

# Science of the Total Environment

## USE OF A WASTEWATER RECOVERY PRODUCT (STRUVITE) TO ENHANCE SUBTROPICAL SEAGRASS RESTORATION

--Manuscript Draft--

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<b>Keywords:</b>	Halodule wrightii; Seagrass; Marine restoration; Fertilizer; struvite; Phosphorus
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<b>Abstract:</b>	<p>Seagrasses are in decline worldwide, and their restoration is relatively expensive and unsuccessful compared to other coastal systems. Fertilization can improve seagrass growth in restoration but can also release nutrients and pollute the surrounding ecosystem. A slow-release fertilizer may reduce excessive nutrient discharge while still providing resources to the seagrass’s rhizosphere. In this study, struvite (magnesium ammonium phosphate), a relatively insoluble, sustainable compound harvested in wastewater treatment plants, was compared to Osmocote™ (14:14:14 Nitrogen: Phosphorus: Potassium, N:P:K), a popular polymer coated controlled release fertilizer commonly used in seagrass restoration. Two experiments compared the effectiveness of both fertilizers in a subtropical flow-through mesocosm setup. In the first experiment, single 0.5 mg of P per g dry weight (DW) doses of Osmocote™ and struvite fertilizers were added to seagrass plots. Seagrass shoot counts were significantly higher in plots fertilized with struvite than both the Osmocote™ and unfertilized controls (<math>p &lt; 0.0001</math>). A significant difference in total P concentrations was observed in porewater samples of Osmocote™ vs struvite and controls (<math>p &lt; 0.0001</math>), with struvite fertilized plots emitting more than controls (<math>p &lt; 0.0001</math>), but less than 2% of the total dissolved P (TDP) of Osmocote™ fertilized plots (100+ mg/L versus <math>x &gt; 5</math> mg/L). A subsequent experiment, using smaller doses (0.01 and 0.025 mg of P per gram DW added), also found that the struvite treatments performed better than Osmocote™, with 16-114% more aboveground biomass (10-60% higher total biomass) while releasing less N and P. These results indicate the relatively rapid dissolution of Osmocote™ may pose problems to restoration efforts, especially in concentrated doses and possibly leading to seagrass stress. In contrast, struvite may function as a slow-release fertilizer applicable in seagrass and other coastal restoration efforts.</p>
<b>Response to Reviewers:</b>	<p>Reviewer 1                  Page 1, Paragraph 1, Line 11 (Abstract): In the first experiment, single 0.5 mg of P per g DW doses of Osmocote™ and struvite fertilizers - What do you mean by single 0.5... and spell out DW. Changed to “dry weight (DW),” 0.5 mg of P per g DW means that for every gram of sediment 0.5 mg of P was added via fertilizer.                  Page 6, Paragraph 15, Line 130: spell out PCF. Done.                  Page 6, Paragraph 15, Lines 128-132: Please explain your decision of 60 and 70-day experiment. Added “the length of each experiment was based on typical H. wrightii</p>

transplantation recovery times combined with time limitations on the use of the mesocosms at the marine lab.”

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Figure 4: Very nice data analysis but please spell out the title of the axis and their statistical meaning. A more detailed graph/caption has been produced.

However, too often researchers making a huge theoretical leap to the real world where conditions are not in favor of their mesocosms results. The authors touched on this point rather lightly by acknowledging the fact the sea currents, redox potential and other factors may obliterate the advantage of struvite over commercial fertilizer. I would have support the publication of this manuscript if the authors were able to show that their findings are applicable in a real world conditions. Added two new paragraphs within a new section “Field Applications of the Study” addressing the applicability of the mesocosm study to field conditions.

#### Reviewer 3

Page 1, Paragraph 1, Line 9: or N-P2O5-K2O? Yes, however the composition is referred to as 14:14:14 NPK.

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but P2O5 for sure.... We did not analyze the composition of Osmocote, however, Osmocote has an ingredients list stating that its fertilizer is composed of P as P2O5. P2O5 is the standardized terminology for P in traditional fertilizers (Tisdale, Page 9, Paragraph 22, Lines 201, 206: Blank, don't know how to respond. Page 11, Paragraph 27, Line 233: What is this unit? Please specify. Parts per thousand, added the unabbreviated name.

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**Submission date: 1/11/22**

Professor Damià Barceló  
Co-Editor in Chief  
Science of the Total Environment

Dear Professor Damià Barceló,

We are pleased to submit our manuscript entitled: “Use of wastewater recovery product (struvite) to enhance subtropical seagrass restoration”, for consideration as an original research paper. This study consists of two comparative mesocosm experiments testing the effectiveness of *in situ* mineral fertilization on seagrass restoration. Seagrass is one of the most ecologically valuable marine ecosystems, providing estimated \$29,000 ha<sup>-1</sup> yr<sup>-1</sup> in ecosystems services. Yet, despite its value, seagrass beds are declining worldwide in alarming rate.

*In situ* fertilizer application is a commonly applied seagrass restoration technique, characterized with high rates of success. However, the key drawback of the process is the use of unsustainable fertilizers such as phosphate rock or commercially manufactured controlled release NPK fertilizers. Such an approach can largely offset potential ecological benefits of successful restoration projects. Therefore, we propose the use of a wastewater recovery product, struvite, as an alternative to manufactured commercial fertilizers. Struvite is a widely recognized and applied slow release fertilizer obtained during the wastewater treatment process. Use of struvite introduces elements of sustainability and circular economy in seagrass restoration efforts. In our study, we compared the effects of a popular polymer coated fertilizer (Osmocote) commonly used in seagrass restoration with struvite on seagrass growth and porewater nutrient release. The experiments found that struvite at equivalent doses to Osmocote produced significantly higher seagrass metrics (shoot count and biomass) while emitting significantly fewer nutrients (total dissolved nitrogen and phosphorus). These results demonstrate the potential effectiveness of struvite in seagrass restoration and the relatively rapid dissolution of Osmocote, a potential issue for restoration efforts/local water quality. These findings may be important to providing an effective but low nutrient emission fertilizer for applications in coastal restoration. Within the aims and scope of the Science of the Total Environment, the results of this study are relevant to the subjects of stress ecology in marine ecosystems (or attempts to reduce stress in a sensitive ecosystem) and water quality. This study applies a novel compound in a semi-controlled environment that interconnects with multiple spheres (including the hydrosphere, biosphere, and lithosphere).

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other peer-reviewed media. Thank you for your consideration.

Sincerely,

Dr. Conor MacDonnell

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11 **Authors:** MacDonnell, C.<sup>a,1</sup>, Bydalek, F.<sup>b,2</sup>, Osborne, T.Z.<sup>c</sup>, Beard, A.<sup>c</sup>, Barbour, S.<sup>a</sup>,  
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## Response to Reviewers

### Reviewer 1

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#### **Other edits**

##### **Title Page**

T. Osborne changed to T.Z. Osborne.

P. Inglett added as corresponding author.

##### **Manuscript:**

Figure 2: Adjusted color scheme of 2B to match 2A.

Tables: Moved all tables to supplementary material, added two tables for sediment nutrients/significant differences.

Mesocosm conditions: Moved from the results section to methods (site description) for greater clarity of environmental conditions.

## 1 Abstract

2 Seagrasses are in decline worldwide, and their restoration is relatively expensive and  
3 unsuccessful compared to other coastal systems. Fertilization can improve seagrass  
4 growth in restoration but can also ~~excessively~~ release nutrients and pollute the  
5 surrounding ecosystem. A slow-~~release~~ fertilizer may reduce excessive nutrient  
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7 struvite (magnesium ammonium phosphate), a relatively insoluble ~~fertilizer sustainably,~~  
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15 higher in plots fertilized with struvite than both the Osmocote™ and unfertilized controls  
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23 less nitrogenN and phosphorusP. These results indicate the relatively rapid dissolution

24 of Osmocote™ may pose problems to restoration efforts, especially in concentrated  
25 doses and possibly leading to seagrass stress. In contrast, struvite may function as a  
26 slow-release fertilizer applicable in seagrass and other coastal restoration efforts.

27 **Keywords:** *Halodule wrightii*; seagrass; marine restoration; fertilizer; struvite;  
28 Osmocote™; phosphorus

## 29 1. **4-Introduction**

30 In many environments, restoration ~~can be is~~ improved by fertilization, lessening  
31 nutrient limitations and improving growth of desired species (Armitage et al., 2011;  
32 Balestri & Lardicci, 2014; Fereidooni et al., 2013; Holmes, 2001; Jaquetti et al., 2014;  
33 Reed et al., 2007). ~~For example, Jaquetti et al. (2014) found that fertilization more than~~  
34 ~~doubled the absolute growth rate and significantly improved the photosynthetic~~  
35 ~~response of trees applied in a degraded rainforest restoration site.~~ However, in some  
36 environments, fertilizers can have a negative effect on species diversity and in extreme  
37 cases may even pollute the surrounding environment (Fonseca et al., 1998; Hill & Heck,  
38 2015; Zedler, 2000). ~~For example, nitrogen fertilization often contributes to coastal~~  
39 ~~hypoxia and nitrous oxide emissions (Robertson & Vitousek, 2009). Therefore,~~  
40 ~~consideration of the ecosystem, nutrient needs, and type of fertilizer is important to~~  
41 ~~maximizing the benefits of fertilization approaches while minimizing the environmental~~  
42 ~~impact of fertilizer use. Therefore, consideration of the ecosystem, nutrient needs, and~~  
43 ~~type of fertilizer is important to maximizing the benefits of fertilization approaches while~~  
44 ~~minimizing the environmental impact of fertilizer use.~~

45 ~~Balancing the positive and negative~~The ramifications of fertilizer use ~~is are~~  
46 especially relevant in coastal seagrass ~~ecosystem restoration. Seagrass ecosystems~~  
47 ~~are systems, which are both~~ important ~~coastal ecosystemshabitats and currently~~ facing

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48 global declines due to ~~direct~~ human disturbance and climate change (Bayraktarov et al.,  
49 2016). Seagrasses are a comparatively difficult and expensive coastal ecosystem to  
50 restore, partially due to eutrophication, competition from algae and other nutrient related  
51 issues (ibid). However, fertilizers have been consistently found to improve seagrass  
52 health and restoration success (~~Armitage et al., 2011; Kenworthy et al., 2018~~)(Armitage  
53 et al., 2011; Kenworthy et al., 2018). ~~Therefore, it is critical to provide a fertilizer that~~  
54 ~~directs nutrients toward seagrass growth and minimizes the release of nutrients to the~~  
55 ~~surrounding environment.~~

56 Traditionally, both the direct application of controlled release fertilizers (Armitage  
57 & Fourqurean, 2016; Fonseca et al., 1998; Peralta et al., 2003; Sheridan et al., 1998)  
58 and the deployment of bird roosting stakes (Fonseca et al., 1994; Furman et al., 2019)  
59 have positive effects on seagrass ~~above and belowground biomass in multiple systems.~~  
60 Ecosystem, and can accelerate ecosystem succession for seagrass ~~also appears to be~~  
61 ~~accelerated by the addition of nutrients in the short and long term~~ (Bourque &  
62 Fourqurean, 2014; Armitage et al., 2011). However, the use of traditional fertilization  
63 techniques in seagrass restoration may result in variable levels of nutrients or over-  
64 fertilization (~~Fonseca et al., 1998; Kenworthy et al., 2018~~)(Fonseca et al., 1998;  
65 Kenworthy et al., 2018), with consequences for the succession of seagrass species  
66 (ibid).

67 One of the main issues with fertilization in aquatic seagrass systems is ~~the~~  
68 ~~difficulty~~ that immersion and hydrodynamics can lead to rapid dissolution of fertilizers,  
69 increasing short term nutrient availability to the desired plant species, but at the  
70 expense of nutrient loss, ecosystem disruption, and pollution (Fonseca et al., 1998; Hill

71 & Heck, 2015; Olsen & Valiela, 2010). For example, Hall et al. (2006) had to replace  
72 buried fertilizer pellets every three to four months in a macrophyte restoration effort,  
73 while Herbert and Fourqurean (2008) found that bird ~~stake fertilization~~stakes (bird  
74 roosting structures that promote feces accumulation, Fonseca et al., 1994; Furman et  
75 al., 2019) can overfertilize seagrass ~~restoration~~ sites, disrupting succession and  
76 increasing epiphytic biomass. These drawbacks are due either to the fertilizers being  
77 adapted for terrestrial applications, releasing nutrients too rapidly after flushing with  
78 water, or in the case of bird stakes, due to variable rates of feces deposition combined  
79 with diffusion of nutrients in the water during precipitation and settling (Hill & Heck,  
80 2015). Applying multiple doses of traditional mineral fertilizers ~~incurs a significant~~  
81 ~~financial and labor cost~~ (Ferdie & Fourqurean, 2004; Hall et al., 2006; Olsen & Valiela,  
82 2010). ~~Similarly, the or monitoring~~ bird stake ~~approach requires extra labor to~~  
83 ~~monitor~~treated beds for symptoms of excess fertilization ~~and remove the stakes after~~  
84 ~~about 18 months (Kenworthy et al., 2018). Thus, a slower dissolving fertilizer resistant~~  
85 ~~to leaching may reduce overfertilization with less labor inputs while still providing~~  
86 ~~benefits toward seagrass growth and survival. (Kenworthy et al., 2018) also incurs a~~  
87 significant financial and labor cost. Thus, a slower dissolving fertilizer that resists  
88 leaching may reduce overfertilization and labor expenses while still providing benefits  
89 toward seagrass growth and survival.

90 Struvite (magnesium ammonium phosphate, or  $MgNH_4PO_4 \cdot 6H_2O$ ) is a by-  
91 product of wastewater treatment that is harvested in separated, side-stream sludge  
92 management processes (Ghosh et al., 2019). ~~Struvite forms when equal molar ratios of~~  
93  ~~$Mg^{2+}$ ,  $NH_4^+$ ,  $PO_4^{3-}$  occur in the solution, thus the feeding sources are typically nutrient-~~

94 ~~rich sludge dewatering liquors or digestate often dosed with an external source of~~  
95 ~~magnesium (Kumar & Pal, 2015; Martí et al., 2010).~~ Struvite is poorly soluble in water,  
96 but releases P more rapidly in the presence of organic acids exuded from roots, making  
97 it a potentially ideal fertilizer for direct plant uptake (Cabeza et al., 2011; Robles-Aguilar  
98 et al., 2019). Past studies have supported both high performance of struvite for  
99 terrestrial plant applications as well as its resistance to flushing (Lee et al., 2009;  
100 Rahman et al., 2014). ~~Struvite application for restoration purposes would also support a~~  
101 ~~more sustainable wastewater management through the increased use of recovered~~  
102 ~~resources (Mayer et al., 2016) and introduced restoration activities into a circular~~  
103 ~~economy.~~

104 While the utilization of struvite in aquatic systems appears very promising, to  
105 date there is an absence of studies investigating this fertilizer in marine restoration  
106 projects, especially in combination with other fertilization techniques. While it has been  
107 demonstrated that struvite is poorly soluble fertilizer except when exposed to acidic  
108 conditions (Cabeza et al., 2011; Talboys et al., 2016), experiments determining the  
109 availability of struvite to submerged aquatic vegetation do not currently exist. Thus, the  
110 goals of this study were to 1) assess potential differences in seagrass performance (~~e.g.~~  
111 ~~metrics like~~ shoot count, growth, length, and biomass as defined by Arrington, 2008,  
112 Herbeck et al., 2014, Rezek et al., 2019, Short & Coles, 2001, and Thomsen et al.,  
113 2012, ~~among others~~) after addition of struvite versus a polymer coated, 'slow  
114 ~~release~~' controlled release fertilizer (PCF, Osmocote™) commonly used in seagrass  
115 restoration, and 2) to determine shifts in sediment and porewater nutrients caused by  
116 the introduction of the fertilizers in plots with and without seagrass. We hypothesized





117 that seagrass in plots fertilized with struvite would have increased performance  
118 compared to plots fertilized with Osmocote™, and that struvite would be dissolved at a  
119 slower rate than Osmocote™ (~~determined by measuring based on~~ porewater total  
120 dissolved nutrients).

## 121 **2. 2- Materials and Methods**

### 122 **2.1.2-1 Site Description and Design**

123 To minimize the variability found in field experiments and more accurately  
124 investigate nutrient levels related to fertilization, a mesocosm experiment was  
125 conducted at the Whitney Laboratory of Marine Biosciences in St. Augustine, FL.  
126 Seawater (filtered through a shelly sand and activated charcoal biofilter) pumped from  
127 offshore entered a 6.5 m diameter mesocosm (approximately 1 m deep), to emulate the  
128 natural environment. Water flow was constant into the mesocosm. Experiments were  
129 based on the methods explained in the propagation guide for *Halodule wrightii*,  
130 ~~prepared by the University of Southern Mississippi~~ (Biber et al., 2013). Seagrass was  
131 collected directly from donor sites off St. Martins Marsh Aquatic Preserve, FL. Shoots  
132 were removed from the donor sediment and maintained in cool conditions until they  
133 were transplanted into plastic pot containers (10 cm depth), buried in approximately 5  
134 cm of coarse, shell-dominated sand taken from the local St. Augustine area (rinsed to  
135 reduce organics and residual nutrients). The sediment used ~~comprised at least 99%~~  
136 ~~sand with had~~ a mean grain size of 706 microns (not including particles greater than 2  
137 mm).

#### 138 **2.1.1 Mesocosm Conditions**

139 ~~2.2- Mesocosm temperature and salinity remained between 27-31 °C and 33-38~~  
140 ~~parts per thousand respectively during the periods sampled (between 9 am and 3 pm)~~

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141 for both studies. The hydraulic residence time was variable at 0.5-2 days, due to a  
 142 limited saltwater supply. The mean TDN of surface water was  $0.44 \pm 0.06 \text{ mg N L}^{-1}$ ,  
 143 while the mean TDP was  $0.035 \pm 0.001 \text{ mg P L}^{-1}$  (or  $0.029 \text{ mg P L}^{-1}$  when excluding a  
 144 day of low inflow). The level of flow was great enough to prevent significant cross  
 145 contamination of the plots studied, as well as prevent significant swings in temperature  
 146 and salinity that could stress the plants.

147 **2.2. Experiments**

148 Two separate experiments were conducted in the summer and fall of 2018. The  
 149 first 60-day experiment consisted of six different treatment options, including bare sand  
 150 with or without fertilizers (terrestrial **PCF polymer coated fertilizer** or struvite) and  
 151 seagrass with or without fertilizers. A second 70-day experiment was conducted  
 152 consisting of multiple lower doses of both fertilizers.

153 **2.2.1. 2.2.1 Single Dose/First Experiment**

154 For the **PCF polymer coated** controlled release fertilizer treatment, Osmocote™  
 155 14:14:14 NPK (Scotts Miracle-Gro Company, Marysville, OH, USA) was chosen due to  
 156 its commercial availability, composition (containing both N and P), and past use in  
 157 seagrass restoration experiments (Peralta et al., 2003; Sheridan et al., 1998; Tanner &  
 158 Parham, 2010). Struvite used in the experiment was produced in a pilot scale fluidized  
 159 bed reactor fed with sludge dewatering liquor. Detailed morphological and elemental  
 160 characteristics are described elsewhere (Bydalek et al., 2018). Unlike the mostly  
 161 homogenous struvite, each Osmocote™ prill has a porous outer layer that gradually  
 162 releases a contained water-soluble nutrient dose through diffusion. The composition of  
 163 elements is also different between the two compounds; with  $\text{NH}_4^+/\text{NO}_3^-$ -N comprising

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164 14% of Osmocote™ versus  $\text{NH}_4^+\text{-N}$  comprising only 6% of struvite ([Osmocote™](#)  
165 manufacturer information, Kenworthy & Fonseca, 1992; Rahman et al., 2014). The P  
166 composition of both fertilizers is also different, with struvite ([13% P as  \$\text{PO}\_4^{3-}\$](#) ) versus  
167 having a higher concentration by weight versus Osmocote™ ([13% P as  \$\text{PO}\_4^{3-}\$](#)  versus  
168 6.1% P as  $\text{P}_2\text{O}_5$ ) ([Osmocote™ manufacturer information](#), Rahman et al., 2014).

