added P despite defoliation if increases in mangrove plant biomass were more significant than soil C losses (Lovelock et al. 2011).

Methods

Study Area and Experimental Wetland Facility

We collected twenty-four peat cores from a coastal mangrove forest near lower Shark River Slough in Everglades National Park (25°21′52.7” N, 81°4′40.6” W; Chambers et al. 2014). We transported soil cores (approximately 25 cm deep × 28 cm diameter) to the Florida Bay Interagency Science Center Outdoor Mesocosm Facility in Key Largo, FL (25°5′9.21” N, 80°27′6.9″). We planted a single red mangrove (*Rhizophora mangle*) propagule in the center of each soil core, and planted saplings grew for two years before the start of our experiment. At the beginning of our study, the mangrove saplings were 56.36 ± 1.51 cm tall from the soil surface to the top branch. Mangrove-peat soil monoliths were randomly assigned to and placed in concrete mesocosms (0.7 m D × 0.8 m W × 2.2 m L) containing saltwater from nearby Florida Bay (see below). The composition of the initial soils was as follows 24.9 ± 0.9 %C, 1.4 ± 0.1%N, and 0.06 ± 0.00 %P.

Experimental Design

Seawater was pumped from nearby Florida Bay and stored in water holding tanks (7.6 m^3), which released water into each concrete mesocosm at a constant flow-through rate of 60 mL min^{-1}. To simulate natural conditions under a canopy of full-size trees, we covered mesocosms with nylon mesh shade cloth at the start of the experiment, and they remained shaded for the duration of our study. The shade cloth reduced photosynthetic active radiation by 70%. We measured water levels weekly using a meter stick affixed to each mesocosm to ensure a consistent water level of 27 cm, relative to the bottom of each mesocosm, throughout the experiment, ensuring that the soil surface of each mangrove monoliths was submerged. We drained the mangrove-peat monoliths once each week to measure soil CO_2 efflux.

We manipulated two factors of added P and defoliation. Within each concrete mesocosm, four mangrove-peat monoliths were housed in individual 25-L plastic containers (0.42 m D × 0.5 m W × 0.7 m L; Fig. 1). Collectively, we characterized

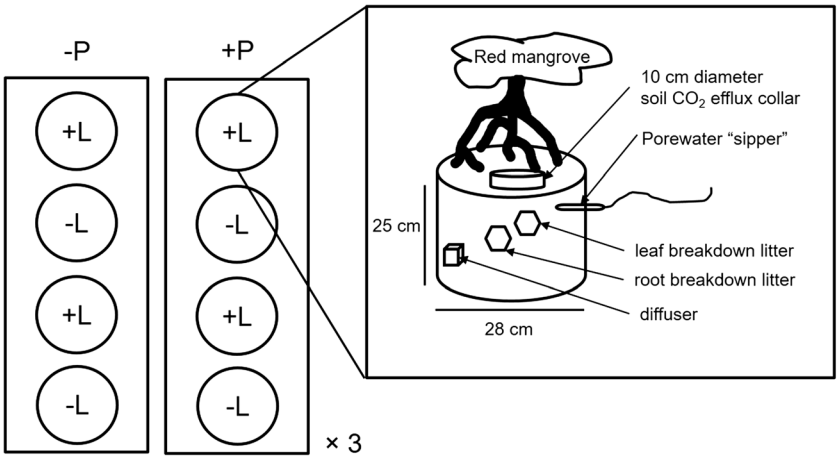

Fig. 1 Diagram of the outdoor experimental wetlands and mangrove-peat monolith structure. Six experimental wetland mesocosms contained four mangrove-peat monoliths each. Each mesocosm was designated as phosphorus added (+P) or no phosphorus added (-P). Within each mesocosm, mangrove-peat monoliths were designated as defoliated (−L) or non-defoliated (+L). Each mangrove-Peat monolith was contained in a 25 × 28 cm perforated bucket equipped with a porewater sipper, 10 cm diameter collar for CO_2 soil efflux measurements, diffusers that were either empty or contained granular orthophosphate in the +P treatments, and root and leaf decomposition material

the four treatments as: (i) control, (ii) -L, (iii) + P, (iv) + P/−L for no added P with leaves (i), no added P and defoliation (ii), P addition with leaves (iii), and P addition/defoliation (iv), respectively. We added 30 g of granular orthophosphate (Hoffman®, Lancaster, New York, USA) to 125-μm mesh containers (hereafter diffusers) inserted at 20 cm within the soil monolith to ensure added P stayed within the soil and was not transported out of the mangrove-peat monolith and to help control the rate of P release. Inorganic P diffused over the course of the 42-d experiment. Empty diffusers were added to the -P monoliths. We measured the mass of orthophosphate remaining in diffusers by retrieving all diffusers, rinsing off the residual soil, and drying the remaining orthophosphate. The defoliation treatment consisted of a single event of complete removal of leaves at the start of the experiment. The leaves in the defoliated treated mangroves were removed on day 0 and began the experiment without leaves. We used the leaves removed to measure leaf litter breakdown all treatment conditions (see below). Litter for root breakdown was collected by clipping prop roots that had grown beyond soil monoliths.