169 In total, there were 30 plots, with an unplanted, untreated/unfertilized control  
170 (labelled control,  $n=4$ ), sediment-only treatments (labelled Control-Osmo and Control-  
171 Struv,  $n=4$ ), and seagrass control and treatments (labelled Seagrass, Seagrass-Osmo,  
172 and Seagrass-Struv,  $n=6$ ). Nutrient treatments were fertilized [with approximately by](#)  
173 [adding the Osmocote™ or struvite equivalent of](#) 3 g of P mixed into approximately 6 kg  
174 of sand (equivalent to 0.5 mg P  $\text{g}^{-1}$  DW sand), which was about half of what was  
175 considered "[lightly low](#) fertilized" according to Peralta et al. (2003). ~~Each seagrass plot~~  
176 ~~had exactly three individuals, each with five shoots. The first experiment was~~  
177 ~~conducted for 60 days (Figure A.1). During this period, the levels of dissolved total P).~~  
178 [The dosing was equilibrated to P as tropical seagrass systems are primarily P limited](#)  
179 [\(Brodersen et al., 2017; Gras et al., 2003\). In this experiment, serving as pilot study, N](#)  
180 [concentrations were not equilibrated, however given the actual fertilizer dosages,](#)  
181 [concentrations were still below the low fertilized treatment in Peralta et al.'s study \(0.23](#)  
182 [mg N  \$\text{g}^{-1}\$  DW sand for struvite and 1.16 mg N  \$\text{g}^{-1}\$  DW sand for Osmocote respectively\).](#)  
183 [Each seagrass plot had exactly three individuals, each with five shoots. The first](#)  
184 [experiment was conducted for 60 days. During this period, the levels of dissolved total](#)  
185 [P porewater](#) concentrations were excessively high, exceeding 100 mg P  $\text{L}^{-1}$  in the  
186 Osmocote™ treatments and 5 mg P  $\text{L}^{-1}$  for struvite.

187 **2.2.2. 2.2.2. Multi-Dose/Second Experiment**

188 In this second experiment, struvite doses were 0.0125 (low dose struvite or  
 189 Seagrass-Struv-Lo), 0.025 (medium dose struvite or Seagrass-Struv-Med) and 0.05 mg  
 190 P g<sup>-1</sup> DW sand (high dose struvite or Seagrass-Struv-Hi). For Osmocote™, 0.0125 (low  
 191 dose Osmocote™ or Seagrass-Osmo-Lo) and 0.025 mg P g<sup>-1</sup> DW (medium dose  
 192 Osmocote™ or Seagrass-Osmo-Med) doses were used. Unplanted, fertilized controls  
 193 had a 0.0250 mg P g<sup>-1</sup> DW dose of Osmocote™ (Osmocote™ control or Control-Osmo)  
 194 and struvite (struvite control or Control-Struv). Unfertilized, unplanted plots were  
 195 labelled “control” while unfertilized, planted plots were labelled “unfertilized seagrass” or  
 196 “Seagrass-Control”. There were four replicates for all controls/treatments. A high dose  
 197 of Osmocote™ was not used due to space limitations in the mesocosm and concerns of  
 198 overfertilization based on the results of the single dose/first experiment. There were  
 199 three individuals with five shoots per plot (initially two individuals with the third added 10  
 200 days post deployment to match the starting shoot count of the previous experiment).

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201 **2.3.2.3. Plant and Nutrient Measurements**

202 Seagrass shoot count (seagrass shoots defined as a unit of several leaves or  
 203 blades according to Short & Coles, (2001)), were quantified ~~in both experiments~~  
 204 approximately every 10 days in both experiments. During the second experiment,  
 205 blade/leaf lengths (substrate to leaf tip according to Arrington, (2008)) were also  
 206 quantified. Surface water was sampled for temperature, salinity, and total dissolved  
 207 nutrients (Total Dissolved N/TDN, Total Dissolved P/TDP), while porewater was only  
 208 sampled for total nutrients and (randomly) sulfide presence. Surface and porewater  
 209 samples were collected using a syringe sampler fashioned out of a 60 mL syringe  
 10 attached to a plastic tube and 1 mL serological ~~4 mL~~ pipette with an attached air stone.

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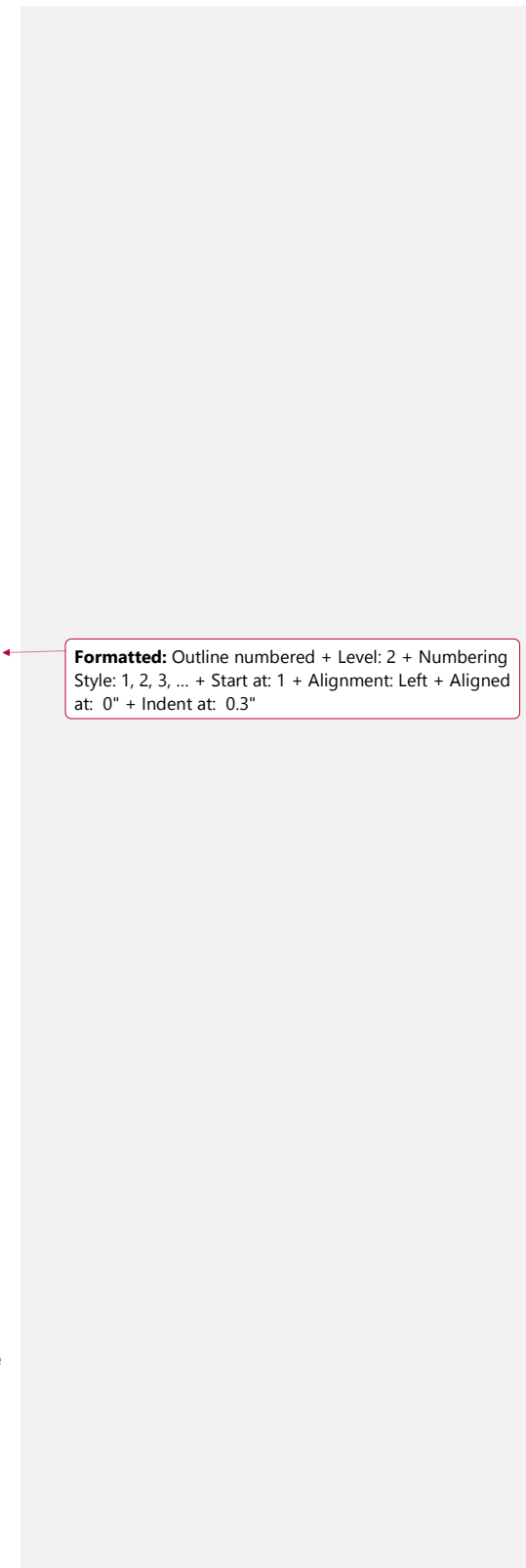
211 The samples were filtered through a 0.45  $\mu\text{m}$ -~~pore size~~ filter (Whatman, Maidstone,  
212 United Kingdom), preserved with sulfuric acid to a pH < 2, and stored at 4 °C until  
213 analysis in the Wetland Biogeochemistry Laboratory (USEPA, 1974, 1993). Porewater  
214 was also tested for the presence of sulfide, ~~which is toxic to seagrasses~~ (Calleja et al.,  
215 2007; Carlson et al., 1994) using a Hach ~~testing test~~ kit (product number 2537800). No  
216 measurable sulfide was found in any plots sampled (detection limit 0.1 mg L<sup>-1</sup>). DOC  
217 and TDN samples were analyzed on a Shimadzu TOC-L analyzer fitted with a N module  
218 (Shimadzu Scientific Instruments, Durham, NC, USA) according to EPA method 415.1  
219 for TOC and ASTM D 8083 for total nitrogen (TN) (ASTM International, 2016; Nevins et  
220 al., 2020; USEPA, 1974). TDP was digested with persulfate in an autoclave and  
221 analyzed via a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto,  
222 Japan) using EPA method 365.1 (~~Tootoonchi et al., 2018; USEPA, 1993~~)(Irick et al.,  
223 2015; USEPA, 1993).

224 At the end of the experiment, plant biomass and sediment were destructively  
225 sampled. Plants were rinsed to clean off sediments, and promptly frozen. ~~Once at~~In  
226 the lab, tissue samples were cleaned of epiphytes and rinsed with de-ionized water.  
227 Plant tissue and sediment samples were dried for 72 hours at 65 °C, and ground using a  
228 ball mill, ~~and~~. Sediment was analyzed for total carbon (TC), and nitrogen (TN), while  
229 tissue was analyzed for TC, TN, and phosphorus (TP). Bulk sediment TC/TN were run  
230 on an ECS 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia,  
231 CA, USA) (~~dry combustion method~~)(Nevins et al., 2020). Tissue TP was determined by  
232 ashing the sample followed by dissolution with 6 M HCL (following Andersen, 1976) and  
233 analysis for soluble P using a Shimadzu UV-1800 spectrophotometer (Shimadzu

234 Corporation, Kyoto, Japan) (~~Liao et al., 2016; USEPA, 1993~~)(Liao et al., 2019; USEPA,  
 235 1993). Due to low and variable weights found after drying seagrass samples, plant dry  
 236 biomass was calculated using a 10% wet weight conversion used for *H. wrightii* and  
 237 *Thalassia testudinum* in Heck et al., (2015) and outlined in Short & Coles, (2001). A  
 238 sediment particle analysis was also conducted to determine the distribution of particle  
 239 sizes and possible changes over time. These samples were analyzed by the Soil and  
 240 Water Sciences Environmental Pedology and Land Use Laboratory using laser  
 241 diffraction (LD) with a Beckman Coulter LS-13320 multi-wave particle size analyzer  
 242 (Beckman Coulter Diagnostics, Brea, CA, USA).

243 **2.4.2.4 Statistical Analyses**

244 Differences in seagrass metrics (shoot count and shoot length) and porewater  
 245 nutrients for both experiments were calculated using a linear mixed model, followed by  
 246 a post hoc multiple comparison significant (Fisher's Least Significant Difference test).  
 247 Factors included the treatment type, date, and the interaction between treatment and  
 248 date. A linear mixed model analysis was also conducted on sediment and biomass  
 249 measurements from the second experiment, testing the effect of treatment type. The  
 250 tests were run using JMP 15.2.1 (SAS Software, Cary, NC, USA) with significance set  
 251 to  $\alpha = 0.05$ . To determine the fit of the model predictions to the measured data,  
 252 residuals and qq-plots were visually inspected and data was log transformed as  
 253 necessary (shoot counts, shoot lengths, and total dissolved nutrients). To differentiate  
 254 between the effects of fertilization methods, K-means clustering was applied to classify  
 255 all observations in the multi-dose/second experiment. K-means were computed using  
 256 the `kmeans` function in R (version R-4.0.2.). Given the number of observations ( $n = 6$ ) the  
 257 data was predefined into two clusters (`centers = 2`). Prior to the analysis, the data was



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258 standardized using the `scale` function (each element is subtracted by the mean value of  
 259 the vector and divided by standard deviation of the vector). The results were visualized  
 260 using the `fviz_cluster` function (factoextra package) based on function's encoded  
 261 principal component analysis (PCA) (Kassambara & Mundt, 2017)(Kassambara &  
 262 Mundt, 2017).

263 **3. 3. Results**

264 ~~2.1.1~~ **3.1 Mesocosm Conditions**

265 ~~Mesocosm temperature and salinity remained between 27-31 °C and 33-38 ppt~~  
 266 ~~respectively during the periods sampled (between 9 am and 3 pm) for both studies. The~~  
 267 ~~hydraulic residence time was variable at 0.5-2 days, due to a limited saltwater supply.~~  
 268 ~~The mean TDN of surface water was  $0.44 \pm 0.06 \text{ mg N L}^{-1}$ , while the mean TDP was~~  
 269  ~~$0.035 \pm 0.001 \text{ mg P L}^{-1}$  (or  $0.029 \text{ mg P L}^{-1}$  when excluding a day of low inflow). The~~  
 270 ~~level of flow was great enough to prevent significant cross-contamination of the plots~~  
 271 ~~studied, as well as prevent significant swings in temperature and salinity that could~~  
 272 ~~stress the plants.~~

273 **3.1.3.2 Single Dose/First Experiment**

274 **3.1.1 Plant Metrics**

275 Increases in shoot counts occurred one month after transplantation for the  
 276 struvite treatment. However, this was not the case with the unfertilized control or the  
 277 Seagrass-Osmo treatment, which both slowly declined on average. At the end of the  
 278 first experiment, mean ~~seagrass~~ shoot counts ranged from  $6.33 \pm 0.87$  shoots in the  
 279 Seagrass-Osmo treatment to  $52.33 \pm 5.49$  shoots in the Seagrass-Struv treatment  
 280 (Figure 1). ~~The effects of fertilizer treatment, date, and the treatment x date interaction~~  
 31 ~~were significant for the shoot count (Table 1).~~ Seagrasses in ~~Seagrass-Struvstruvite~~

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282 fertilized plots had significantly higher shoot counts than the seagrass control and  
283 ~~Seagrass-Osmo overall ( $t \geq 6.83$ , Osmocote treatment ( $p < 0.0001$ ), while there were no~~  
284 ~~significant differences between the seagrass control and Seagrass-Osmo plots.01).~~

285 More specifically, the Seagrass-Struv treatment had a significantly higher shoot count in  
286 mid-July, just one month after planting ( ~~$t = 2.30$ ,  $p = \leq 0.024305$~~ ), becoming greater over  
287 the next month (by end of the study  ~~$t = 19.71$ ,  $p < 0.0001001$~~ ). By the end of the study,  
288 the unfertilized seagrass also had a significantly higher number of shoots than the  
289 Seagrass-Osmo treatment ( $t = 2.56$ ,  $p = \leq 0.012405$ ).

290 ~~The effects of treatment, date, and the interaction between treatment and date were~~  
291 ~~significant for porewater TDP (Table 1).~~ **3.1.2 Water Chemistry**

292 The TDP levels were significantly higher in the Seagrass-Osmo plots than the  
293 unfertilized controls and Seagrass-Struv treatments ( ~~$t > 15.12$ ,  $p < 0.0001$ , table S1~~).  
294 By the end of the study, the average TDP concentration for the Seagrass-Osmo  
295 porewater plots was  $136.09 \pm 15.71$  mg P L<sup>-1</sup> for the unplanted plots (Control-Osmo)  
296 and  $109.53 \pm 19.96$  mg P L<sup>-1</sup> for the planted plots (Seagrass-Osmo), more over ten  
297 times higher than the struvite plots, which was  $2.43 \pm 0.61$  mg P L<sup>-1</sup> in the unplanted  
298 plots and  $0.76 \pm 0.19$  mg P L<sup>-1</sup> in the Seagrass-Struv plots.

299 Porewater TDP in the Control-Struv treatment was significantly higher than the  
300 control, unfertilized seagrass, and the Seagrass-Struv treatments ( ~~$t > 4.72$ ,  $p <$~~   
301  ~~$0.0001001$~~ ), indicating that significant uptake of TDP by seagrasses likely occurred.  
302 There were no significant differences in TDP between the unplanted and planted  
303 Seagrass-Osmo plots, overall or during any specific sampling date. ~~Over time, TDP~~  
304 ~~concentrations in both the Control-Struv and Seagrass-Struv treatments significantly~~

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305 increased by the end of the study ( $t > 2.44$ ,  $p < 0.02$ ) (Figure 1). Overall, the  
 306 concentration of TDP in the Seagrass-Osmo plot porewater significantly increased over  
 307 time ( $t > 4.29$ ,  $p < 0.0001$ ), with the Seagrass-Osmo fertilized plots increasing  
 308 significantly in TDP between the first and second sample dates ( $t > 3.74$ ,  $p \leq 0.0005$ ).  
 309 Subsequent sampling periods showed no statistically significant changes in TDP  
 310 concentrations for the Control-Osmo or Seagrass-Osmo plots over time. There were no  
 311 significant differences within or between the control and unfertilized seagrass plots.