A 10-cm diameter polyvinyl chloride (PVC) collar (10 cm height) was inserted in each of the mangrove-peat monoliths and left for the duration of the experiment to place a gas chamber for CO_2 efflux measurements. To measure porewater, we installed a sipper through the side bucket perforation into the center of each monolith at a depth of 10 cm from the soil surface.

Experimental measurements were conducted at various intervals throughout 42 d. This timeframe coincided with when all removed leaves from the defoliation treatment fully regenerated and when we expected the majority of added P would have been released from experimental diffusers.

Physicochemical Conditions

We measured water temperature and salinity biweekly (n = 24). During the biweekly measurements, we also collected surface water samples (filtered and unfiltered) and filtered pore water samples from each plant-soil monolith (n = 72). Unfiltered surface water samples were collected in 60 mL HDPE sample bottles. Filtered surface water samples were collected in a plastic syringe and filtered onsite through a 0.45-μm membrane filter into a 60 mL HDPE sample bottle. Filtered porewater samples were collected by extracting water from the sipper embedded in each monolith. Then the porewater was filtered using a 0.45-μm membrane filter and released into a 60 mL HDPE sample bottle. All water samples were stored at −20 °C until analysis at the Southeast Environmental Research Center, Nutrient Analysis Laboratory. Unfiltered surface water was analyzed for total N (TN), total P (TP), and total organic C (TOC). Filtered porewater and filtered surface water samples were analyzed for dissolved organic C (DOC), dissolved inorganic nitrogen (DIN, NO_3^-, NO_2^-, NH_4^+), and soluble reactive P (SRP). Dissolved inorganic N, total N, TP and SRP parameters were analyzed on an Alpkem RFA 300 auto-analyzer (OI Analytical, College Station, TX, USA) and TOC and DOC were analyzed with a Shimadzu 5000 TOC Analyzer (Shimadzu Scientific Instruments, Columbia, MD, USA).

Plant Biomass

We counted the total number of leaves present on all 24 mangrove saplings before administering the defoliation treatment, just after applying the defoliation treatment, and at the end of the experiment. We calculated the change in the number of

leaves as the final leaf count minus the leaf count immediately after applying the defoliation treatment. Finally, at the end of the experiment, we destructively sampled mangrove plants ($n = 24$) to quantify above and belowground plant biomass. We collected all the leaves from each plant to quantify total leaf biomass for each plant ($n = 24$). We quantified total aboveground woody biomass by clipping the prop roots and stems at the soil surface after the leaf biomass had been removed. We also determined belowground coarse root biomass from each entire core (0.015 m^3) by washing away soil and collecting intact coarse roots, attached to the shoot, small fine, and unattached roots were not included in the coarse root biomass estimate. Leaf, woody, and coarse root tissue samples were dried in an oven at 60 °C for 48 h. Completely dried samples were weighed, then finely ground and homogenized using an 800-D mixer/mill (spex SamplePrep, Metuchen, New Jersey, USA). Ground plant material was subsampled, oven-dried (60 °C) for 48 h, weighed, combusted (550 °C for 4 h), and re-weighed to determine ash-free dry mass (g AFDM).

Plant and Soil Elemental Stoichiometry

We collected initial and final soil cores (2 cm diameter × 20 cm depth) from ($n = 12$ initial, $n = 24$ final). We dried soil samples in an oven at 60 °C for 48 h. Ground soil material was subsampled, oven-dried (60 °C) for 48 h, weighed, combusted (550 °C for 4 h), and re-weighed to determine AFDM. Carbon and N content was measured using a Carlo Erba NA 1500 CHN Analyser (Carlo Erba, Milan, Italy). Phosphorus content was measured using the ash/acid extraction method followed by spectrophotometric analysis using the ascorbic acid method (Allen 1974; APHA 1998). We estimated elemental composition (%C, %N, and %P) and elemental stoichiometry (C:N, C:P, and N:P) at three soil depths (0–2 cm, 2–10 cm, and 10–20 cm). All elemental compositions were calculated from molar mass, and elemental stoichiometry is reported in molar ratios.

Root and Leaf Litter Breakdown Rates

Within each mangrove-peat core, two mesh containers were deployed with oven-dried leaf (1.30 ± 0.03 g) and prop root litter (1.30 ± 0.02 g) material of known initial mass. We retrieved incubated litter at the end of the six-week study to quantify mass loss. We used oven-dried green litter which best represents organic matter inputs deposited during storms. By using the green leaf and prop root litter we were also able to better control for variation in initial litter chemical composition. We estimated breakdown rates (k) by ln-transforming the proportion of AFDM remaining (using same methods for plant and soil AFDM above). We used the exponential decay model: $M_{42} = M_0 \times e^{-k42}$, where M_0 is the initial litter mass on day 0, M_{42} is the litter mass on day 42. The slope of the linear regression of the ln-transformed AFDM remaining versus incubation time is k (Benfield 2006). We also estimated elemental composition (%C, %N, and %P) and elemental stoichiometry (C:N, C:P, and N:P) of decomposing root and leaf litter following the same methods described above.

Soil CO_2 Efflux

Weekly soil CO_2 efflux was measured during the day from all monoliths, using a portable infrared gas analyzer (LI-COR 8100, Lincoln, NE, USA) equipped fit onto the embedded PVC pipe installed in each core. Each efflux measurement lasted for 75 s. In addition to daytime soil CO_2 efflux, we also measured nighttime CO_2 efflux during the final week of the experiment. Nighttime CO_2 efflux was measured following the same procedure used for daytime efflux.

Data Analyses

We performed all statistical analyses using R Studio (R Core Team 2017 version 3.3.3). We ran repeated measures ANOVAs followed by Tukey HSD *post-hoc* tests to test variation in effects of defoliation and P addition on changes in surface and porewater chemistry, and daytime CO_2 efflux over time. For the variables measured only at the beginning and end of the experiment (soil organic matter content, the stoichiometry of soil, leaf and root litter, and root and litter k), we used ANOVA followed by a Tukey HSD test to determine differences among treatments. We considered results with an alpha less than 0.05 statistically significant.

Results

Experimental Treatments

Initial mean leaf count for mangroves in control and P addition was 92 ± 48 and 86 ± 36, respectively. In administering the defoliation treatment, we removed an average of 137 ± 36 leaves from both the defoliation and P addition/defoliation treatments. At the end of the experiment, we measured the amount of orthophosphate remaining in the diffusers. Of the original 30 g of orthophosphate that was added to each diffuser in P addition treatments, an average of 1.21 ± 0.11 mg cm^{-3} of orthophosphate diffused into the system over 42 d.

Physicochemical Conditions

Surface water temperature (28.8 ± 1.1 °C, mean ± SE) and salinity (31.2 ± 3.7 ppt) were not affected by treatments and did not vary over time except for lower water temperature measured on July 19, 2013 (all, ANOVA $P > 0.05$).

Surface water SRP and TOC consistently increased with P addition throughout the study. Surface water DOC did not change with defoliation or P addition but steadily increased over time in all monoliths. Surface water DIN increased for all monoliths over time but was consistently lower with P addition. Finally, surface water TN varied with time and was not affected by defoliation or P addition, whereas TP increased with P addition and remained elevated over time (Table S1).

Porewater DOC was not affected by defoliation or P addition and did not change over time. However, porewater DIN varied with sample date and was higher in the defoliation, P addition, and P addition/defoliation treatments. Porewater SRP changed over time and was higher in the P addition treatments. Soil CO_2 efflux varied over time and was suppressed by defoliation but was unaffected by P addition (Table S1).

Plant Biomass

After the 42-d experiment, there were no differences in final mangrove leaf count among the four treatments (Fig. 2a; ANOVA, $P = 0.24$). However, the change in leaf number showed a net decrease in the number of leaves on control plants (-36 ± 24) and no net change in the P addition treatment (-4 ± 11). Compared to the control, the defoliation and P addition/defoliation treatments had significant net increases in the number of leaves present and ended the experiment with 49 ± 6 and 58 ± 13 net increases in the number of leaves respectively (Fig. 2b; ANOVA, $P < 0.01$),

Final leaf biomass AFDM was highest in the control and P addition and significantly lower in the defoliation and P addition/defoliation treatments (Fig. 3a; ANOVA, $P < 0.01$). Individual leaf biomass (final leaf count/final leaf biomass AFDM) at 42-d was lower for the defoliation and P addition/defoliation treatments (ANOVA, $P < 0.01$).

Aboveground woody biomass (g AFDM) was the same across all treatments at the end of the experiment (Fig. 3b; ANOVA, $P > 0.05$). Belowground coarse root biomass was the same across all treatments at the end of the experiment (Fig. 3c; ANOVA, $P > 0.05$). The average root-to-shoot biomass ratio was 1.13 ± 0.18 and was the same across all treatments at the end of the experiment (ANOVA, $P > 0.05$).

Plant and Soil Elemental Stoichiometry

Soil %C, %N, and %P were not different at any soil depth for either defoliation or P addition (all responses, $P > 0.05$, Table S2). Soil C:N, C:P, and N:P were not different at any soil depth for either defoliation or P addition (all responses, $P > 0.05$, Table S3). Final leaf %C and %N was not different among the four treatments (ANOVA, $P > 0.05$); however, final leaf %P was higher in the P addition treatments compared to control (ANOVA, $P < 0.01$). Plants increased % P in live leaf and root tissue with P addition (Fig. 4, $P = 0.01$). Final leaf C:N was not different among the four treatments (ANOVA, $P > 0.05$); however, leaf C:P and N:P were lower with defoliation (ANOVA, both $P = 0.02$) and added P (ANOVA, both $P < 0.01$; Table S3). Final root C:N increased within the P addition treatments (ANOVA, $P = 0.04$) and final root C:P was lower within the P addition treatments (ANOVA, $P < 0.01$; Table S3). Final root N:P was lower with P addition compared to controls (ANOVA, $P < 0.01$) and there was an interaction between P addition and defoliation which lowered N:P compared to controls (ANOVA, $P = 0.04$; Table S3).