### 3.2.3.3 Multi-Dose/Second Experiment

#### 3.2.1. Plant Metrics

314 At the end of the second experiment, the average seagrass shoot counts ranged  
 315 from  $8.00 \pm 0.41$  shoots in the Seagrass-Control to  $14.50 \pm 3.10$  shoots in the  
 316 ~~Seagrass~~Seagrass-Struv-Med treatment (Figure 2). There was relatively less growth in  
 317 the second experiment versus the first/single dose experiment, however the effects of  
 318 date and its interaction with the treatment type were still significant for shoot count  
 319 (Table 2). ~~The shoot count of the Seagrass-Control was significantly lower than the~~  
 320 ~~Seagrass-Osmo-Low treatment ( $t = 2.61$ ,  $p = 0.0117$ ), Seagrass-Osmo-Med ( $t = 3.01$ ,  $p =$~~   
 321  ~~$0.0040$ ), and the Lo/Med/Hi doses of struvite ( $t = 3.88$ ,  $p = 0.0003$ ,  $t = 3.88$ ,  $p = 0.0003$ ,~~  
 322 ~~and  $t = 3.06$ ,  $p = 0.0034$  for the Lo, Med, and Hi doses, respectively) during the 10/05 or~~  
 323 ~~Day 31 sampling. During the final sampling period (10/25 or 74 days after deployment),~~  
 324 ~~struvite plots had significantly higher shoot counts than the Seagrass-Control ( $t = 3.42$ ,~~  
 325  ~~$p = 0.0012$ ,  $t = 3.50$ ,  $p = 0.0009$ , and  $t = 3.35$ ,  $p = 0.0015$ , for the Lo, Med, and Hi doses,~~  
 326 ~~respectively), while there were not significant differences between Osmocote™ and the~~  
 327 ~~Seagrass-Control (Figure 2). In addition, there were no significant differences between~~

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328 ~~the struvite and Osmocote™ treatments for shoot count~~(S-2). After 53 days, seagrass  
329 ~~shoot count started showing signs of treatment effect in comparison to control seagrass~~  
330 ~~plot which showed significant shoot count declines ( $p < 0.05$ ) in comparison to the rest~~  
331 ~~of the fertilized seagrass plots. By the end of the experiment (74 days) only the~~  
332 ~~Seagrass-Struv-Med treated seagrass plots maintained plant density ( $14.50 \pm 3.10$~~   
333 ~~shoots) close to the original coverage of 15 shoots per plot indicating high~~  
334 ~~transplantation survival rate. At the conclusion of the study, only the struvite fertilized~~  
335 ~~plots were statistically higher in shoot count than unfertilized plots.~~

336 The effects of both treatment and date were significant for blade length (Table  
337 S-2). All fertilized treatments became significantly greater in length than the Seagrass-  
338 Control ~~during and~~ after 39 days post deployment (Figure 2). The average seagrass  
339 blade length ranged from  $9.1 \pm 1.02$  cm in the unfertilized seagrass to  $19.1 \pm 1.74$  cm in  
340 the medium dose struvite. ~~Seagrass blade length in the Seagrass-Struv-Med was~~  
341 ~~significantly higher than the Seagrass-Osmo-Lo ( $t = 2.84, p = 0.0065, t = 3.62, p = 0.0007,$~~   
342 ~~and  $t = 4.40, p < 0.0001$  for the Seagrass-Osmo-Lo 39, 53, and 74 days after~~  
343 ~~deployment, respectively) and Seagrass-Osmo-Med ( $t = 3.13, p = 0.0029, t = 3.07, p =$~~   
344  ~~$0.0036,$  and  $t = 3.02, p = 0.004$  for the Seagrass-Osmo-Med at 39, 53, and 74 days after~~  
345 ~~deployment, respectively). The Seagrass-Struv-Med treatment also had a significantly~~  
346 ~~higher shoot length than the Seagrass-Struv-Lo 53 ( $p = 0.0276$ ) and 74 days after~~  
347 ~~deployment ( $p = 0.0257$ ).~~ ~~by the end of the experiment. The highest increase in blade~~  
348 ~~length was observed in struvite treatments. The Seagrass-Struv-Med treatment showed~~  
349 ~~a significantly ( $p < 0.005$ ) higher blade growth than the Seagrass-Osmo-Lo/Med~~  
350 ~~treatments.~~

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351 The mean aboveground biomass ranged from  $0.012 \pm 0.004$  g DW in the  
352 Seagrass-Control to  $0.080 \pm 0.011$  g DW in the Seagrass-Struv-Hi treatment (Figure  
353 [43](#)), with the effect of treatment type being significant (Table [3](#)). ~~For S-3). All fertilized~~  
354 ~~plots had significantly higher~~ aboveground biomass, ~~the Seagrass-Control was~~  
355 ~~significantly lower~~ than the Seagrass-Osmo-Med ( $t = 1.53, p = 0.045$ ), low ( $t = 2.60, p =$   
356  $0.018$ ), medium ( $t = 4.07, p = 0.0007$ ), and high dose struvite ( $t = 4.03, p = 0.0008$ ) (Figure  
357 [3](#)). ~~Med and Hi dose struvite treatments were significantly higher in aboveground~~  
358 ~~biomass than control, except for the Seagrass-Osmo-Lo ( $t = 2.54, p = 0.0205$  and  $t =$~~   
359  $2.50, p = 0.0225$  for medium and high dose struvite). ~~Marginal treatment ( $p < 0.05$ ).~~  
360 ~~There was a marginal~~ significance ~~was also~~ found for the Med and Hi struvite  
361 ~~treatments/doses~~ having a higher aboveground biomass than the Seagrass-Osmo-Med  
362 ( $t = 1.92, p = 0.0713$  and  $t = 1.87, p = 0.0775$  for Med and Hi dose struvite, respectively).  
363  $p < 0.08$ . Belowground biomass ranged from  $0.11 \pm 0.02$  g DW in the Seagrass-  
364 Control to  $0.20 \pm 0.01$  g DW in the ~~high dose struvite~~ Seagrass-Struv-Med treatment  
365 (Figure [43](#)). The ~~effect of treatment type was also significant for~~ belowground biomass  
366 ~~of control plots was significantly lower compared to all fertilized plots except for the~~  
367 Seagrass-Osmo-Med (Table [S-3](#)). ~~The Seagrass-Osmo-Lo, Seagrass-Struv-Lo,~~  
368 ~~and Additionally, the~~ Seagrass-Struv-Med ~~were~~ dose had significantly higher ~~than the~~  
369 Seagrass-Control ( $t = 2.47, p = 0.0238, t = 2.57, p = 0.0194, and t = 3.98, p = 0.0009$ , for  
370 belowground biomass compared to the Seagrass-Osmo-Lo, Med and Seagrass-Struv-  
371 Lo, and Seagrass-Struv-Med, respectively). Additionally, the Seagrass-Struv-Med was  
372 significantly higher than both the Seagrass-Struv-Hi ( $t = 2.24, p = 0.0378$ ) and the  
373 Seagrass-Osmo-Med ( $t = 2.93, p = 0.0089$ ).





374 At the end of the experiment, the mean porewater TDN concentration ranged  
375 from  $1.68 \pm 0.15$  mg N L<sup>-1</sup> in the Seagrass-Control to  $17.26 \pm 4.98$  mg N L<sup>-1</sup> in the  
376 Seagrass-Osmo-Med (Table A.1). Only the effect of the treatment type was significant  
377 for TDN (Table 2). Overall, the porewater TDN concentrations for the Control-Osmo  
378 and planted Seagrass-Osmo-Med were significantly higher than the controls and the  
379 struvite treatments ( $t \geq 2.06$ ,  $p \leq 0.0423$ ). The Seagrass-Osmo-Lo was significantly  
380 higher in TDN than all treatments except the Seagrass-Osmo-Med and the Seagrass-  
381 Struv-Hi treatment ( $t \geq 2.01$ ,  $p \leq 0.0473$ ). All struvite doses were significantly higher in  
382 TDN than both the unplanted control and Seagrass-Control ( $t \geq 3.62$ ,  $p \leq 0.0005$ ).

383 The mean porewater TDP concentrations ranged from  $0.084 \pm 0.021$  mg P L<sup>-1</sup> in  
384 the Seagrass-Control to  $0.551 \pm 0.105$  mg P L<sup>-1</sup> in the Seagrass-Osmo-Med at the end  
385 of the experiment. The effects of treatment and date were significant for porewater TDP  
386 (Table 2). Similarly, the unplanted control and Seagrass-Control had significantly lower  
387 TDP than all fertilized plots ( $t \geq 4.68$ ,  $p \leq 0.0001$ ). The Seagrass-Osmo-Med was  
388 significantly higher than all other controls/treatments except the equivalently dosed  
389 unplanted Osmocote™ treatment ( $t \geq 3.38$ ,  $p \leq 0.001$ ), while Seagrass-Osmo-Lo was  
390 significantly higher than the controls and the equivalently dosed (P basis) struvite  
391 treatment ( $t \geq 4.05$ ,  $p \leq 0.0001$ ). Marginal significance was also found for the Seagrass-  
392 Osmo-Lo being higher in porewater TDP than the Seagrass-Struv-Med ( $t = 1.92$ ,  $p =$   
393  $0.0569$ ). Over time, TDP concentrations appeared to fluctuate greatly between fertilized  
394 treatments, while remaining stable for unfertilized controls. Between the two highest  
395 peaks (Day 6 and Day 31), Osmocote™ plots had a significant reduction in porewater

396 ~~TDP concentration ( $t = 3.06$ ,  $p = 0.0028$ ,  $t = 2.81$ ,  $p = 0.0059$ , and  $t = 2.44$ ,  $p = 0.0164$  for~~  
397 ~~the Osmo-Control and Lo/Med Osmocote™ treatments, respectively).~~

398 Hi doses ( $p < 0.05$ ). Aboveground tissue %TN ranged from 1.9% in the  
399 unfertilized seagrass (one sample) to  $2.34 \pm 0.23\%$  in the Seagrass-Struv-Lo treatment,  
400 while tissue %TP ranged from  $0.236 \pm 0.007\%$  in the Seagrass-Struv-Lo treatment to  
401  $0.258 \pm 0.016\%$  in the Seagrass-Osmo-Lo treatment (Table [A.3S-7](#)). There was no  
402 significant effect of treatment on aboveground %TN or %TP (Table 4). ~~This lack of~~  
403 ~~significant difference is possibly due to the absence of available control replicates. For~~  
404 ~~example, the aboveground control only had a single combined sample (from  $n=4$ ).~~ The  
405 mean aboveground N:P ratios ranged between  $8.3 \pm 0.57$  for the Seagrass-Osmo-Lo  
406 and  $10.0 \pm 1.20$  for Seagrass-Struv-Lo treatment. ~~The N:P ratio and the mean~~  
407 ~~aboveground TN and TP weights in the seagrasses (calculated by multiplying the~~  
408 ~~biomass with the tissue %TN or %TP) yielded no significant differences (Tables [5S-4](#)~~  
409 ~~and [A.3](#)).~~

410 7). Belowground tissue %TN ranged from  $0.53 \pm 0.07\%$  for the Seagrass-Osmo-  
411 Lo treatment to  $0.84 \pm 0.06\%$  in the Seagrass-Osmo-Med treatment, while tissue %TP  
412 ranged from  $0.154 \pm 0.009\%$  for the Seagrass-Osmo-Lo treatment to  $0.179 \pm 0.008\%$   
413 for the Seagrass-Osmo-Med treatment (~~Table A.3~~). The effect of treatment type was  
414 significant for belowground %TN (Table 4), with the Seagrass-Osmo-Med being  
415 significantly higher than the Seagrass-Osmo-Lo ( ~~$t = 3.43$ ,  $p = 0.003705$~~ ). No effects  
416 were significant for belowground %TP. The mean belowground N:P ratio ranged from  
417  $3.4 \pm 0.47$  for the Seagrass-Osmo-Lo and  $4.7 \pm 0.36$  for the Seagrass-Osmo-Med  
418 treatment (~~Table A.3~~). ~~Similarly, no significant differences in the belowground N:P~~



419 ratios were found. However, the The effect of treatment type was significant for both  
420 the belowground mass of TN and TP (% total nutrient x biomass, Table 5). ~~For~~  
421 ~~belowground TN weight, the Seagrass-Struv-Med was significantly higher than the~~  
422 ~~Seagrass-Control ( $t=3.38$ ,  $p=0.0041$ ) and the Seagrass-Osmo-Lo ( $t=2.66$ ,  $p=0.0177$ ).~~  
423 ~~For the belowground TP weight, the Seagrass-Struv-Med was significantly higher than~~  
424 ~~the Seagrass-Control ( $t=3.67$ ,  $p=0.0023$ ) and the Seagrass-Osmo-Med ( $t=2.22$ ,  $p=$   
425  $0.0421$ ). Additionally, the Seagrass-Struv-Lo was significantly higher than the~~  
426 ~~Seagrass-Control ( $t=2.59$ ,  $p=0.0205$ ).~~S-5).

### 427 **3.2.2. Water, Tissue, and Sediment Chemistry**

428 Nutrient dynamics in porewater differ significantly between the fertilizer types  
429 indicating different dissolution kinetics and plant and substrate interaction. Unfertilized  
430 control plots (planted and unplanted) showed variable TDN concentrations throughout  
431 the experiment however, never surpassing 2 mg TDN L<sup>-1</sup>. Background porewater TDP  
432 content in observed controls varied within 0.05-0.15 mg TDP L<sup>-1</sup>. The biggest nutrient  
433 release was observed at plots fertilized with Osmocote with peak nutrient  
434 concentrations occurring at 6<sup>th</sup> day of experiment reaching 26.8 ± 7.53 mg TDN L<sup>-1</sup> and  
435 17.68 ± 6.74 mg TDP L<sup>-1</sup> for medium Osmocote dose. TDP dynamics in struvite  
436 seagrass treatments were highly variable throughout the time and showed alternating  
437 pulses of TDP release. However, by the end of the experiment porewater TDP content  
438 in struvite fertilized plots was 2-3 times lower than in respective Osmocote treatments.  
439 DOC measured at the end of the study was between 12.26 ± 0.67 mg DOC L<sup>-1</sup> for  
440 Seagrass-Struv-Lo and 14.71 ± 1.23 mg DOC L<sup>-1</sup> for Seagrass-Osmo-Lo.

441 The average TC content of sediment ranged from  $48.7 \pm 5.02$  g C kg<sup>-1</sup> in the  
442 medium dose struvite to  $58.2 \pm 5.63$  g C kg<sup>-1</sup> in the Seagrass-Struv-Hi, while the  
443 average TN content ranged from  $2.02 \pm 0.032$  g N kg<sup>-1</sup> in the Seagrass-Control to  $2.10 \pm$   
444  $0.020$  g kg<sup>-1</sup> in the Seagrass-Struv-Hi treatment (Table A.5). ~~The TP content of~~  
445 ~~sediment was not measured due to the high variability of replicates (possibly caused by~~  
446 ~~the large grain size of the sediment and/or the granular nature of the fertilizers, creating~~  
447 ~~regions of low/high nutrients). S-6).~~ There were no significant differences in the TC or  
448 TN contents between treatments (Table A.6S-7).

449 ~~Nutrient dynamics (TN, TOC and TDP), above and belowground biomass, and~~  
450 ~~shoot count data~~ Porewater nutrients and seagrass metrics were used to further assess  
451 the global effect of fertilization dose and method based on multivariate analysis. K-  
452 means clustering detected two separate groups. The struvite treatment was clearly  
453 distinguished from the Osmocote™ treatment and control plot, occupying separated,  
454 non-overlapping clusters on the PCA plane (Figure 4), reinforcing the significant effects  
455 of struvite on seagrass and its surrounding environment.

## 456 **4. Discussion**

### 457 **4.1.4.1 Factors in Seagrass Performance**

458 ~~In all but the Seagrass-Osmo in the first experiment, fertilizer~~ Fertilizer application  
459 improved seagrass metrics compared to the unfertilized control, ~~including in all but the~~  
460 ~~Seagrass-Osmo treatment of the first experiment. This included average~~ shoot count  
461 (more than six times higher vs the control at the end of the first experiment, and ~~41% or~~  
462 ~~more~~ up to 81% at the end of the second experiment), length (~~32% or greater~~ up to 110%  
463 at the end of the second experiment, Figure 2), and biomass (~~52% or greater~~ up to  
464 138% at the end of the second experiment, Figure 3). In general, these results support

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465 past findings examining the effects of fertilizer ~~in the restoration techniques in of~~  
466 seagrass ecosystems (~~Armitage et al., 2011; Kenworthy et al., 2018~~)(~~Armitage et al.,~~  
467 ~~2011; Kenworthy et al., 2018~~). Additionally, the results of this study found that  
468 ~~compared to equivalent P dosages with Osmocote,~~ fertilization using struvite resulted in  
469 higher average seagrass shoot count (more than ~~sixeight~~ times higher by the end of the  
470 first experiment, and ~~60% or more compared to the Seagrass-Struv-Med~~29% at the end  
471 of the second experiment), length (~~36% or more compared to the Seagrass-Struv-~~  
472 ~~Med~~up to 36% at the end of the second experiment, Figure 2), and biomass (~~40-up to~~  
473 60% higher total biomass ~~compared to the Seagrass-Struv-Med~~ at the end of the  
474 second experiment, Figure 3) ~~compared to equivalent doses of Osmocote™.~~ The  
475 significant multivariate improvements in plant metrics ~~produced~~ in both experiments are  
476 promising towards the use of struvite as a fertilizer to rapidly establish seagrass species  
477 in future restoration efforts.

478 In addition to improving seagrass metrics, struvite consistently ~~appeared to~~  
479 ~~releaser~~released less nutrients than Osmocote™. Porewater TDN was excessive in the  
480 Osmocote™ treatment in the first experiment (~~the sample readings were out of range, x~~  
481 ~~> 10 (> 100 mg/L without dilutions).~~). In the second experiment, TDN in struvite  
482 ~~introduced TDN treated plots~~ was only as low as 12% of Osmocote™ ~~released TDN~~  
483 ~~experiment treated plots~~ (Table S-48). Porewater TDP in equivalent struvite doses was  
484 less than 2% TDP of Osmocote™ in the first experiment (Figure 1), and as low as 10%  
485 P of Osmocote™ in equivalent struvite doses in the second experiment (Table S-48).  
486 The speed of nutrient release by Osmocote™ was so high, that it may have contributed  
487 to the decreased performance of the Osmocote™ treatments through excessive N

488 levels, as evidenced by roots that appeared stunted from possible root burn (observed  
489 in the first experiment, [Figure S-1](#)), commonly associated with N exposure (NC State,  
490 2018; Schönau & Herbert, 1983). ~~This~~

491 ~~The possible root burn in Osmocote™ treated seagrass~~ may be the result of  
492 nitrate ~~specifically, as it is included in the Osmocote™ blend and was found to inhibit~~  
493 ~~seagrass biomass in past fertilization studies~~ (Peralta et al., 2003; Statton et al., 2014).  
494 ~~Alternatively, toxicity may have been caused by the ammonia fraction in the Osmocote,~~  
495 ~~as NH<sub>x</sub> forms are also toxic to seagrasses, especially at low biomass levels or ammonia~~  
496 (van der Heide et al., 2008) ~~fractions in the fertilizer~~. However, previous seagrass  
497 (*Zostera marina*) mesocosm studies have detected increased seagrass metrics  
498 following Osmocote™ fertilization. For example, *Zostera marina* plants were found to  
499 have increased shoot counts after one month of Osmocote™ 14:14:14 NPK fertilizer  
500 exposure compared to unfertilized plots (Wang et al., 2020). Similarly, another study  
501 found significant differences in shoot length in *Z. marina* over a period of two months  
502 when exposed to fertilizer doses higher than those used in this study (Peralta et al.,  
503 2003). In these cases, it should be noted that *Z. marina* exhibited a “remarkable  
504 tolerance” of N and P fertilization, and many species of seagrass may not be as flexible  
505 regarding higher levels of nutrient exposure.

506 Another factor affecting the difference between struvite and Osmocote™ could  
507 be the balance of N versus P. In the second experiment, the aboveground tissue N:P  
508 ratios ([8.3 ± 0.57 to 10.0 ± 1.20, Table S-9](#)) consistently exceeded the traditionally  
509 accepted threshold for a balanced nutrient supply. ~~The mean N:P ratios ranged~~  
510 ~~between 8.3 ± 0.57 to 10.0 ± 1.20 (Table S-2), while a for seagrasses (14 weight N:P~~







511 ratio calculated from the 30:1 molar N:P ratio as provided by Atkinson & Smith, [1983] ~~is~~  
512 ~~considered balanced for seagrass-]).~~ A study of *H. wrightii* found that in a natural  
513 system (Florida Bay) the molar N:P was over 20, while in ~~an enriched scenario (a~~  
514 fertilized ~~with scenario (using~~ bird roosting stakes) the ratio was approximately 13  
515 (Powell et al., 1989). Thus, the authors argued that *H. wrightii* was P limited in ~~an~~  
516 ~~unenriched a natural~~ setting, and N limited ~~in the enriched setting when fertilized.~~  
517 Another study in Florida Bay found that *H. wrightii* was “released” from P limitation  
518 ~~had a~~ tissue ~~weight to N:P~~ weight ratios ~~of~~ between 9.7 and 21 (Armitage et al., 2011).  
519 Generally, the *H. wrightii* in all fertilized plots did not appear to be strongly limited by a  
520 specific nutrient, exceeding the 1.8% TN/ 0.2% TP tissue nutrient requirement defined  
521 by Duarte (1990). The exception to this may have been the control, which was closer to  
522 N limitation than all plots with a 1.9% TN tissue content, although this conclusion is  
523 tenuous because only one replicate was able to be analyzed due to a lack of biomass.

524 The lack of significant differences in tissue nutrient content between fertilized and  
525 non-fertilized treatments may be due to delays in nutrient response by the plants. For  
526 example, one study found that it took *Thalassia testudinum* four months to acquire  
527 elevated N levels after fertilizer exposure, while elevated P levels in plants took up to 14  
528 months to develop (Ferdie & Fourqurean, 2004). While *H. wrightii* is a faster growing  
529 species, and higher growth was demonstrated in fertilized vs non-fertilized plots, the  
530 limited experiment duration may not have fully captured long-term increases in tissue  
531 content. However, significant differences in belowground nutrient content (i.e. medium  
532 dose struvite vs. non-fertilized control, Table S-4) and tissue nutrient weight ~~may~~ (Table  
533 S-5) indicate uptake of nutrients by the seagrass.