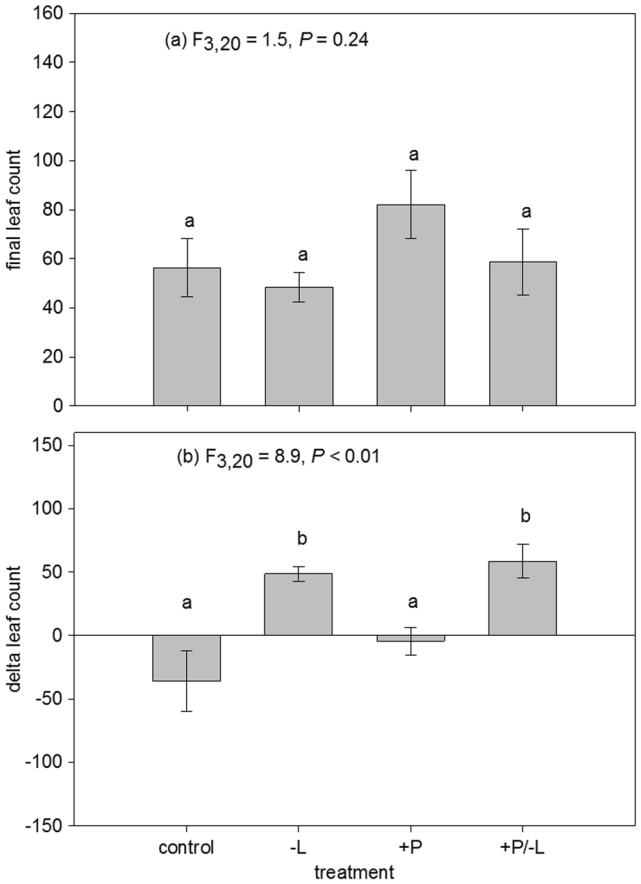

Fig. 2 Final leaf count (**a**) and delta leaf count (**b**) for the four treatments control, leaves removed (−L), phosphorus addition (+P), and leaves removed and phosphorus addition (+P/−L). Final leaf count (**a**) was determined by counting the total number of live leaves present on each mangrove plant on the last day of the experiment. Delta leaf count (**b**) was determined by subtracting the number of leaves after the defoliation treatment was administered from the final leaf count. Treatments were compared using an ANOVA followed by a Tukey HSD for comparison. *P*-values less than 0.05 were considered significant

Root and Leaf Litter Breakdown Rates

Decomposing root and leaf litter deployed in each experimental core did not differ in %C, %N, or %P. Decomposing leaf k was not different among treatments (Fig. 5a; ANOVA, $P > 0.05$). However, decomposing root material showed

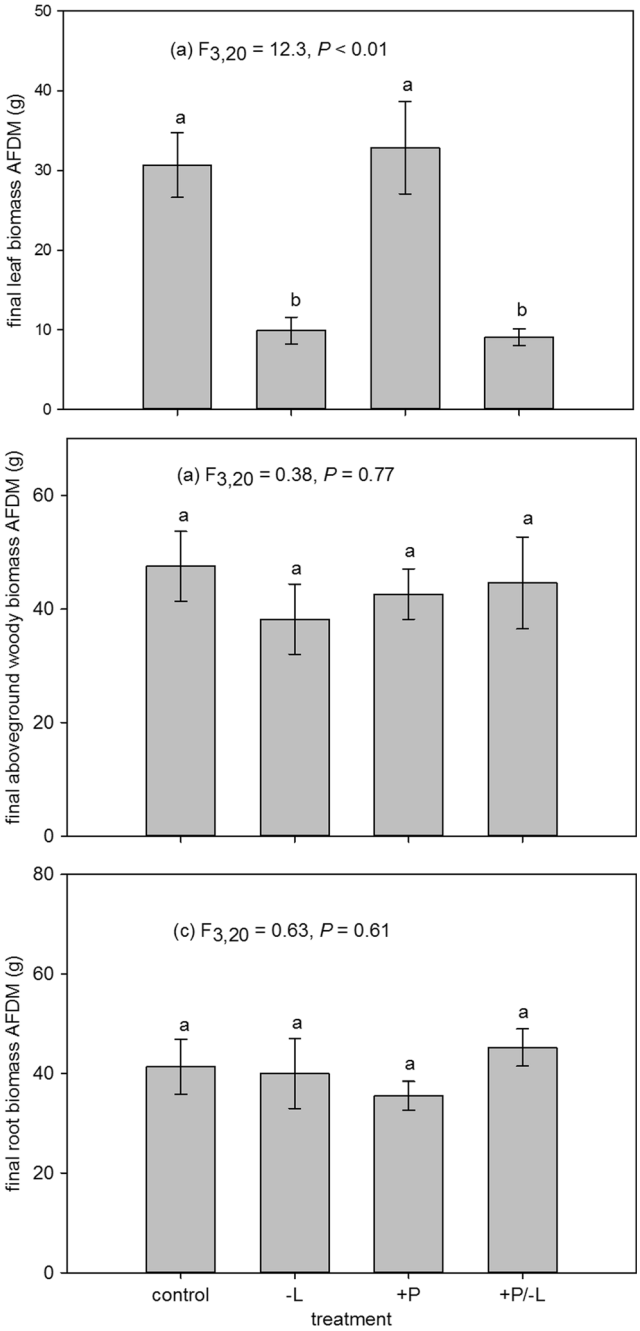

Fig. 3 Final aboveground leaf biomass (**a**), final aboveground woody biomass (**b**), and final belowground coarse woody biomass (**c**) for the four treatments control, leaves removed (−L), phosphorus addition (+P), and leaves removed and phosphorus addition (+P/−L). Biomass is reported as ash-free dry mass (AFDM). Treatments were compared using an ANOVA followed by a Tukey HSD for comparison. P-values less than 0.05 were considered significant

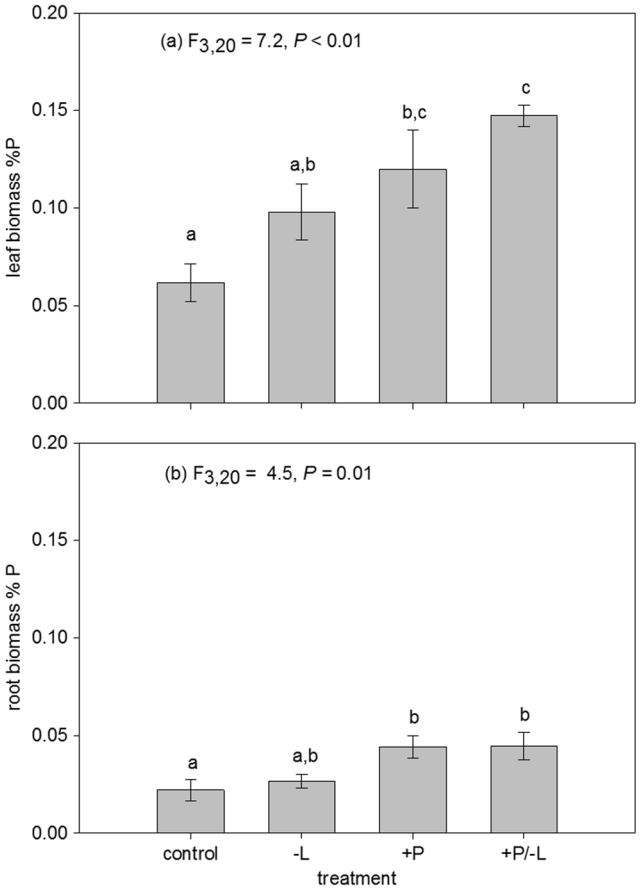

Fig. 4 Final leaf (**a**) and root (**b**) live biomass % phosphorus (P) for the four treatments control, leaves removed (−L), P addition (+P), and leaves removed and P addition (+P/−L). Treatments were compared using an ANOVA followed by a Tukey HSD for comparison. P-values less than 0.05 were considered significant

slower k in defoliation treatments (Fig. 5b; ANOVA, P = 0.03). At the end of the experiment, %C and %N of decomposing root and leaf litter were not different among the four treatments; however, the %P in decomposing root litter, but not leaf litter, was higher with P addition (Table S2).

Decomposing root litter C:N, C:P, and N:P were not different among treatments (all responses, $P > 0.05$, Table S3). However, P addition decreased decomposing leaf litter C:P (ANOVA, $P = 0.03$) and N:P (ANOVA, $P = 0.01$; Table S3). Decomposing leaf litter C:N was not different among treatments (ANOVA, $P > 0.05$).

Soil CO_2 Efflux

Weekly soil CO_2 efflux was suppressed in the defoliation and P addition/defoliation treatments and varied over time (Fig. 6; ANOVA, $P < 0.05$). We also plotted the response of day and nighttime CO_2 efflux against the total amount of P released from each P addition treatment. Daytime CO_2 efflux was not correlated to increased P released over the whole six weeks (Fig. 7a; $R^2 = 0.16$, $P = 0.19$). Nighttime CO_2 was positively associated with the total amount of P released over the course of the experiment (Fig. 7b; $R^2 = 0.37$, $P = 0.04$). However, both trends were positive.