534 ~~The surface and porewater results appear to support N limitation of the~~  
535 ~~mesocosm environment, with all controls/treatments having a TDN/TDP ratio of less~~  
536 ~~than 20 (most notably porewater TDN/TDP ratio of the unplanted control at  $12.7 \pm 1.71$ ,~~  
537 ~~and the unfertilized seagrass at  $10.0 \pm 1.89$ , Table S-1). Thus, the results of the~~  
538 ~~experiment and past studies appear to support the argument that seagrasses in this~~  
539 ~~study were either nutrient balanced or slightly N limited. In this case, the likely lack of~~  
540 ~~severe N limitation, combined with the high levels of porewater nutrients, potentially~~  
541 ~~indicates that the N content of struvite (6% by weight) is sufficient to improve seagrass~~  
542 ~~growth in this system.~~

543 Furthermore, the size of the mesocosm plots may have been a factor in the high  
544 porewater nutrient levels ~~found in the experiment~~ by preventing lateral flow of porewater  
545 and limiting diffusion. The current flow and increased sediment depth may dilute  
546 porewater, increase diffusion, and reduce the effectiveness of fertilizers in a natural  
547 environment, requiring more fertilizer for field studies. This potential problem may be  
548 partially compensated by the relatively large grain size of the shelly sand used in the  
549 study, compared to the often silty sand found in seagrass systems (a property produced  
550 by seagrass beds as discussed in Folmer et al., 2012). The lack of sulfide present in  
551 the experiment also indicates a higher redox potential that is likely not present in field  
552 experiments.

553 This study demonstrated that struvite and Osmocote™ both released N and P  
554 ~~unabated~~ for at least two months (Table S-4~~8~~). Based off on longer studies ~~using~~  
555 ~~Osmocote™~~, it is expected that ~~the~~ Osmocote™ would provide N and P for ~~a couple~~  
556 ~~more months, totaling 4-6 months based on~~ (Hall et al., (2006) ~~and~~ Olsen and Valiela (

557 2010). Struvite may be able to provide nutrients for longer periods, indicated by its  
558 slower release rate. After the second experiment, ~~the~~selected fertilized plots were  
559 moved to another mesocosm and left submerged. A year after the experiment was  
560 deployed, the only evidence found of the Osmocote™ fertilizer were the outer  
561 membranes of the prills, whereas struvite granules were still found in the mesocosm  
562 plots, indicating a potential continued release of nutrients. ~~While~~Thus, while the effects  
563 of struvite were only measured for up to nine weeks, the presence of struvite after this  
564 extended period indicates that struvite could be effective throughout a whole growing  
565 season or longer. The ability of struvite to produce higher seagrass metrics while  
566 emitting less nutrients (indicating a more sustained release of nutrients over a longer  
567 period of time) is promising toward the future applications of struvite in future coastal  
568 restoration efforts.

#### 569 4.2. Field Applications of the Study

570 The controlled environment of the mesocosm study allowed tests to be done with  
571 minimal interference from the confounding variables of a field study. However, several  
572 external factors may still have affected the results of the two experiments. The first  
573 experiment was conducted at the peak of the seagrass growing season (June through  
574 August), whereas the second experiment occurred during the end of the season  
575 (August through October, with the season typically ending in September; Choice et al.,  
576 2014). The later date of deployment could help explain why ~~the~~ differences between  
577 shoot counts were not as apparent in the second experiment ~~compared to the first.~~  
578 Based on the declining seagrass performance ~~found when exceeding above~~ the  
579 medium/0.025 mg P g<sup>-1</sup> DW dose, there may have been even larger differences in the



580 first experiment between struvite and Osmocote™ if the second experiment was begun  
581 earlier in the summer.

582 4.2 When considering the broad applicability of the results, it is important to note  
583 how close the conditions in the mesocosm were mimicking the natural environment.  
584 First, the local sediment substrate was not sterilized and contained a representative  
585 microbial population. Similarly, seawater for the mesocosm was only prefiltered to  
586 minimize inputs of algae or debris, and largely maintained the natural composition and  
587 physiochemistry. The mesocosm environment was sheltered from hydrodynamic  
588 disturbance and herbivory which are significant problems in field restoration efforts  
589 (Bourque & Fourqurean, 2013; W. Kenworthy et al., 2018; Tuya et al., 2017). However,  
590 there are numerous techniques such as protective cages, or biodegradable lattices,  
591 artificial seagrass, in ground fertilizer application, and sediment tubes that aim to  
592 minimize environmental disturbances and which can be successfully integrated into  
593 restoration projects utilizing fertilizers (Hall et al., 2006; Hammerstrom et al., 1998; W. J.  
594 Kenworthy et al., 2018; Li et al., 2019; MacDonnell et al., 2022; Temmink et al., 2020;  
595 Tuya et al., 2017).

596 Multiple field and mesocosm seagrass studies investigating the use of  
597 Osmocote™ have yielded generally similar results (Peralta et al., 2003; Pereda-Briones  
598 et al., 2018; Tanner & Parham, 2010). Both struvite/Osmocote™ experiments could be  
599 considered extensions of these previous investigations with real world applications.  
600 However, it must be noted that a successful mesocosm scale study such as this one  
601 cannot simply be scaled up to field applications. Rather, it would require the additional  
602 understanding of local environmental conditions and applied restoration techniques that

603 enhance the success rate. Therefore, a future field study would be recommended to  
 604 optimize the dose of struvite in different biogeochemical conditions and assess  
 605 associated operational efforts and costs.

606 **4.3. Implications/Applications of Struvite**

607 The integration of struvite in restoration projects could have multiple advantages  
 608 ~~concerning for~~ both ~~future~~ environmental management and sustainability of wastewater  
 609 treatment. First, more research is needed, but struvite is potentially less harmful for the  
 610 environment than ~~traditionally available, traditional~~ commercial fertilizers. For example,  
 611 struvite is sourced from wastewater, a source of eutrophication for many coastal  
 612 systems (Mayer et al., 2016). The N content of struvite is also relatively low, and while  
 613 it still provides plants with nutrients, it limits excess fertilization and resulting nitrous  
 614 oxide emissions (Rahman et al., 2014). Second, ~~that~~ struvite ~~has the potential to be a~~  
 615 ~~sustainable, and~~ locally sourced ~~fertilizer. This could have~~ has global implications as P  
 616 resources are being depleted in an accelerating rate, and there are indications that  
 617 demand will surpass supply within the next 20 years ~~(Nedelciu et al., 2020)(Nedelciu et~~  
 618 ~~al., 2020)~~. The processing of struvite allows for the production of a P fertilizer without  
 619 dealing with the instability and increasing costs of importing fertilizer ~~(Rufi-Salis et al.,~~  
 620 ~~2020; Ye et al., 2020)(Rufi-Salis et al., 2020; Ye et al., 2020)~~. Finally, the feasibility of  
 621 using struvite on multiple scales has been demonstrated in experiments and industrial  
 622 applications, indicating a practical and readily available treatment process ~~(Ghosh et al.,~~  
 623 ~~2019).(Ghosh et al., 2019)~~.

624 The advantages of struvite in reducing pollution and phosphate shortages,  
 625 combined with its feasibility, make it an attractive ~~option as an~~ alternative P and N  
 626 fertilizer. Struvite is a ~~widely~~-recognized slow--release terrestrial nutrient amendment

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627 ~~characterized~~ with a low environmental footprint. However, struvite application ~~in~~  
628 ~~agriculture~~ is still limited due to its high price in comparison to conventional mineral  
629 fertilizers and availability. Therefore, extending application of struvite into ~~non-~~  
630 ~~agricultural applications~~ areas such as restoration could potentially create a new market  
631 ~~and consequently lower the price~~, thus making struvite more affordable and available.  
632 This is particularly important since struvite represents a very important aspect of circular  
633 economy in water management.

## 634 5. 5. Summary and Conclusions

635 Because of the current need for effective fertilization methods that minimize  
636 environmental risk, this study evaluated the wastewater by-product struvite and its  
637 potential to enhance seagrass growth under simulated natural conditions. ~~Within the~~  
638 ~~fertilizer types, seagrass~~ Seagrass growth metrics (shoots, length, biomass) in plots  
639 fertilized with struvite were consistently equal to or better than ~~a common~~ the  
640 commercial fertilizer Osmocote™. This improvement in seagrass performance was  
641 provided while also producing lower porewater nutrient release from equal P fertilization  
642 doses, likely due to the slower release of nutrients from struvite delivering a low but  
643 sustained load of N and P to the rhizosphere. Excessive N inputs from the Osmocote™  
644 treatment in the first experiment may have even reduced performance of treated plots  
645 compared to the unfertilized control. Measurements of porewater nutrients and visual  
646 observations indicated that struvite has a lower solubility and is therefore longer lasting  
647 compared to Osmocote™ in marine conditions. Other possible factors in plant  
648 performance, including the effects of specific nutrients (i.e. temporal delays in N/P  
649 tissue concentration, micronutrient differences), current flow (possibly increasing

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650 nutrient diffusion), and sediment particle size (affecting dissolution rates and redox  
651 potential), will require further investigation.

652 ~~In the future, Future~~ studies should apply the results of this experiment ~~in the field~~  
653 in multiple coastal systems, ensuring that results are not constrained to a seagrass  
654 mesocosm setting. Testing the solubility of struvite in different environments may reveal  
655 more applications for the fertilizer ~~in different environments. Ideally, Experiments~~  
656 ~~should include other seagrass species with diverse nutrient requirements, and ideally, a~~  
657 restoration experiment would take place over multiple growing seasons to determine  
658 how long struvite remains effective. Special consideration should also be given toward  
659 testing the effectiveness of struvite ~~versus Osmocote™~~ in a heavily more N-limited  
660 environment, ~~as Osmocote™ where other fertilizers~~ may have ana better advantage ~~due~~  
661 ~~to higher N content~~. This study was a first ever attempt to apply struvite in marine  
662 restoration project, serving as an example of interdisciplinary ~~merger~~ between  
663 wastewater treatment engineering and restoration ecology. The ~~obtained~~ positive  
664 results here should encourage future research and field activities to further explore  
665 ~~struvite~~ the application of struvite and similar materials for restoration projects ~~in both~~  
666 ~~terrestrial and aquatic environment~~.

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681





682

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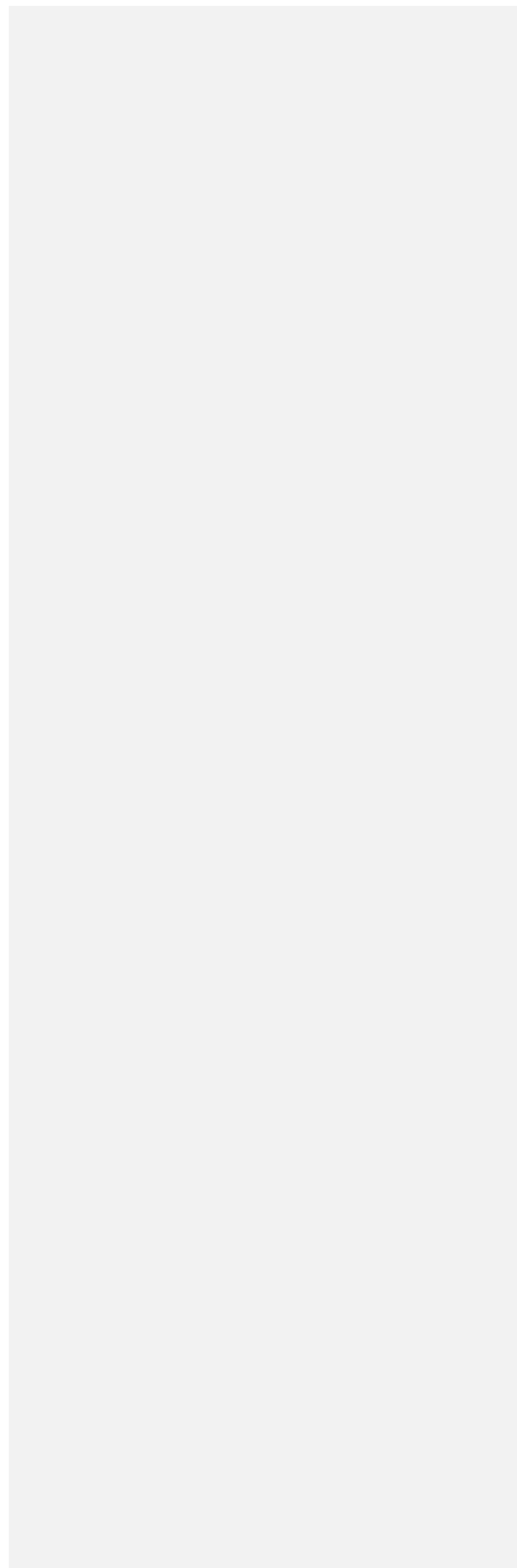
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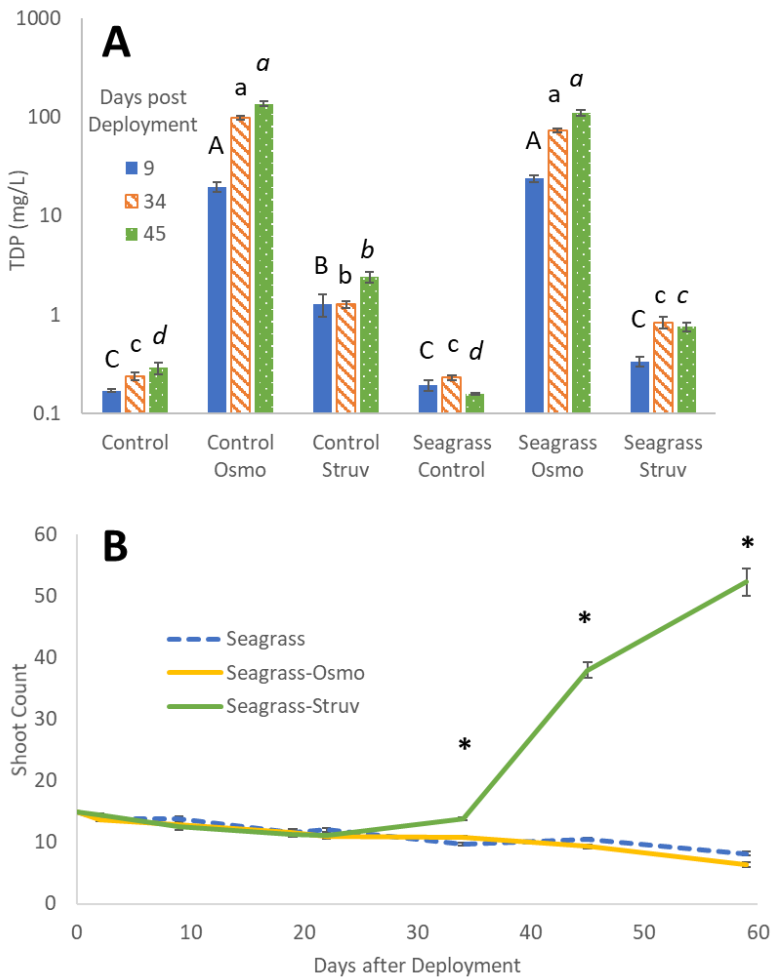
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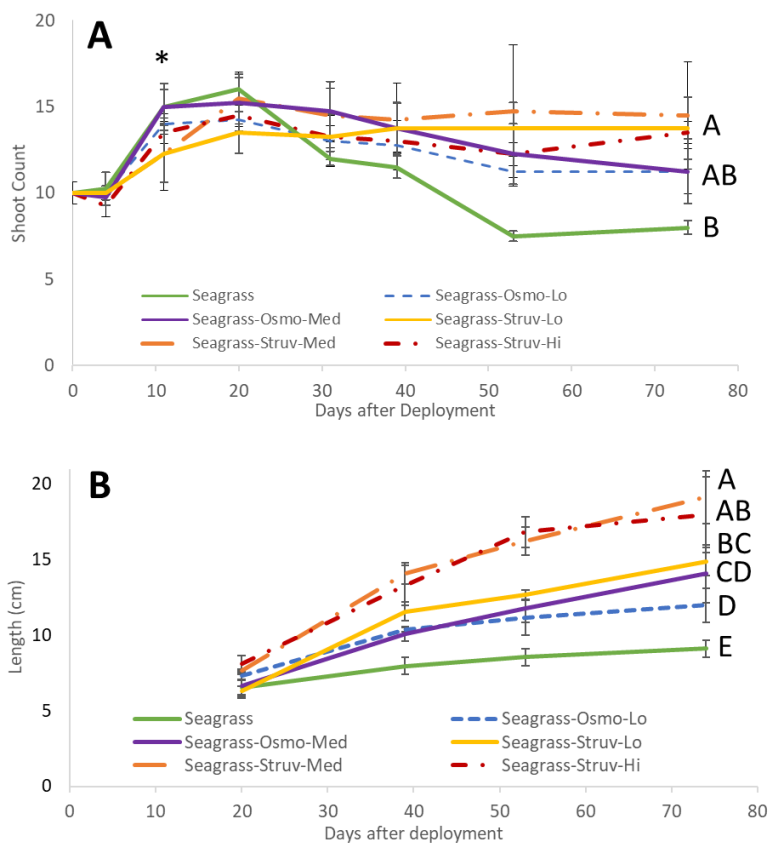




957 Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count  
 958 (B) taken during the first mesocosm experiment. The treatments were labelled  
 959 Seagrass (for the unfertilized seagrass plots), Seagrass-Osmocote™ (for planted plots  
 960 fertilized with Osmocote™), and Seagrass-Struv (planted plots fertilized with struvite).  
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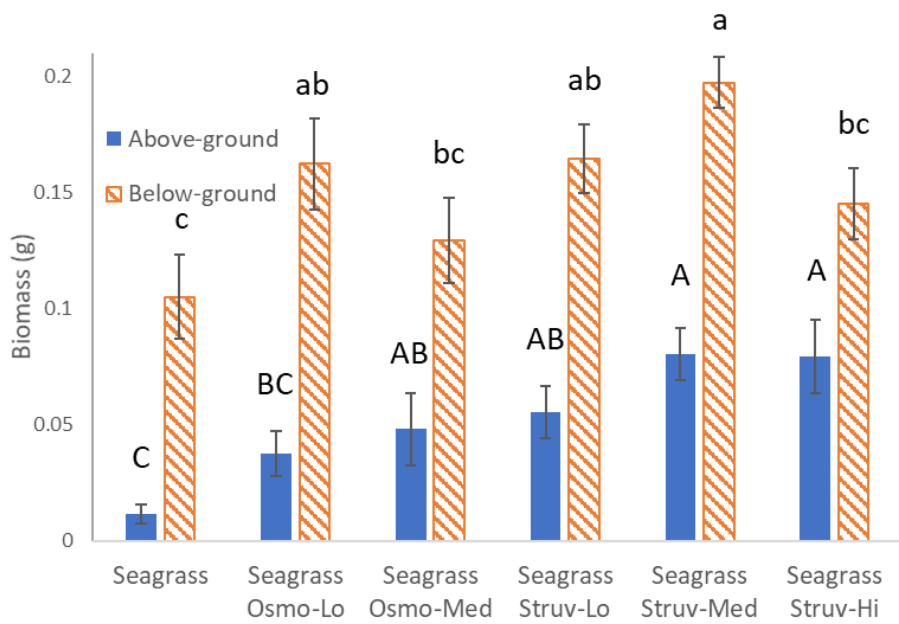
962 The asterisks designate significant differences between treatments for the same sample  
 963 dates. Points represent the mean of six replicates ( $\pm$  SE).