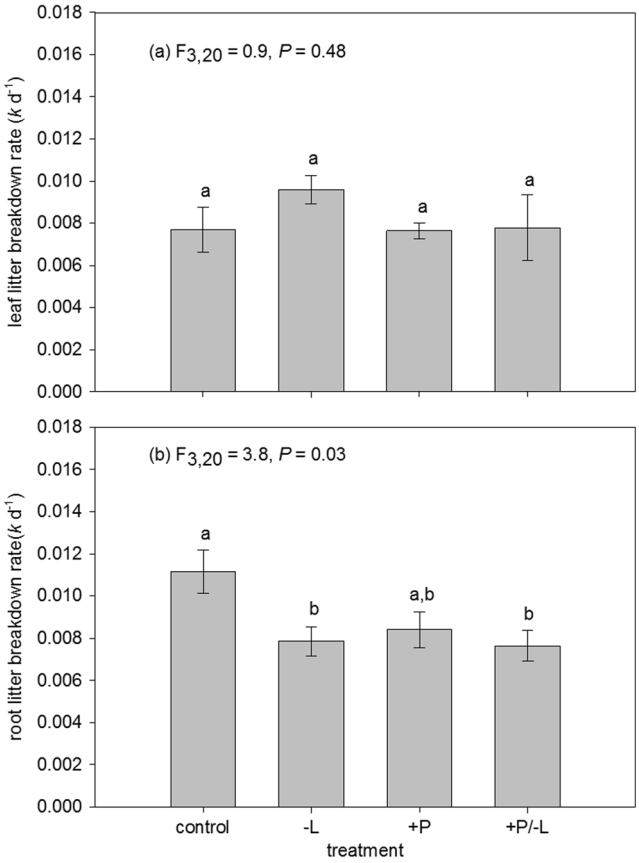

Fig. 5 Decomposing leaf (**a**) and root (**b**) breakdown rates (k d^{-1}) for the four treatments control, leaves removed (−L), phosphorus addition (+P/−L), and leaves removed and phosphorus addition (+P/−L). Treatments were compared using an ANOVA followed by a Tukey HSD for comparison. *P*-values less than 0.05 were considered significant

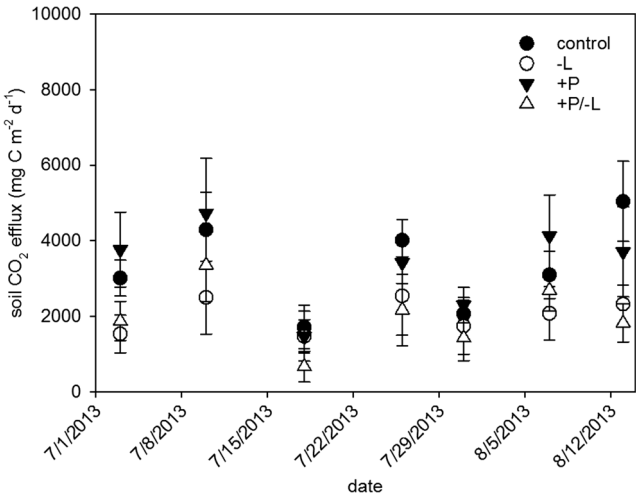

Fig. 6 Weekly daytime soil CO_2 efflux (CO_2 – C, mg C m^{-2} d^{-1}) for all for the four treatments control, leaves removed (−L), phosphorus addition (+P/−L), and leaves removed and phosphorus addition (+P/−L). Treatments were compared using a repeated measures ANOVA followed by a Tukey HSD for comparison. P-values less than 0.05 were considered significant

Discussion

Our objective was to understand how exposure to a subsidy (P addition) can influence effects of episodic stress (defoliation) on C and nutrient cycling of mangrove plants and soils. We predicted that addition of P would increase mangrove leaf and root tissue P content, soil P content, litter breakdown rates, and soil CO_2 efflux. We found that P addition resulted in net increases in plant tissue P storage, but soil P did not change. We detected increases in nighttime soil CO_2 efflux attributed to P addition; however, this effect was not present during daytime measurements. We expected defoliation would increase soil CO_2 efflux because of increased soil microbial and root respiration, however, we found defoliation decreased soil CO_2 efflux. Decreased soil CO_2 efflux indicates a potential belowground pathway to maintain soil C storage following disturbance. Finally, we predicted that plants with added P would regenerate leaves more quickly than those without added P, but our results indicate that P addition does not enhance mangrove sapling leaf growth following defoliation after 42-d.

Nutrient subsidies can influence ecosystem recovery following disturbances if plants can retain and incorporate nutrients into biomass. We measured rapid assimilation of added P by *R. mangle*, and added P was incorporated mangrove roots and leaves. Our results only partially supported our prediction that P addition would lead to increases in P content soil and mangrove leaves and roots. Phosphorus addition increased P content in leaf and root tissue, but did not affect soil total P. Mangrove saplings in our experiment were likely more competitive than soil microbes and consequentially able to incorporate more P into live leaf and root tissue, especially since we added inorganic P (Schachtman et al. 1998; Reef et al. 2010). Incorporating the available P in live leaf and root tissue resulted in stoichiometric changes within leaves which had decreased C:P and N:P ratios. Mangroves removed and sequestered added P from the soil or water column where P was dissolved, incorporating it into living leaf and root tissue. Phosphorus inputs following storm surges may result in plant uptake of available P.

The degree of nutrient limitation among ecosystems determines the relative demand for internal and external nutrient sources to maintain plant growth (Bloom et al. 1985). Previous research has shown that nutrient exposure increases mangrove leaf biomass (Feller et al. 2015), decreases the proportion of belowground relative to aboveground biomass (Castañeda-Moya et al. 2012), and promotes mangrove productivity following a storm (Lovelock et al. 2011). However, contrary to our initial prediction that P addition would help mangroves recover from defoliation, we did not see effects of P addition on living leaf biomass. Interestingly, P addition did not result in increased number of leaves nor did it enhance the recovery of leaf count in the P addition/defoliation treatment. Instead, P addition treatment allowed mangrove plants to