964  
 965 Figure 2. Shoot count (A) and blade length (B) from the second mesocosm experiment.  
 966 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-  
 967 Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-  
 968 Struv-Low, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with  
 969 struvite). \*: Five shoots were added to each plot to match the first/single dose

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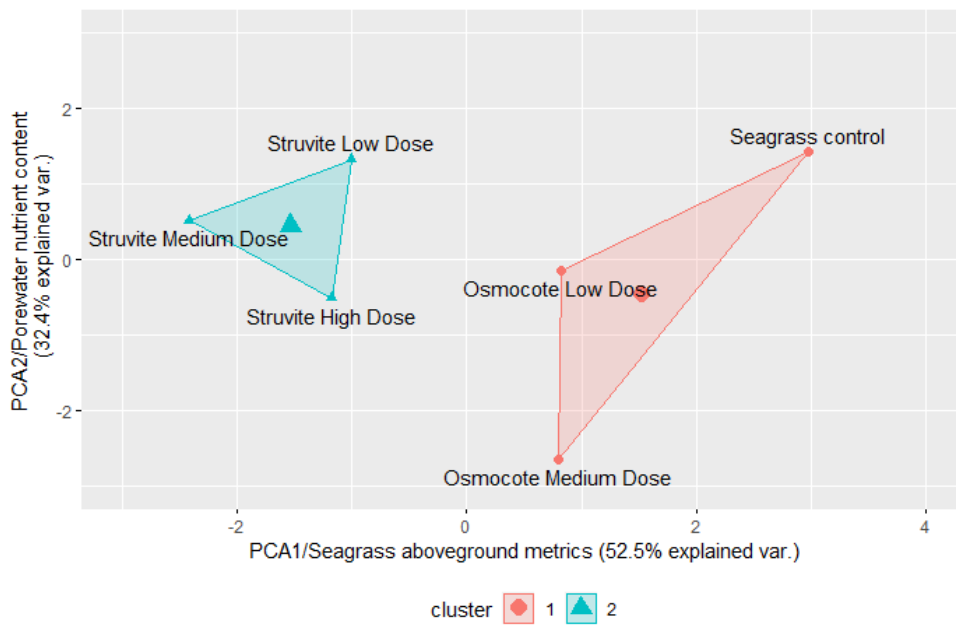
970 experiment. Letters designate significant differences between treatments for the same  
 971 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-  
 972 ground biomass, which had two to four replicates.



973 Figure 3. Above and belowground biomass from the second mesocosm experiment.  
 974 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-  
 975 Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-  
 976 Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with  
 977 struvite). Letters designate significant differences between treatments for the same  
 978 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-  
 979 ground biomass, which had two to four replicates.

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984 Figure 4. Clustering results of treatment methods from the second experiment. No

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985 overlapping clusters were formed, indicating a significantly different global effect of

986 struvite onto the water chemistry and plant growth characteristics compared to

987 Osmocote™ treatment or control plot. Seagrass aboveground metrics (shoot count, blade

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988 length and aboveground biomass) were heavily correlated ( $r > 95\%$ ,  $p < 0.001$ ) with first

989 principal component which explained 52.5% of the variance in the dataset. Porewater

990 nutrient dynamics such as TDN and TDP were most correlated ( $p < 0.05$ ) and contributing

991 to second principal component which explained 32.4% of the variance in the dataset. The

992 treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and

993 Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo,

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994 Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite).

995 Table S-1. Two-way linear mixed effects test results for shoot count and TDP from the  
 996 single dose experiment/Experiment #1. Factors include treatment, date sampled, and  
 997 the interaction between these factors.

Source	Variable					
	Shoot			TDP		
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	2	35.91	< 0.0001	5	246.7	< 0.0001
Date	7	10.07	< 0.0001	2	19.50	< 0.0001
Treatment x Date	14	27.95	< 0.0001	10	2.201	0.0328

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1012 Table S-2. Two-way linear mixed effects test results for seagrass metrics and  
 1013 porewater nutrients from the multi-dose experiment/Experiment #2. Factors include  
 1014 treatment, date sampled, and the interaction between these factors.

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Source	Variable											
	Shoot			Length			TDN			TDP		
Parameter	-----count-----			-----cm-----			-----mg L <sup>-1</sup> -----					
	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	5	0.5703	0.7219	5	13.57	< 0.0001	8	22.37	< 0.0001	8	42.30	< 0.0001
Date	6	18.83	< 0.0001	3	83.75	< 0.0001	2	1.820	0.1686	3	128.2	< 0.0001
Type x Date	30	1.683	0.0289	15	2.118	0.0265	16	1.106	0.3636	24	6.341	< 0.0001

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1016 Table S-3. Linear mixed effects test table for biomass taken at the end of the multi-  
 1017 dose experiment/Experiment #2.

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Variable						
Aboveground Biomass				Belowground Biomass		
Source	-----g-----					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Type	4	5.231	0.0077	4	4.560	0.0131

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1035 Table S-4. Linear mixed effects test table for tissue nutrients (percent weight) taken at the end of the multi-dose  
 1036 experiment/Experiment #2.

Variable												
Aboveground %TN				Aboveground %TP			Belowground %TN			Belowground %TP		
Source	-----Percent-----											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	4	0.784	0.560	3	0.583	0.639	4	3.072	0.049	4	0.539	0.709

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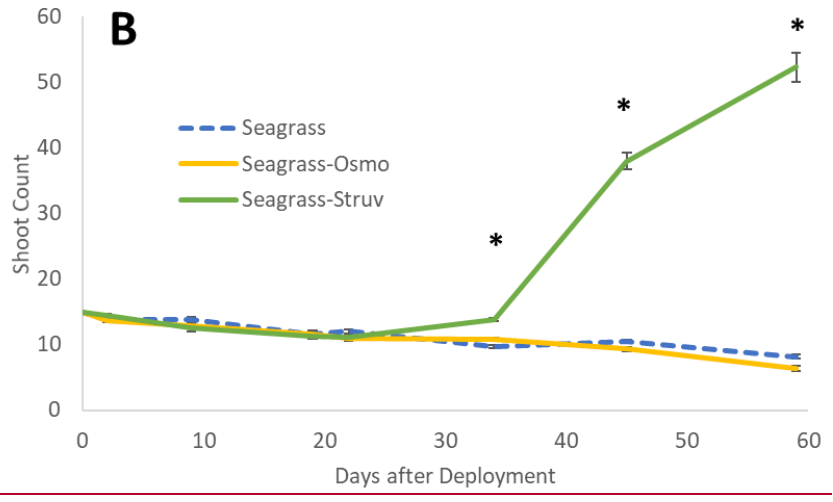
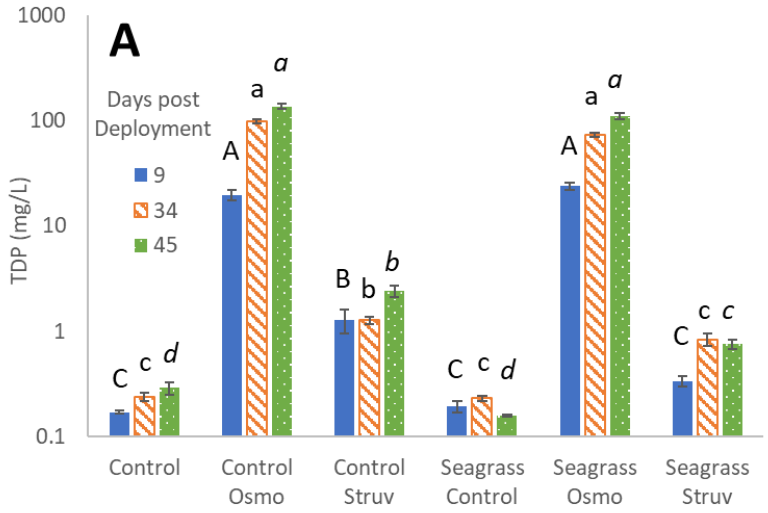


1038 Table S-5. Linear mixed effects test table for tissue nutrient mass (biomass x % nutrients) taken at the end of the multi-  
 1039 dose experiment/Experiment #2. Combined weights present the aboveground TN and TP weights were removed for the  
 1040 analysis (n=2 for Osmocote™ treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did  
 1041 not have combined samples.

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Variable													
Aboveground TN Mass				Aboveground TP Mass			Belowground TN Mass			Belowground TP Mass			
Source -----g-----													
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	P value
Type	3	1.619	0.2370	3	2.524	0.1070	4	3.276	0.0406	4	3.705	0.0273	

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Table S-6. Mean sediment TC/TN values taken at the end of the multi-dose experiment/Experiment #2. The treatments were labelled control (unfertilized, unplanted plots), Osmo (unplanted fertilized with Osmocote), Struv (unplanted fertilized with struvite), S-Control (unfertilized seagrass plots), S-Osmo-Lo and S-Osmo-Med (planted plots fertilized with Osmocote), S-Struv-Lo, S-Struv-Med, and S-Struv-Hi (planted plots fertilized with struvite).

Variable	TC		TN	
	Mean	SE	Mean	SE
Treatment	-----mg/kg-----			
Control	52.55 <sup>NS</sup>	2.22	2.058 <sup>NS</sup>	0.009
Control-Osmo	57.55 <sup>NS</sup>	2.24	2.073 <sup>NS</sup>	0.015



<u>Control-Struv</u>	<u>53.53<sup>NS</sup></u>	<u>6.73</u>	<u>2.036<sup>NS</sup></u>	<u>0.016</u>
<u>Seagrass</u>	<u>52.09<sup>NS</sup></u>	<u>4.33</u>	<u>2.020<sup>NS</sup></u>	<u>0.032</u>
<u>Seagrass-Osmo-Lo</u>	<u>50.56<sup>NS</sup></u>	<u>3.28</u>	<u>2.0259<sup>NS</sup></u>	<u>0.005</u>
<u>Seagrass-Osmo-Med</u>	<u>49.46<sup>NS</sup></u>	<u>3.56</u>	<u>2.053<sup>NS</sup></u>	<u>0.027</u>
<u>Seagrass-Struv-Lo</u>	<u>52.02<sup>NS</sup></u>	<u>6.17</u>	<u>2.057<sup>NS</sup></u>	<u>0.027</u>
<u>Seagrass-Struv-Med</u>	<u>48.70<sup>NS</sup></u>	<u>5.02</u>	<u>2.023<sup>NS</sup></u>	<u>0.026</u>
<u>Seagrass-Struv-Hi</u>	<u>58.16<sup>NS</sup></u>	<u>5.63</u>	<u>2.096<sup>NS</sup></u>	<u>0.02</u>

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Table S-7. Mixed effects test table for sediment TC/TN taken at the end of the multi-dose experiment/Experiment #2.

		<u>Variable</u>					
		<u>TC</u>			<u>TN</u>		
<u>Source</u>		<u>---mg/kg---</u>					
<u>Parameter</u>	<u>DF</u>	<u>F statistic</u>	<u>P value</u>	<u>DF</u>	<u>F statistic</u>	<u>P value</u>	
<u>Treatment</u>	<u>8</u>	<u>0.5028</u>	<u>0.8435</u>	<u>8</u>	<u>1.376</u>	<u>0.2514</u>	

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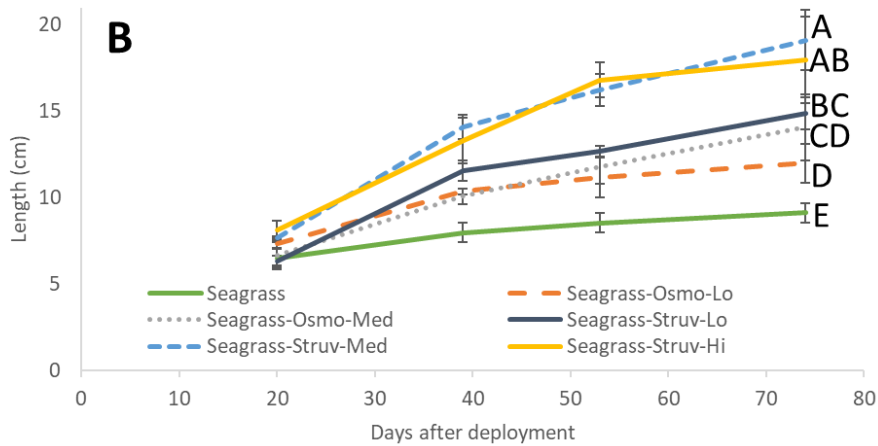
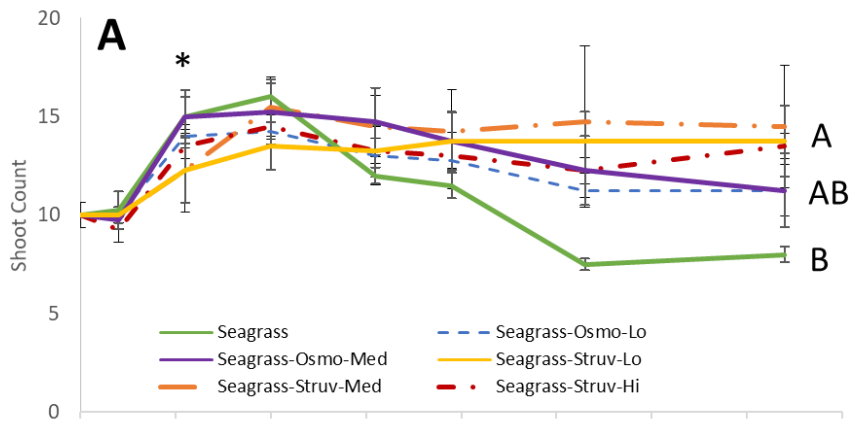


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**Table S-8.** Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count (B) taken during the first mesocosm experiment. The treatments were labelled Seagrass (for the unfertilized seagrass plots), Seagrass Osmocote™ (for planted plots fertilized with Osmocote™), and Seagrass Struv (planted plots fertilized with struvite). The asterisks designate significant differences between treatments for the same sample dates. Points represent the mean of six replicates ( $\pm$  SE).

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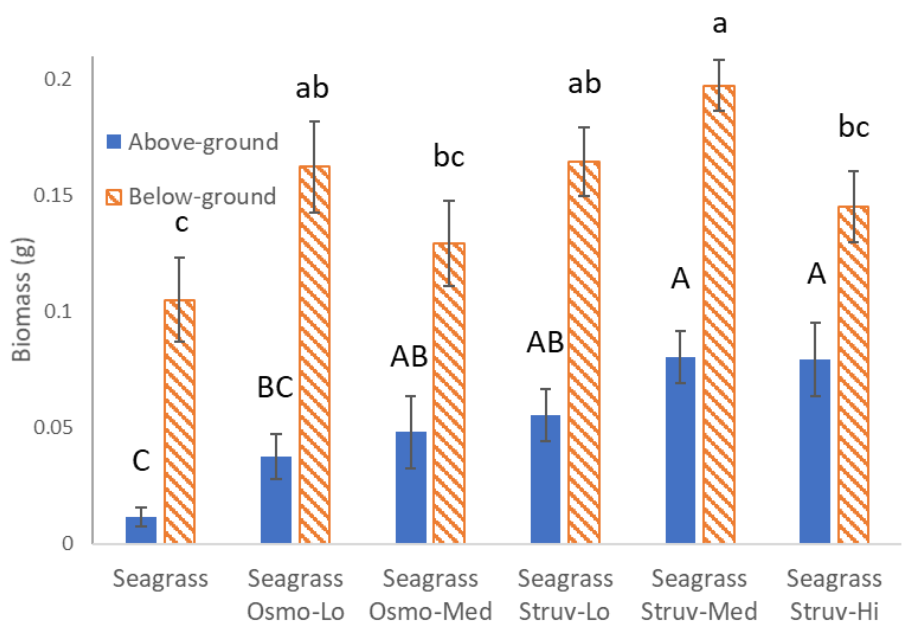




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 1124 **Figure 2.** Shoot count (A) and blade length (B) from the second mesocosm experiment.  
 1125 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass Osmo-  
 1126 Lo and Seagrass Osmo Med (planted plots fertilized with Osmocote™), Seagrass-  
 1127 Struv Lo, Seagrass Struv Med, and Seagrass Struv Hi (planted plots fertilized with  
 1128 struvite). \*: Five shoots were added to each plot to match the first/single dose  
 1129 experiment. Letters designate significant differences between treatments for the same

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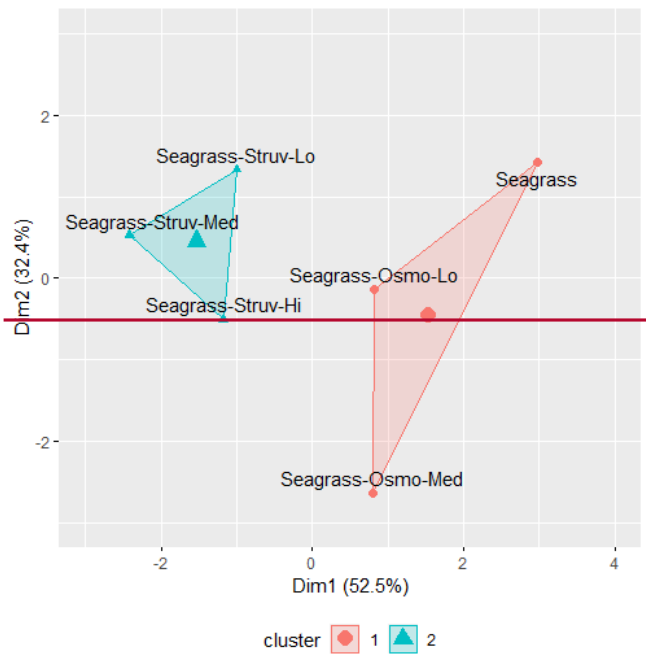
1130 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-  
 1131 ground biomass, which had two to four replicates.  
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1133 **Figure 3.** Above and belowground biomass from the second mesocosm experiment.  
 1134 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass Osmo-  
 1135 Lo and Seagrass Osmo Med (planted plots fertilized with Osmocote™), Seagrass  
 1136 Struv Lo, Seagrass Struv Med, and Seagrass Struv Hi (planted plots fertilized with  
 1137 etruvite). Letters designate significant differences between treatments for the same  
 1138 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-  
 1139 ground biomass, which had two to four replicates.  
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~~Figure 4. Clustering results of treatment methods from the second experiment. No overlapping clusters were formed, indicating a significantly different global effect of struvite onto the water chemistry and plant growth characteristics compared to Osmocote™ treatment or control plot. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass Osmo Lo and Seagrass Osmo Med (planted plots fertilized with Osmocote™), Seagrass Struv Lo, Seagrass Struv Med, and Seagrass Struv Hi (planted plots fertilized with struvite).~~

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**Table S-4.** Mean porewater nutrient measurements from the second experiment. The treatments were labelled Control (unfertilized, unplanted plots), Control-Osmo (unplanted fertilized with Osmocote™), Control-Struv (unplanted fertilized with struvite), Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Only TDP was analyzed for Day 31, therefore TDN values at that date are designated “NA.” Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

Variable		TDN		TDP	
Days after Deployment	Treatment	Mean	SE	Mean	SE
6	Control	1.51 <sup>NS</sup>	0.38	0.128 <sup>D</sup>	0.004
	Control-Osmo	12.03 <sup>NS</sup>	5.16	9.640 <sup>AB</sup>	3.685
	Control-Struv	4.35 <sup>NS</sup>	1.52	2.093 <sup>C</sup>	0.276
	Seagrass	1.41 <sup>NS</sup>	0.45	0.182 <sup>D</sup>	0.021
	Seagrass-Osmo-Lo	7.89 <sup>NS</sup>	4.34	4.750 <sup>BC</sup>	1.169
	Seagrass-Osmo-Med	26.8 <sup>NS</sup>	7.53	17.68 <sup>A</sup>	6.738
	Seagrass-Struv-Lo	4.33 <sup>NS</sup>	1.60	2.795 <sup>C</sup>	0.962
	Seagrass-Struv-Med	3.32 <sup>NS</sup>	0.88	1.943 <sup>C</sup>	0.211
	Seagrass-Struv-Hi	4.79 <sup>NS</sup>	0.86	2.620 <sup>C</sup>	0.351
20	Control	1.10 <sup>NS</sup>	0.16	0.133 <sup>C</sup>	0.010
	Control-Osmo	19.8 <sup>NS</sup>	2.14	0.508 <sup>A</sup>	0.102
	Control-Struv	5.59 <sup>NS</sup>	1.91	0.303 <sup>ABC</sup>	0.138
	Seagrass	0.78 <sup>NS</sup>	0.14	0.134 <sup>C</sup>	0.016
	Seagrass-Osmo-Lo	6.20 <sup>NS</sup>	2.33	0.289 <sup>ABC</sup>	0.094
	Seagrass-Osmo-Med	12.02 <sup>NS</sup>	0.82	0.472 <sup>AB</sup>	0.279

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Seagrass-Struv-Lo	2.47 <sup>NS</sup>	0.42	0.272 <sup>ABC</sup>	0.107
Seagrass-Struv-Med	2.58 <sup>NS</sup>	0.80	0.187 <sup>BC</sup>	0.021
Seagrass-Struv-Hi	6.81 <sup>NS</sup>	1.73	0.322 <sup>ABC</sup>	0.113

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**Table S.8. Continued.**

<u>Variable</u>		<u>TDN</u>		<u>TDP</u>	
<u>Days after Deployment</u>	<u>Treatment</u>	<u>Mean</u>	<u>SE</u>	<u>Mean</u>	<u>SE</u>
-----mg/L-----					
31	Control	NA	NA	0.110 <sup>D</sup>	0.009
	Control-Osmo	NA	NA	2.455 <sup>BC</sup>	0.560
	Control-Struv	NA	NA	2.923 <sup>ABC</sup>	0.966
	Seagrass	NA	NA	0.114 <sup>D</sup>	0.016
	Seagrass-Osmo-Lo	NA	NA	1.47 <sup>C</sup>	0.375
	Seagrass-Osmo-Med	NA	NA	9.733 <sup>AB</sup>	6.667
	Seagrass-Struv-Lo	NA	NA	0.163 <sup>D</sup>	0.038
	Seagrass-Struv-Med	NA	NA	1.353 <sup>C</sup>	0.166

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**Table S.1. Continued.**

<u>Variable</u>		<u>TDN</u>		<u>TDP</u>	
<u>Days after Deployment</u>	<u>Treatment</u>	<u>Mean</u>	<u>SE</u>	<u>Mean</u>	<u>SE</u>
-----mg L <sup>-1</sup> -----					
-	Seagrass-Struv-Hi	NA	NA	8.220 <sup>A</sup>	3.634
74	Control	1.97 <sup>NS</sup>	0.19	0.085 <sup>C</sup>	0.007
	Control-Osmo	12.2 <sup>NS</sup>	7.54	0.488 <sup>A</sup>	0.135
	Control-Struv	7.75 <sup>NS</sup>	2.00	0.159 <sup>BC</sup>	0.054
	Seagrass	1.68 <sup>NS</sup>	0.15	0.084 <sup>C</sup>	0.021
	Seagrass-Osmo-Lo	5.39 <sup>NS</sup>	0.54	0.261 <sup>AB</sup>	0.067
	Seagrass-Osmo-Med	17.3 <sup>NS</sup>	4.98	0.551 <sup>A</sup>	0.105
	Seagrass-Struv-Lo	4.04 <sup>NS</sup>	1.23	0.162 <sup>BC</sup>	0.064
	Seagrass-Struv-Med	4.74 <sup>NS</sup>	1.03	0.143 <sup>BC</sup>	0.023
	Seagrass-Struv-Hi	9.32 <sup>NS</sup>	1.62	0.156 <sup>BC</sup>	0.017

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Table S-29. Mean percent tissue nutrient content and ratio taken at the end of the second experiment. The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite). Combined weights present the aboveground TN and TP weights were removed for the analysis (n= 2 for Osmocote™ treatments, unfertilized seagrass removed). Belowground weights for both TN and TP did not have combined samples. Letters within biomass type represent a significantly different mean based on linear mixed model analyses (NS= not significant).

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Biomass Type	Treatment	TN		TP		TN:TP	
		Mean	SE	Mean	SE	Mean	SE
		-----Percent-----				Wt/Wt Ratio	
Above-ground	Seagrass	1.90 <sup>NS</sup>	NA	NA	NA	NA	NA
	Seagrass-Osmo-Lo	2.14 <sup>NS</sup>	0.11	0.258 <sup>NS</sup>	0.016	8.33 <sup>NS</sup>	0.57
	Seagrass-Osmo-Med	2.08 <sup>NS</sup>	0.11	0.249 <sup>NS</sup>	0.017	8.47 <sup>NS</sup>	0.88
	Seagrass-Struv-Lo	2.34 <sup>NS</sup>	0.23	0.236 <sup>NS</sup>	0.007	10.01 <sup>NS</sup>	1.20
	Seagrass-Struv-Med	2.31 <sup>NS</sup>	0.14	0.246 <sup>NS</sup>	0.010	9.38 <sup>NS</sup>	0.29





	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA
Below-ground	Seagrass	0.65 <sup>AB</sup>	0.06	0.166 <sup>NS</sup>	0.018	3.90 <sup>NS</sup>	0.36
	Seagrass-Osmo-Lo	0.53 <sup>B</sup>	0.07	0.154 <sup>NS</sup>	0.009	3.44 <sup>NS</sup>	0.47
	Seagrass-Osmo-Med	0.84 <sup>A</sup>	0.06	0.179 <sup>NS</sup>	0.008	4.71 <sup>NS</sup>	0.36
	Seagrass-Struv-Lo	0.66 <sup>AB</sup>	0.05	0.168 <sup>NS</sup>	0.011	3.94 <sup>NS</sup>	0.29
	Seagrass-Struv-Med	0.71 <sup>AB</sup>	0.07	0.164 <sup>NS</sup>	0.011	4.32 <sup>NS</sup>	0.45
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA

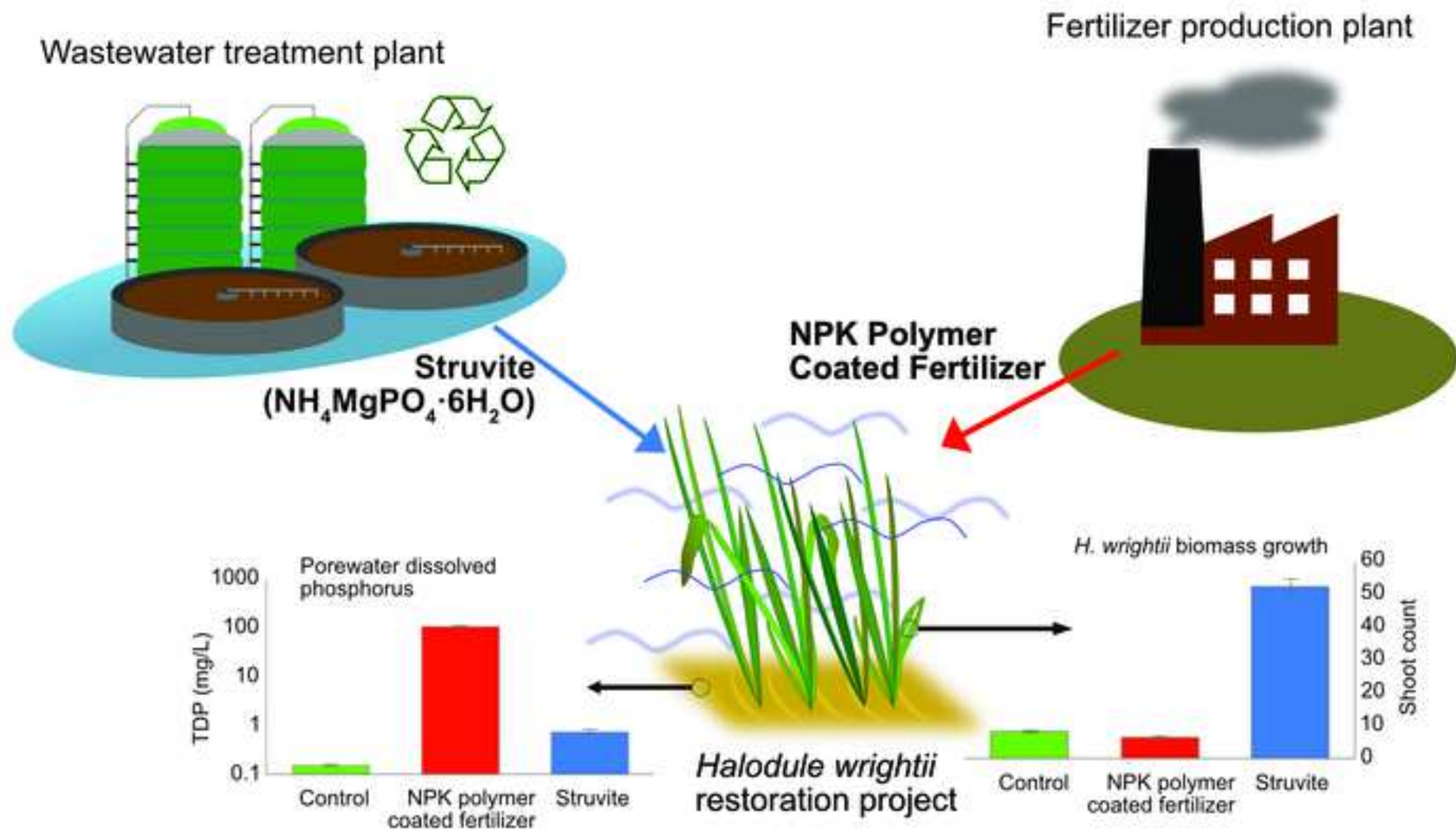
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1209 Figure S-1. Image showing an example of the differences in above and belowground  
1210 biomass in seagrasses from the first experiment. Observable stunted roots (possibly  
1211 root burn) are visible in the Osmocote™ treated plots (SP) versus the control (S) and  
1212 struvite treated plots (SS).

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**Highlights:**

Seagrass restoration is currently expensive and often unsuccessful.

Fertilizers improve restoration but can release excess nutrients.

Osmocote™ and struvite fertilizers were investigated for plant and nutrient metrics.

Struvite produced higher seagrass metrics and released less nutrients.

1 **Abstract**

2 Seagrasses are in decline worldwide, and their restoration is relatively expensive and  
3 unsuccessful compared to other coastal systems. Fertilization can improve seagrass  
4 growth in restoration but can also release nutrients and pollute the surrounding  
5 ecosystem. A slow-release fertilizer may reduce excessive nutrient discharge while still  
6 providing resources to the seagrass's rhizosphere. In this study, struvite (magnesium  
7 ammonium phosphate), a relatively insoluble, sustainable compound harvested in  
8 wastewater treatment plants, was compared to Osmocote™ (14:14:14 Nitrogen:  
9 Phosphorus: Potassium, N:P:K), a popular polymer coated controlled release fertilizer  
10 commonly used in seagrass restoration. Two experiments compared the effectiveness  
11 of both fertilizers in a subtropical flow-through mesocosm setup. In the first experiment,  
12 single 0.5 mg of P per g dry weight (DW) doses of Osmocote™ and struvite fertilizers  
13 were added to seagrass plots. Seagrass shoot counts were significantly higher in plots  
14 fertilized with struvite than both the Osmocote™ and unfertilized controls ( $p < 0.0001$ ).  
15 A significant difference in total P concentrations was observed in porewater samples of  
16 Osmocote™ vs struvite and controls ( $p < 0.0001$ ), with struvite fertilized plots emitting  
17 more than controls ( $p \leq 0.0001$ ), but less than 2% of the total dissolved P (TDP) of  
18 Osmocote™ fertilized plots (100+ mg/L versus  $x > 5$  mg/L). A subsequent experiment,  
19 using smaller doses (0.01 and 0.025 mg of P per gram DW added), also found that the  
20 struvite treatments performed better than Osmocote™, with 16-114% more  
21 aboveground biomass (10-60% higher total biomass) while releasing less N and P.  
22 These results indicate the relatively rapid dissolution of Osmocote™ may pose  
23 problems to restoration efforts, especially in concentrated doses and possibly leading to

24 seagrass stress. In contrast, struvite may function as a slow-release fertilizer applicable  
25 in seagrass and other coastal restoration efforts.

26 **Keywords:** *Halodule wrightii*; seagrass; marine restoration; fertilizer; struvite;  
27 Osmocote™; phosphorus

## 28 **1. Introduction**

29 In many environments, restoration is improved by fertilization, lessening nutrient  
30 limitations and improving growth of desired species (Armitage et al., 2011; Balestri &  
31 Lardicci, 2014; Fereidooni et al., 2013; Holmes, 2001; Jaquetti et al., 2014; Reed et al.,  
32 2007). However, in some environments, fertilizers can have a negative effect on  
33 species diversity and in extreme cases may even pollute the surrounding environment  
34 (Fonseca et al., 1998; Hill & Heck, 2015; Zedler, 2000). Therefore, consideration of the  
35 ecosystem, nutrient needs, and type of fertilizer is important to maximizing the benefits  
36 of fertilization approaches while minimizing the environmental impact of fertilizer use.

37 The ramifications of fertilizer use are especially relevant in coastal seagrass  
38 systems, which are both important habitats and currently facing global declines due to  
39 human disturbance and climate change (Bayraktarov et al., 2016). Seagrasses are a  
40 comparatively difficult and expensive coastal ecosystem to restore, partially due to  
41 eutrophication, competition from algae and other nutrient related issues (ibid).  
42 However, fertilizers have been consistently found to improve seagrass health and  
43 restoration success (Armitage et al., 2011; Kenworthy et al., 2018). Traditionally, both  
44 the direct application of controlled release fertilizers (Armitage & Fourqurean, 2016;  
45 Fonseca et al., 1998; Peralta et al., 2003; Sheridan et al., 1998) and the deployment of  
46 bird roosting stakes (Fonseca et al., 1994; Furman et al., 2019) have positive effects on  
47 seagrass biomass, and can accelerate ecosystem succession for seagrass (Bourque &

48 Fourqurean, 2014; Armitage et al., 2011). However, the use of traditional fertilization  
49 techniques in seagrass restoration may result in variable levels of nutrients or over-  
50 fertilization (Fonseca et al., 1998; Kenworthy et al., 2018), with consequences for the  
51 succession of seagrass species (ibid).

52 One of the main issues with fertilization in aquatic seagrass systems is that  
53 immersion and hydrodynamics can lead to rapid dissolution of fertilizers, increasing  
54 short term nutrient availability to the desired plant species, but at the expense of nutrient  
55 loss, ecosystem disruption, and pollution (Fonseca et al., 1998; Hill & Heck, 2015;  
56 Olsen & Valiela, 2010). For example, Hall et al. (2006) had to replace buried fertilizer  
57 pellets every three to four months in a macrophyte restoration effort, while Herbert and  
58 Fourqurean (2008) found that bird stakes (bird roosting structures that promote feces  
59 accumulation, Fonseca et al., 1994; Furman et al., 2019) can overfertilize seagrass  
60 sites, disrupting succession and increasing epiphytic biomass. These drawbacks are  
61 due either to the fertilizers being adapted for terrestrial applications, releasing nutrients  
62 too rapidly after flushing with water, or in the case of bird stakes, due to variable rates of  
63 feces deposition combined with diffusion of nutrients in the water during precipitation  
64 and settling (Hill & Heck, 2015). Applying multiple doses of traditional mineral fertilizers  
65 (Ferdie & Fourqurean, 2004; Hall et al., 2006; Olsen & Valiela, 2010) or monitoring bird  
66 stake treated beds for symptoms of excess fertilization (Kenworthy et al., 2018) also  
67 incurs a significant financial and labor cost. Thus, a slower dissolving fertilizer that  
68 resists leaching may reduce overfertilization and labor expenses while still providing  
69 benefits toward seagrass growth and survival.



70 Struvite (magnesium ammonium phosphate, or  $MgNH_4PO_4 \cdot 6H_2O$ ) is a by-  
71 product of wastewater treatment that is harvested in separated, side-stream sludge  
72 management processes (Ghosh et al., 2019). Struvite is poorly soluble in water, but  
73 releases P more rapidly in the presence of organic acids exuded from roots, making it a  
74 potentially ideal fertilizer for direct plant uptake (Cabeza et al., 2011; Robles-Aguilar et  
75 al., 2019). Past studies have supported both high performance of struvite for terrestrial  
76 plant applications as well as its resistance to flushing (Lee et al., 2009; Rahman et al.,  
77 2014).

78 While the utilization of struvite in aquatic systems appears very promising, to  
79 date there is an absence of studies investigating this fertilizer in marine restoration  
80 projects, especially in combination with other fertilization techniques. While it has been  
81 demonstrated that struvite is poorly soluble fertilizer except when exposed to acidic  
82 conditions (Cabeza et al., 2011; Talboys et al., 2016), experiments determining the  
83 availability of struvite to submerged aquatic vegetation do not currently exist. Thus, the  
84 goals of this study were to 1) assess potential differences in seagrass performance  
85 (shoot count, growth, length, and biomass as defined by Arrington, 2008, Herbeck et al.,  
86 2014, Rezek et al., 2019, Short & Coles, 2001, and Thomsen et al., 2012) after addition  
87 of struvite versus a polymer coated, controlled release fertilizer (Osmocote™)  
88 commonly used in seagrass restoration, and 2) to determine shifts in sediment and  
89 porewater nutrients caused by the introduction of the fertilizers in plots with and without  
90 seagrass. We hypothesized that seagrass in plots fertilized with struvite would have  
91 increased performance compared to plots fertilized with Osmocote™, and that struvite



92 would be dissolved at a slower rate than Osmocote™ (based on porewater total  
93 dissolved nutrients).

## 94 **2. Materials and Methods**

### 95 **2.1. Site Description and Design**

96 To minimize the variability found in field experiments and more accurately  
97 investigate nutrient levels related to fertilization, a mesocosm experiment was  
98 conducted at the Whitney Laboratory of Marine Biosciences in St. Augustine, FL.  
99 Seawater (filtered through a shelly sand and activated charcoal biofilter) pumped from  
100 offshore entered a 6.5 m diameter mesocosm (approximately 1 m deep), to emulate the  
101 natural environment. Water flow was constant into the mesocosm. Experiments were  
102 based on the methods explained in the propagation guide for *Halodule wrightii* (Biber et  
103 al., 2013). Seagrass was collected directly from donor sites off St. Martins Marsh  
104 Aquatic Preserve, FL. Shoots were removed from the donor sediment and maintained  
105 in cool conditions until they were transplanted into plastic pot containers (10 cm depth),  
106 buried in approximately 5 cm of coarse, shell-dominated sand taken from the local St.  
107 Augustine area (rinsed to reduce organics and residual nutrients). The sediment used  
108 had a mean grain size of 706 microns (not including particles greater than 2 mm).