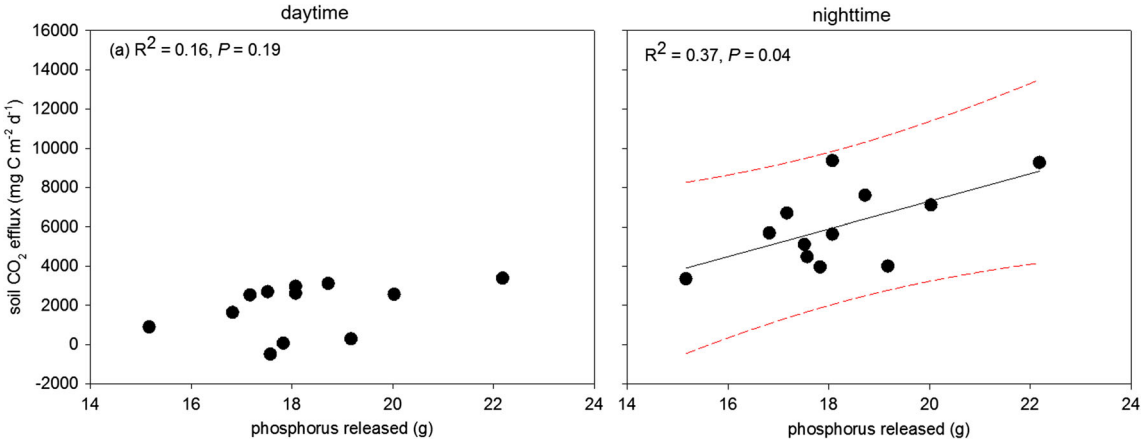

Fig. 7 Final **a** daytime and **b** nighttime soil CO_2 efflux (CO_2 – C, mg C m^{-2} d^{-1}) plotted against the total amount of phosphorus (g) released by diffusion by the end of the 42-d experiment. Phosphorus released was calculated by subtracting the amount of granular orthophosphate remaining in the diffusers at the end of the experiment from the starting amount of orthophosphate. Linear regression (solid line) and 95% confidence interval (dashed lines) are plotted when significant ($P < 0.05$)

maintain their leaves throughout the experiment whereas mangroves in the control treatment on average lost 0.85 leaves per day. Although mangrove plants typically have the highest leaf fall during summer months, when our study was conducted, only the controls had net decreases in the number of leaves (Lugo and Snedaker 1974). The growth of mangroves in the control treatment was likely P limited indicating that marine-derived P subsidies are essential for mangroves growth.

Mangroves rapidly regenerate leaves following defoliation but regaining pre-disturbance leaf biomass requires months to years. Even though the final leaf count was similar across treatments, the total biomass measured after the 42-d study was reduced in the defoliation and the P addition/defoliation treatment. Leaves regenerated quickly following the defoliation as was observed by Danielson et al. (2017); however, the new leaves were smaller and did not reach pre-treatment sizes within 42-d. Phosphorus exposure following defoliation did not promote leaf biomass recovery. The lack of a response to P addition in aboveground biomass production is another potential mechanism behind mangrove forest resilience as the overproduction of leaf biomass, without similar increases in belowground biomass, enhances mangrove susceptibility to hurricane-induced damage (Feller et al. 2015). Chronic nutrient loading in coastal ecosystems may exacerbate the damage caused by storm surge by disproportionally increasing aboveground biomass relative to belowground biomass (Lovelock et al. 2009); whereas, storm-delivered pulses of nutrient subsidies, occurring in tandem to the disturbance, may aid in forest recovery (Herbert et al. 1999). Perhaps the timing of nutrient addition, either long before or following hurricane disturbance, regulates the recovery process.

Soil processes were more sensitive to defoliation stress than P subsidy. Despite our prediction that P would stimulate litter *k* and soil respiration rates, we did not detect effects of added P on soil microbial processes except for soil CO_2-efflux. Microbial communities in reduced environments, like wetland soils, may be unable to use excess P because of oxygen limitation, suggesting greater thermodynamic than nutrient limitation in these ecosystems (Helton et al. 2015). It is also important to note that there was no difference in %P in leaf litter and bulk soil and only a slight increase 0.01% in root liter P with the addition of P (Table S2). However, leaf litter C:P and N:P was lower with P addition indicating increased P availability resulted in higher P content relative to C and N. Previous work has shown that the effects of long-term nutrient addition on mangrove litter breakdown is mediated by changes in litter quality instead of direct effects of nutrient addition on breakdown (Keuskamp et al. 2015). We did not measure indirect effects of P enrichment on litter breakdown. However, if we were to use the P-enriched biomass for breakdown litter, as is observed in coastal mangroves of Shark River Slough, we would potentially have found increased breakdown rates (Keuskamp et al. 2015). Episodic P deliveries that co-occur with disturbance may be less likely to enhance litter breakdown, which may help maintain C storage in wetlands. Defoliation reduced breakdown rates of the more recalcitrant root litter, which may have been caused by decreases in root exudates (Vančura and Staněk 1975). In our experiment, root litter breakdown was likely more dependent on priming from plant exudates than the inorganic addition of P (Kuzyakov 2002). The release of root exudates and rhizosphere priming provide energy sources to the soil microbial community to enhance organic matter breakdown but also represent an energy investment for the plant (Dijkstra et al. 2013). Therefore, the reduction of decomposition in the defoliation treatment may indicate that defoliated mangroves invest available energy into the production of leaves and decrease root exudate release. The suppression of below-ground breakdown following a loss of above-ground C is a potential pathway towards mangrove forest recovery of the valuable ecosystem service of storing C.