#### 109 **2.1.1 Mesocosm Conditions**

110 Mesocosm temperature and salinity remained between 27-31 °C and 33-38 parts  
111 per thousand respectively during the periods sampled (between 9 am and 3 pm) for  
112 both studies. The hydraulic residence time was variable at 0.5-2 days, due to a limited  
113 saltwater supply. The mean TDN of surface water was  $0.44 \pm 0.06$  mg N L<sup>-1</sup>, while the  
114 mean TDP was  $0.035 \pm 0.001$  mg P L<sup>-1</sup> (or 0.029 mg P L<sup>-1</sup> when excluding a day of low  
115 inflow). The level of flow was great enough to prevent significant cross contamination of



116 the plots studied, as well as prevent significant swings in temperature and salinity that  
117 could stress the plants.

## 118 **2.2. Experiments**

119 Two separate experiments were conducted in the summer and fall of 2018. The  
120 first 60-day experiment consisted of six different treatment options, including bare sand  
121 with or without fertilizers (terrestrial polymer coated fertilizer or struvite) and seagrass  
122 with or without fertilizers. A second 70-day experiment was conducted consisting of  
123 multiple lower doses of both fertilizers.

### 124 **2.2.1. Single Dose/First Experiment**

125 For the polymer coated controlled release fertilizer treatment, Osmocote™  
126 14:14:14 NPK (Scotts Miracle-Gro Company, Marysville, OH, USA) was chosen due to  
127 its commercial availability, composition (containing both N and P), and past use in  
128 seagrass restoration experiments (Peralta et al., 2003; Sheridan et al., 1998; Tanner &  
129 Parham, 2010). Struvite used in the experiment was produced in a pilot scale fluidized  
130 bed reactor fed with sludge dewatering liquor. Detailed morphological and elemental  
131 characteristics are described elsewhere (Bydalek et al., 2018). Unlike the mostly  
132 homogenous struvite, each Osmocote™ prill has a porous outer layer that gradually  
133 releases a contained water-soluble nutrient dose through diffusion. The composition of  
134 elements is also different between the two compounds; with  $\text{NH}_4^+/\text{NO}_3^-$ -N comprising  
135 14% of Osmocote™ versus  $\text{NH}_4^+$ -N comprising only 6% of struvite (Osmocote™  
136 manufacturer information, Kenworthy & Fonseca, 1992; Rahman et al., 2014). The P  
137 composition of both fertilizers is also different, with struvite (13% P as  $\text{PO}_4^{3-}$ ) versus



138 having a higher concentration by weight versus Osmocote™ (6.1% P as P<sub>2</sub>O<sub>5</sub>)  
139 (Osmocote™ manufacturer information, Rahman et al., 2014).

140 In total, there were 30 plots, with an unplanted, untreated/unfertilized control  
141 (labelled control, n= 4), sediment-only treatments (labelled Control-Osmo and Control-  
142 Struv, n= 4), and seagrass control and treatments (labelled Seagrass, Seagrass-Osmo,  
143 and Seagrass-Struv, n= 6). Nutrient treatments were fertilized by adding the  
144 Osmocote™ or struvite equivalent of 3 g of P mixed into approximately 6 kg of sand  
145 (equivalent to 0.5 mg P g<sup>-1</sup> DW sand), which was about half of what was considered  
146 “low fertilized” according to Peralta et al. (2003). The dosing was equilibrated to P as  
147 tropical seagrass systems are primarily P limited (Brodersen et al., 2017; Gras et al.,  
148 2003). In this experiment, serving as pilot study, N concentrations were not equilibrated,  
149 however given the actual fertilizer dosages, concentrations were still below the low  
150 fertilized treatment in Peralta et al.’s study (0.23 mg N g<sup>-1</sup> DW sand for struvite and 1.16  
151 mg N g<sup>-1</sup> DW sand for Osmocote respectively). Each seagrass plot had exactly three  
152 individuals, each with five shoots. The first experiment was conducted for 60 days.  
153 During this period, the levels of dissolved total P porewater concentrations were  
154 excessively high, exceeding 100 mg P L<sup>-1</sup> in the Osmocote™ treatments and 5 mg P L<sup>-1</sup>  
155 for struvite.

### 156 **2.2.2. Multi-Dose/Second Experiment**

157 In this second experiment, struvite doses were 0.0125 (low dose struvite or  
158 Seagrass-Struv-Lo), 0.025 (medium dose struvite or Seagrass-Struv-Med) and 0.05 mg  
159 P g<sup>-1</sup> DW sand (high dose struvite or Seagrass-Struv-Hi). For Osmocote™, 0.0125 (low  
160 dose Osmocote™ or Seagrass-Osmo-Lo) and 0.025 mg P g<sup>-1</sup> DW (medium dose

161 Osmocote™ or Seagrass-Osmo-Med) doses were used. Unplanted, fertilized controls  
162 had a 0.0250 mg P g<sup>-1</sup> DW dose of Osmocote™ (Osmocote™ control or Control-Osmo)  
163 and struvite (struvite control or Control-Struv). Unfertilized, unplanted plots were  
164 labelled “control” while unfertilized, planted plots were labelled “unfertilized seagrass” or  
165 “Seagrass-Control”. There were four replicates for all controls/treatments. A high dose  
166 of Osmocote™ was not used due to space limitations in the mesocosm and concerns of  
167 overfertilization based on the results of the single dose/first experiment. There were  
168 three individuals with five shoots per plot (initially two individuals with the third added 10  
169 days post deployment to match the starting shoot count of the previous experiment).

### 170 **2.3. Plant and Nutrient Measurements**

171 Seagrass shoot count (seagrass shoots defined as a unit of several leaves or  
172 blades according to Short & Coles, 2001), were quantified approximately every 10 days  
173 in both experiments. During the second experiment, blade/leaf lengths (substrate to  
174 leaf tip according to Arrington, 2008) were also quantified. Surface water was sampled  
175 for temperature, salinity, and total dissolved nutrients (Total Dissolved N/TDN, Total  
176 Dissolved P/TDP), while porewater was only sampled for total nutrients and (randomly)  
177 sulfide presence. Surface and porewater samples were collected using a syringe  
178 sampler fashioned out of a 60 mL syringe attached to a plastic tube and 1 mL  
179 serological pipette with an attached air stone. The samples were filtered through a 0.45  
180 µm filter (Whatman, Maidstone, United Kingdom), preserved with sulfuric acid to a pH <  
181 2, and stored at 4 °C until analysis in the Wetland Biogeochemistry Laboratory (USEPA,  
182 1974, 1993). Porewater was also tested for the presence of sulfide (Calleja et al., 2007;  
183 Carlson et al., 1994) using a Hach test kit (product number 2537800). No measurable

184 sulfide was found in any plots sampled (detection limit 0.1 mg L<sup>-1</sup>). DOC and TDN  
185 samples were analyzed on a Shimadzu TOC-L analyzer fitted with a N module  
186 (Shimadzu Scientific Instruments, Durham, NC, USA) according to EPA method 415.1  
187 for TOC and ASTM D 8083 for total nitrogen (TN) (ASTM International, 2016; Nevins et  
188 al., 2020; USEPA, 1974). TDP was digested with persulfate in an autoclave and  
189 analyzed via a Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto,  
190 Japan) using EPA method 365.1 (Irick et al., 2015; USEPA, 1993).

191 At the end of the experiment, plant biomass and sediment were destructively  
192 sampled. Plants were rinsed to clean off sediments, and promptly frozen. In the lab,  
193 tissue samples were cleaned of epiphytes and rinsed with de-ionized water. Plant  
194 tissue and sediment samples were dried for 72 hours at 65 °C and ground using a ball  
195 mill. Sediment was analyzed for total carbon (TC), and nitrogen (TN), while tissue was  
196 analyzed for TC, TN, and phosphorus (TP). Bulk sediment TC/TN were run on an ECS  
197 4010 CHNSO analyzer (Costech Analytical Technologies, Inc., Valencia, CA, USA)  
198 (Nevins et al., 2020). Tissue TP was determined by ashing the sample followed by  
199 dissolution with 6 M HCL (following Andersen, 1976) and analysis for soluble P using a  
200 Shimadzu UV-1800 spectrophotometer (Shimadzu Corporation, Kyoto, Japan) (Liao et  
201 al., 2019; USEPA, 1993). Due to low and variable weights found after drying seagrass  
202 samples, plant dry biomass was calculated using a 10% wet weight conversion used for  
203 *H. wrightii* and *Thalassia testudinum* in Heck et al., (2015) and outlined in Short &  
204 Coles, (2001). A sediment particle analysis was also conducted to determine the  
205 distribution of particle sizes and possible changes over time. These samples were  
206 analyzed by the Soil and Water Sciences Environmental Pedology and Land Use

207 Laboratory using laser diffraction (LD) with a Beckman Coulter LS-13320 multi-wave  
208 particle size analyzer (Beckman Coulter Diagnostics, Brea, CA, USA).

## 209 **2.4. Statistical Analyses**

210 Differences in seagrass metrics (shoot count and shoot length) and porewater  
211 nutrients for both experiments were calculated using a linear mixed model, followed by  
212 a post hoc multiple comparison significant (Fisher's Least Significant Difference test).  
213 Factors included the treatment type, date, and the interaction between treatment and  
214 date. A linear mixed model analysis was also conducted on sediment and biomass  
215 measurements from the second experiment, testing the effect of treatment type. The  
216 tests were run using JMP 15.2.1 (SAS Software, Cary, NC, USA) with significance set  
217 to  $\alpha = 0.05$ . To determine the fit of the model predictions to the measured data,  
218 residuals and qq-plots were visually inspected and data was log transformed as  
219 necessary (shoot counts, shoot lengths, and total dissolved nutrients). To differentiate  
220 between the effects of fertilization methods, K-means clustering was applied to classify  
221 all observations in the multi-dose/second experiment. K-means were computed using  
222 the `kmeans` function in R (version R-4.0.2.). Given the number of observations ( $n = 6$ ) the  
223 data was predefined into two clusters (`centers = 2`). Prior to the analysis, the data was  
224 standardized using the `scale` function (each element is subtracted by the mean value of  
225 the vector and divided by standard deviation of the vector). The results were visualized  
226 using the `fviz_cluster` function (factoextra package) based on function's encoded  
227 principal component analysis (PCA) (Kassambara & Mundt, 2017).

## 228 **3. Results**

### 229 **3.1. Single Dose/First Experiment**

#### 230 **3.1.1 Plant Metrics**



231 Increases in shoot counts occurred one month after transplantation for the  
232 struvite treatment. However, this was not the case with the unfertilized control or the  
233 Seagrass-Osmo treatment, which both slowly declined on average. At the end of the  
234 first experiment, mean shoot counts ranged from  $6.33 \pm 0.87$  shoots in the Seagrass-  
235 Osmo treatment to  $52.33 \pm 5.49$  shoots in the Seagrass-Struv treatment (Figure 1).  
236 Seagrasses in struvite fertilized plots had significantly higher shoot counts than the  
237 seagrass control and Osmocote treatment ( $p < 0.01$ ). More specifically, the Seagrass-  
238 Struv treatment had a significantly higher shoot count in mid-July, just one month after  
239 planting ( $p < 0.05$ ), becoming greater over the next month (by end of the study  $p <$   
240  $0.001$ ). By the end of the study, the unfertilized seagrass also had a significantly higher  
241 number of shoots than the Seagrass-Osmo treatment ( $t = 2.56, p < 0.05$ ).

### 242 **3.1.2 Water Chemistry**

243 The TDP levels were significantly higher in the Seagrass-Osmo plots than the  
244 unfertilized controls and Seagrass-Struv treatments ( $p < 0.0001$ , table S1). By the end  
245 of the study, the average TDP concentration for the Seagrass-Osmo porewater plots  
246 was  $136.09 \pm 15.71$  mg P L<sup>-1</sup> for the unplanted plots (Control-Osmo) and  $109.53 \pm 19.96$   
247 mg P L<sup>-1</sup> for the planted plots (Seagrass-Osmo), more over ten times higher than the  
248 struvite plots, which was  $2.43 \pm 0.61$  mg P L<sup>-1</sup> in the unplanted plots and  $0.76 \pm 0.19$  mg  
249 P L<sup>-1</sup> in the Seagrass-Struv plots. Porewater TDP in the Control-Struv treatment was  
250 significantly higher than the control, unfertilized seagrass, and the Seagrass-Struv  
251 treatments ( $p < 0.001$ ), indicating that significant uptake of TDP by seagrasses likely  
252 occurred. There were no significant differences in TDP between the unplanted and  
253 planted Seagrass-Osmo plots, overall or during any specific sampling date.

## 254 **3.2. Multi-Dose/Second Experiment**

### 255 **3.2.1. Plant Metrics**

256 At the end of the second experiment, the average seagrass shoot counts ranged  
257 from  $8.00 \pm 0.41$  shoots in the Seagrass-Control to  $14.50 \pm 3.10$  shoots in the  
258 Seagrass-Struv-Med treatment (Figure 2). There was relatively less growth in the  
259 second experiment versus the first/single dose experiment, however the effects of date  
260 and its interaction with the treatment type were still significant for shoot count (Table S-  
261 2). After 53 days, seagrass shoot count started showing signs of treatment effect in  
262 comparison to control seagrass plot which showed significant shoot count declines ( $p <$   
263  $0.05$ ) in comparison to the rest of the fertilized seagrass plots. By the end of the  
264 experiment (74 days) only the Seagrass-Struv-Med treated seagrass plots maintained  
265 plant density ( $14.50 \pm 3.10$  shoots) close to the original coverage of 15 shoots per plot  
266 indicating high transplantation survival rate. At the conclusion of the study, only the  
267 struvite fertilized plots were statistically higher in shoot count than unfertilized plots.

268 The effects of both treatment and date were significant for blade length (Table  
269 S-2). All fertilized treatments became significantly greater in length than the Seagrass-  
270 Control after 39 days post deployment (Figure 2). The average seagrass blade length  
271 ranged from  $9.1 \pm 1.02$  cm in the unfertilized seagrass to  $19.1 \pm 1.74$  cm in the medium  
272 dose struvite by the end of the experiment. The highest increase in blade length was  
273 observed in struvite treatments. The Seagrass-Struv-Med treatment showed a  
274 significantly ( $p < 0.005$ ) higher blade growth than the Seagrass-Osmo-Lo/Med  
275 treatments.



276 The mean aboveground biomass ranged from  $0.012 \pm 0.004$  g DW in the  
277 Seagrass-Control to  $0.080 \pm 0.011$  g DW in the Seagrass-Struv-Hi treatment (Figure 3),  
278 with the effect of treatment type being significant (Table S-3). All fertilized plots had  
279 significantly higher aboveground biomass than the control, except for the Seagrass-  
280 Osmo-Lo treatment ( $p < 0.05$ ). There was a marginal significance found for the Med  
281 and Hi struvite doses having higher aboveground biomass than the Seagrass-Osmo-  
282 Med ( $p < 0.08$ ). Belowground biomass ranged from  $0.11 \pm 0.02$  g DW in the Seagrass-  
283 Control to  $0.20 \pm 0.01$  g DW in the Seagrass-Struv-Med treatment (Figure 3). The  
284 belowground biomass of control plots was significantly lower compared to all fertilized  
285 plots except for the Seagrass-Osmo-Med (Table S-3). Additionally, the Seagrass-Struv-  
286 Med dose had significantly higher belowground biomass compared to the Seagrass-  
287 Osmo-Med and Seagrass-Struv-Hi doses ( $p < 0.05$ ). Aboveground tissue %TN ranged  
288 from 1.9% in the unfertilized seagrass (one sample) to  $2.34 \pm 0.23\%$  in the Seagrass-  
289 Struv-Lo treatment, while tissue %TP ranged from  $0.236 \pm 0.007\%$  in the Seagrass-  
290 Struv-Lo treatment to  $0.258 \pm 0.016\%$  in the Seagrass-Osmo-Lo treatment (Table S-7).  
291 There was no significant effect of treatment on aboveground %TN or %TP (Table 4).  
292 The mean aboveground N:P ratios ranged between  $8.3 \pm 0.57$  for the Seagrass-Osmo-  
293 Lo and  $10.0 \pm 1.20$  for Seagrass-Struv-Lo treatment. The N:P ratio and the mean  
294 aboveground TN and TP weights in the seagrasses (calculated by multiplying the  
295 biomass with the tissue %TN or %TP) yielded no significant differences (Tables S-4 and  
296 7). Belowground tissue %TN ranged from  $0.53 \pm 0.07\%$  for the Seagrass-Osmo-Lo  
297 treatment to  $0.84 \pm 0.06\%$  in the Seagrass-Osmo-Med treatment, while tissue %TP  
298 ranged from  $0.154 \pm 0.009\%$  for the Seagrass-Osmo-Lo treatment to  $0.179 \pm 0.008\%$



299 for the Seagrass-Osmo-Med treatment. The effect of treatment type was significant for  
300 belowground %TN (Table 4), with the Seagrass-Osmo-Med being significantly higher  
301 than the Seagrass-Osmo-Lo ( $p < 0.05$ ). No effects were significant for belowground  
302 %TP. The mean belowground N:P ratio ranged from  $3.4 \pm 0.47$  for the Seagrass-  
303 Osmo-Lo and  $4.7 \pm 0.36$  for the Seagrass-Osmo-Med treatment. The effect of  
304 treatment type was significant for both the belowground mass of TN and TP (% total  
305 nutrient x biomass, Table S-5).

### 306 **3.2.2. Water, Tissue, and Sediment Chemistry**

307 Nutrient dynamics in porewater differ significantly between the fertilizer types  
308 indicating different dissolution kinetics and plant and substrate interaction. Unfertilized  
309 control plots (planted and unplanted) showed variable TDN concentrations throughout  
310 the experiment however, never surpassing  $2 \text{ mg TDN L}^{-1}$ . Background porewater TDP  
311 content in observed controls varied within  $0.05\text{-}0.15 \text{ mg TDP L}^{-1}$ . The biggest nutrient  
312 release was observed at plots fertilized with Osmocote with peak nutrient  
313 concentrations occurring at 6<sup>th</sup> day of experiment reaching  $26.8 \pm 7.53 \text{ mg TDN L}^{-1}$  and  
314  $17.68 \pm 6.74 \text{ mg TDP L}^{-1}$  for medium Osmocote dose. TDP dynamics in struvite  
315 seagrass treatments were highly variable throughout the time and showed alternating  
316 pulses of TDP release. However, by the end of the experiment porewater TDP content  
317 in struvite fertilized plots was 2-3 times lower than in respective Osmocote treatments.  
318 DOC measured at the end of the study was between  $12.26 \pm 0.67 \text{ mg DOC L}^{-1}$  for  
319 Seagrass-Struv-Lo and  $14.71 \pm 1.23 \text{ mg DOC L}^{-1}$  for Seagrass-Osmo-Lo.