We detected decreased soil CO_2 efflux within the defoliated treatment that reveals a potential pathway for maintaining belowground C stocks in mangrove forests following disturbance. In our study, weekly daytime soil efflux of CO_2 was reduced in the defoliation treatments. Previous studies in grassland ecosystems have also measured decreased soil respiration following defoliation (Guitian and Bardgett 2000). Similarly, previous studies within the Everglades have indicated that daytime net ecosystem exchange returns to pre-disturbance levels as soon as two years following a hurricane (Barr et al. 2012). The recovery of daytime CO_2 uptake after a hurricane reflects the resilience of C storage to the frequent disturbance from hurricanes in the Florida Everglades region (Smith et al. 1994; Zhang et al. 2008). Reduced C losses could represent the soil contribution to mitigating C loss from the system through the reduced breakdown of recalcitrant decomposing material; however, more evidence is needed to link soil CO_2 efflux to net ecosystem exchange. Consistent with the expectation that P increases CO_2 loss from the soil, we detected a positive relationship between the total amount of P released and nighttime CO_2 efflux, in the P addition treatments. Increased nighttime CO_2 efflux was evident following Hurricane Wilma (Barr et al. 2012). Our findings suggest that for each additional gram of P diffused into the P addition cores, daily CO_2 efflux was increased by 703 mg C m^{-2} d^{-1}.

Our study illustrates how mangroves differentially respond to interactions of stress (defoliation) and subsidy (P addition) common to coastal wetlands. High-energy storms can defoliate large areas of mangrove forests and often occur in conjunction with P-rich marine sediment deposition. When mangroves are defoliated they regenerate lost leaves, and soil C losses from respiration and breakdown are reduced. When nutrient availability is increased for mangroves they quickly incorporate available P into living biomass, and increase nighttime C losses from respiration. We predicted that P addition would help offset adverse effects of defoliation; however, the P/defoliation treatment often displayed similar patterns to the dominant treatment that was controlling a particular response variable. We only detected an interaction between added P and defoliation in the leaf tissue P content, wherbye the P/defoliation treatment had the highest P in live leaf tissue. However, there are limitations to small-scale, short-term mesocosm studies. We were able to quantify short responses of mangroves to defoliation and P addition, but long-term interactive subsidy-stress effects are likely different. Although our study provides a controlled setting for simulating interactions between disturbance and nutrient enrichment, we were unable to replicate conditions of high-energy storms. For example, we added nutrient subsidies and root litter directly to soils instead of depositing it on the soil surface to isolate belowground soil responses where the majority of C is stored. Despite these limitations, our experimental manipulation enabled us to identify essential mechanisms that help inform how mangroves and their soils respond to combinations of subsidies and stressors as well as the differential sensitivities of plant and soil responses.

Conclusions

Mangrove forests provide critical ecosystem services such as timber, fuel, medicine, habitat for wildlife, wave attenuation, sediment accumulation, and C sequestration (Twilley 1995; Kathiresan and Bingham 2001; Saenger 2002; Manson et al. 2005; Mazda et al. 2007; Zhang et al. 2012; Jerath et al. 2016). The ability of mangrove forests to provide these services depends upon their recovery and adaptation to high-energy disturbance events, such as tropical storms and hurricanes. Changes in storm frequency and intensity combined with altered nutrient delivery may destabilize coastal wetlands if these ecosystems are unable to adapt (Odum et al. 1995). As climate changes allows for mangroves to expand into continental interiors and latitudinally understanding how mangrove saplings, which would dominate newly colonized regions, are susceptible to disturbance is increasingly important (Comeaux et al. 2012; Bianchi et al. 2013). It is essential to understand specific mechanisms behind ecosystem responses to stressors and subsidies to better inform ecosystem management and keep anthropogenic impacts within a "safe operating space" (Green et al. 2017). Our results suggest that mangrove saplings respond differently to defoliation and P addition; however, the P addition/defoliation treatment often displayed similar patterns to the dominant treatment that was controlling a particular response variable. Mangrove saplings controlled P retention, and belowground processes were the dominant control on changes in C retention. Our results highlight the need to better identify potential tipping points following disturbance and how the timing and severity of nutrients can influence ecosystem recovery following disturbance.

Acknowledgements We thank S. Charles, L. Marazzi, D. Mills, B. Wilson, and L. Zhai for providing extensive feedback during the development of the manuscript. Funding for this research was provided by the National Science Foundation (NSF) award (DBI-1237517) to the Florida Coastal Everglades Long Term Ecological Research Program. We thank A. Downey-Wall, I. Giles, the Everglades Section of the South Florida Water Management District and Everglades National Park, for providing facilities and support during this research. This publication is contribution number 891 for the Southeast Environmental Research Center.

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