320 The average TC content of sediment ranged from  $48.7 \pm 5.02 \text{ g C kg}^{-1}$  in the  
321 medium dose struvite to  $58.2 \pm 5.63 \text{ g C kg}^{-1}$  in the Seagrass-Struv-Hi, while the

322 average TN content ranged from  $2.02 \pm 0.032 \text{ g N kg}^{-1}$  in the Seagrass-Control to  $2.10 \pm$   
323  $0.020 \text{ g kg}^{-1}$  in the Seagrass-Struv-Hi treatment (Table S-6). There were no significant  
324 differences in the TC or TN contents between treatments (Table S-7).

325 Porewater nutrients and seagrass metrics were used to further assess the global  
326 effect of fertilization dose and method based on multivariate analysis. K-means  
327 clustering detected two separate groups. The struvite treatment was clearly  
328 distinguished from the Osmocote™ treatment and control plot, occupying separated,  
329 non-overlapping clusters on the PCA plane (Figure 4), reinforcing the significant effects  
330 of struvite on seagrass and its surrounding environment.

## 331 **4. Discussion**

### 332 **4.1. Factors in Seagrass Performance**

333 Fertilizer application improved seagrass metrics compared to the unfertilized  
334 control in all but the Seagrass-Osmo treatment of the first experiment. This included  
335 average shoot count (more than six times higher vs the control at the end of the first  
336 experiment, and up to 81% at the end of the second experiment), length (up to 110% at  
337 the end of the second experiment, Figure 2), and biomass (up to 138% at the end of the  
338 second experiment, Figure 3). In general, these results support past findings  
339 examining the effects of fertilizer in the restoration of seagrass ecosystems (Armitage et  
340 al., 2011; Kenworthy et al., 2018). Additionally, the results of this study found that  
341 compared to equivalent P dosages with Osmocote, fertilization using struvite resulted in  
342 higher average seagrass shoot count (more than eight times higher by the end of the  
343 first experiment, and 29% at the end of the second experiment), length (up to 36% at  
344 the end of the second experiment, Figure 2), and biomass (up to 60% higher total  
345 biomass at the end of the second experiment, Figure 3). The significant multivariate

346 improvements in plant metrics in both experiments are promising towards the use of  
347 struvite as a fertilizer to rapidly establish seagrass species in future restoration efforts.

348 In addition to improving seagrass metrics, struvite consistently released less  
349 nutrients than Osmocote™. Porewater TDN was excessive in the Osmocote™  
350 treatment in the first experiment (> 100 mg/L). In the second experiment, TDN in  
351 struvite treated plots was as low as 12% of Osmocote™ treated plots (Table S-8).  
352 Porewater TDP in equivalent struvite doses was less than 2% TDP of Osmocote™ in  
353 the first experiment (Figure 1), and as low as 10% P of Osmocote™ in equivalent  
354 struvite doses in the second experiment (Table S-8). The speed of nutrient release by  
355 Osmocote™ was so high, that it may have contributed to the decreased performance of  
356 the Osmocote™ treatments through excessive N levels, as evidenced by roots that  
357 appeared stunted from possible root burn (observed in the first experiment, Figure S-1),  
358 commonly associated with N exposure (NC State, 2018; Schönau & Herbert, 1983).

359 The possible root burn in Osmocote™ treated seagrass may be the result of  
360 nitrate (Peralta et al., 2003; Statton et al., 2014) or ammonia (van der Heide et al.,  
361 2008) fractions in the fertilizer. However, previous seagrass (*Zostera marina*)  
362 mesocosm studies have detected increased seagrass metrics following Osmocote™  
363 fertilization. For example, *Zostera marina* plants were found to have increased shoot  
364 counts after one month of Osmocote™ 14:14:14 NPK fertilizer exposure compared to  
365 unfertilized plots (Wang et al., 2020). Similarly, another study found significant  
366 differences in shoot length in *Z. marina* over a period of two months when exposed to  
367 fertilizer doses higher than those used in this study (Peralta et al., 2003). In these  
368 cases, it should be noted that *Z. marina* exhibited a “remarkable tolerance” of N and P

369 fertilization, and many species of seagrass may not be as flexible regarding higher  
370 levels of nutrient exposure.

371 Another factor affecting the difference between struvite and Osmocote™ could  
372 be the balance of N versus P. In the second experiment, the aboveground tissue N:P  
373 ratios ( $8.3 \pm 0.57$  to  $10.0 \pm 1.20$ , Table S-9) consistently exceeded the traditionally  
374 accepted threshold for a balanced nutrient supply for seagrasses (14 weight N:P ratio  
375 calculated from the 30:1 molar N:P ratio as provided by Atkinson & Smith, [1983]). A  
376 study of *H. wrightii* found that in a natural system (Florida Bay) the molar N:P was over  
377 20, while in a fertilized scenario (using bird roosting stakes) the ratio was approximately  
378 13 (Powell et al., 1989). Thus, the authors argued that *H. wrightii* was P limited in a  
379 natural setting, and N limited when fertilized. Another study in Florida Bay found that *H.*  
380 *wrightii* was “released” from P limitation at tissue N:P weight ratios between 9.7 and 21  
381 (Armitage et al., 2011). Generally, the *H. wrightii* in all fertilized plots did not appear to  
382 be strongly limited by a specific nutrient, exceeding the 1.8% TN/ 0.2% TP tissue  
383 nutrient requirement defined by Duarte (1990). The exception to this may have been  
384 the control, which was closer to N limitation than all plots with a 1.9% TN tissue content,  
385 although this conclusion is tenuous because only one replicate was able to be analyzed  
386 due to a lack of biomass.

387 The lack of significant differences in tissue nutrient content between fertilized and  
388 non-fertilized treatments may be due to delays in nutrient response by the plants. For  
389 example, one study found that it took *Thalassia testudinum* four months to acquire  
390 elevated N levels after fertilizer exposure, while elevated P levels in plants took up to 14  
391 months to develop (Ferdie & Fourqurean, 2004). While *H. wrightii* is a faster growing



392 species, and higher growth was demonstrated in fertilized vs non-fertilized plots, the  
393 limited experiment duration may not have fully captured long-term increases in tissue  
394 content. However, significant differences in belowground nutrient content (i.e. medium  
395 dose struvite vs. non-fertilized control, Table S-4) and tissue nutrient weight (Table S-5)  
396 indicate uptake of nutrients by the seagrass.

397 Furthermore, the size of the mesocosm plots may have been a factor in the high  
398 porewater nutrient levels by preventing lateral flow of porewater and limiting diffusion.  
399 The current flow and increased sediment depth may dilute porewater, increase  
400 diffusion, and reduce the effectiveness of fertilizers in a natural environment, requiring  
401 more fertilizer for field studies. This potential problem may be partially compensated by  
402 the relatively large grain size of the shelly sand used in the study, compared to the often  
403 silty sand found in seagrass systems (a property produced by seagrass beds as  
404 discussed in Folmer et al., 2012). The lack of sulfide present in the experiment also  
405 indicates a higher redox potential that is likely not present in field experiments.

406 This study demonstrated that struvite and Osmocote™ both released N and P for  
407 at least two months (Table S-8). Based on longer studies, it is expected that  
408 Osmocote™ would provide N and P for 4-6 months (Hall et al., 2006; Olsen and Valiela,  
409 2010). Struvite may be able to provide nutrients for longer periods, indicated by its  
410 slower release rate. After the second experiment, selected fertilized plots were moved  
411 to another mesocosm and left submerged. A year after the experiment was deployed,  
412 the only evidence found of the Osmocote™ fertilizer were the outer membranes of the  
413 prills, whereas struvite granules were still found in the mesocosm plots, indicating a  
414 potential continued release of nutrients. Thus, while the effects of struvite were only

415 measured for up to nine weeks, the presence of struvite after this extended period  
416 indicates that struvite could be effective throughout a whole growing season or longer.  
417 The ability of struvite to produce higher seagrass metrics while emitting less nutrients  
418 (indicating a more sustained release of nutrients over a longer period of time) is  
419 promising toward the future applications of struvite in future coastal restoration efforts.

#### 420 **4.2. Field Applications of the Study**

421 The controlled environment of the mesocosm study allowed tests to be done with  
422 minimal interference from the confounding variables of a field study. However, several  
423 external factors may still have affected the results of the two experiments. The first  
424 experiment was conducted at the peak of the seagrass growing season (June through  
425 August), whereas the second experiment occurred during the end of the season  
426 (August through October, with the season typically ending in September; Choice et al.,  
427 2014). The later date of deployment could help explain why differences between shoot  
428 counts were not as apparent in the second experiment. Based on the declining  
429 seagrass performance above the medium/0.025 mg P g<sup>-1</sup> DW dose, there may have  
430 been even larger differences in the first experiment between struvite and Osmocote™ if  
431 the second experiment was begun earlier in the summer.

432 When considering the broad applicability of the results, it is important to note how  
433 close the conditions in the mesocosm were mimicking the natural environment. First,  
434 the local sediment substrate was not sterilized and contained a representative microbial  
435 population. Similarly, seawater for the mesocosm was only prefiltered to minimize inputs  
436 of algae or debris, and largely maintained the natural composition and physiochemistry.  
437 The mesocosm environment was sheltered from hydrodynamic disturbance and

438 herbivory which are significant problems in field restoration efforts (Bourque &  
439 Fourqurean, 2013; W. Kenworthy et al., 2018; Tuya et al., 2017). However, there are  
440 numerous techniques such as protective cages, or biodegradable lattices, artificial  
441 seagrass, in ground fertilizer application, and sediment tubes that aim to minimize  
442 environmental disturbances and which can be successfully integrated into restoration  
443 projects utilizing fertilizers (Hall et al., 2006; Hammerstrom et al., 1998; W. J. Kenworthy  
444 et al., 2018; Li et al., 2019; MacDonnell et al., 2022; Temmink et al., 2020; Tuya et al.,  
445 2017).

446 Multiple field and mesocosm seagrass studies investigating the use of  
447 Osmocote™ have yielded generally similar results (Peralta et al., 2003; Pereda-Briones  
448 et al., 2018; Tanner & Parham, 2010). Both struvite/Osmocote™ experiments could be  
449 considered extensions of these previous investigations with real world applications.  
450 However, it must be noted that a successful mesocosm scale study such as this one  
451 cannot simply be scaled up to field applications. Rather, it would require the additional  
452 understanding of local environmental conditions and applied restoration techniques that  
453 enhance the success rate. Therefore, a future field study would be recommended to  
454 optimize the dose of struvite in different biogeochemical conditions and assess  
455 associated operational efforts and costs.

#### 456 **4.3. Implications/Applications of Struvite**

457 The integration of struvite in restoration projects could have multiple advantages  
458 for both environmental management and sustainability of wastewater treatment. First,  
459 more research is needed, but struvite is potentially less harmful for the environment  
460 than traditional commercial fertilizers. For example, struvite is sourced from  
461 wastewater, a source of eutrophication for many coastal systems (Mayer et al., 2016).

462 The N content of struvite is also relatively low, and while it still provides plants with  
463 nutrients, it limits excess fertilization and resulting nitrous oxide emissions (Rahman et  
464 al., 2014). Second, that struvite is sustainable and locally sourced has global  
465 implications as P resources are being depleted in an accelerating rate, and there are  
466 indications that demand will surpass supply within the next 20 years (Nedelciu et al.,  
467 2020). The processing of struvite allows for the production of a P fertilizer without  
468 dealing with the instability and increasing costs of importing fertilizer (Rufí-Salís et al.,  
469 2020; Ye et al., 2020). Finally, the feasibility of using struvite on multiple scales has  
470 been demonstrated in experiments and industrial applications, indicating a practical and  
471 readily available treatment process (Ghosh et al., 2019).

472 The advantages of struvite in reducing pollution and phosphate shortages,  
473 combined with its feasibility, make it an attractive alternative P and N fertilizer. Struvite  
474 is a recognized slow-release terrestrial nutrient amendment with a low environmental  
475 footprint. However, struvite application is still limited due to its high price in comparison  
476 to conventional mineral fertilizers and availability. Therefore, extending application of  
477 struvite into areas such as restoration could potentially create a new market, thus  
478 making struvite more affordable and available. This is particularly important since  
479 struvite represents a very important aspect of circular economy in water management.

## 480 **5. Summary and Conclusions**

481 Because of the current need for effective fertilization methods that minimize  
482 environmental risk, this study evaluated the wastewater by-product struvite and its  
483 potential to enhance seagrass growth under simulated natural conditions. Seagrass  
484 growth metrics (shoots, length, biomass) in plots fertilized with struvite were consistently  
485 equal to or better than the commercial fertilizer Osmocote™. This improvement in



486 seagrass performance was provided while also producing lower porewater nutrient  
487 release from equal P fertilization doses, likely due to the slower release of nutrients from  
488 struvite delivering a low but sustained load of N and P to the rhizosphere. Excessive N  
489 inputs from the Osmocote™ treatment in the first experiment may have even reduced  
490 performance of treated plots compared to the unfertilized control. Measurements of  
491 porewater nutrients and visual observations indicated that struvite has a lower solubility  
492 and is therefore longer lasting compared to Osmocote™ in marine conditions. Other  
493 possible factors in plant performance, including the effects of specific nutrients (i.e.  
494 temporal delays in N/P tissue concentration, micronutrient differences), current flow  
495 (possibly increasing nutrient diffusion), and sediment particle size (affecting dissolution  
496 rates and redox potential), will require further investigation.

497 Future studies should apply the results of this experiment in multiple coastal  
498 systems, ensuring that results are not constrained to a seagrass mesocosm setting.  
499 Testing the solubility of struvite in different environments may reveal more applications  
500 for the fertilizer. Experiments should include other seagrass species with diverse  
501 nutrient requirements, and ideally, a restoration experiment would take place over  
502 multiple growing seasons to determine how long struvite remains effective. Special  
503 consideration should also be given toward testing the effectiveness of struvite in a more  
504 N-limited environment, where other fertilizers may have a better advantage. This study  
505 was a first ever attempt to apply struvite in marine restoration project, serving as an  
506 example of interdisciplinary merger between wastewater treatment engineering and  
507 restoration ecology. The positive results here should encourage future research and

508 field activities to further explore the application of struvite and similar materials for  
509 restoration projects.

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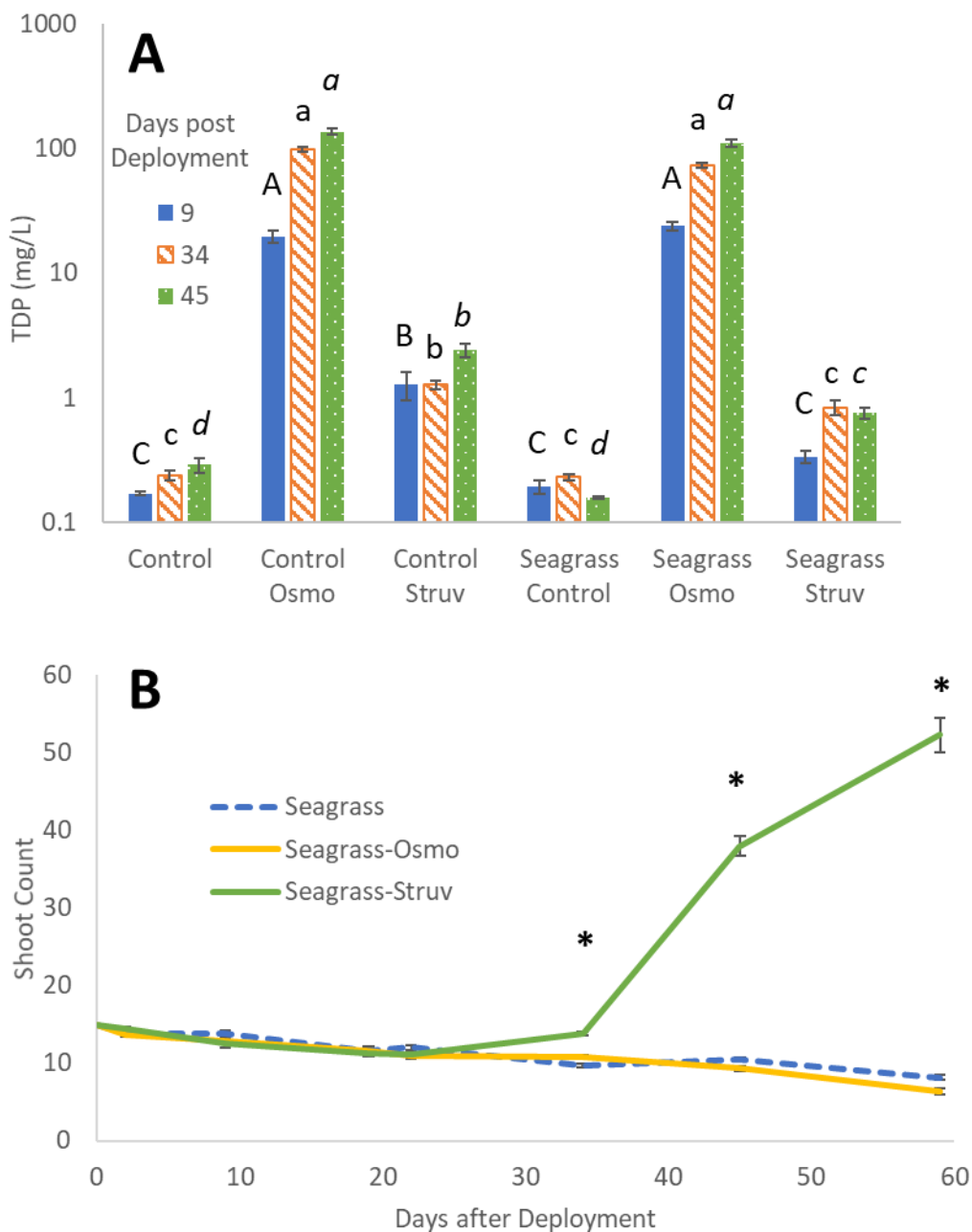
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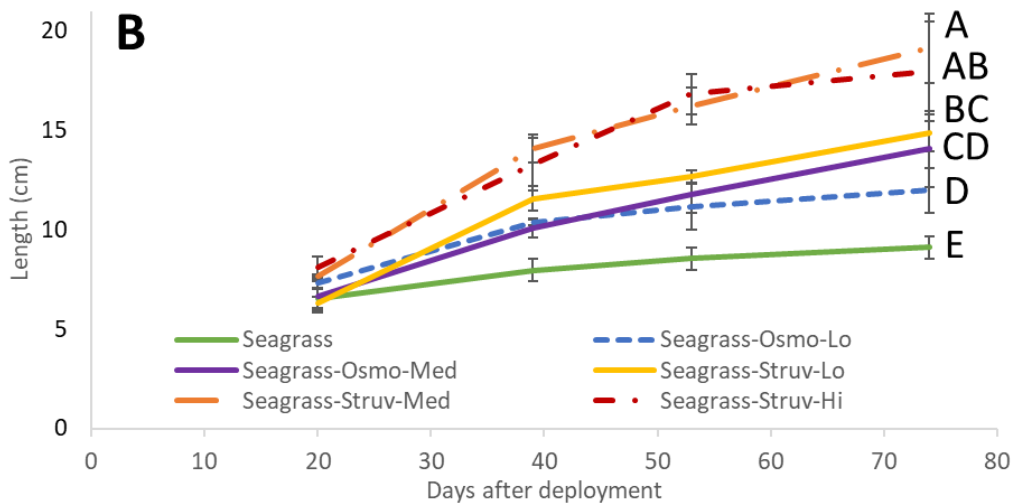
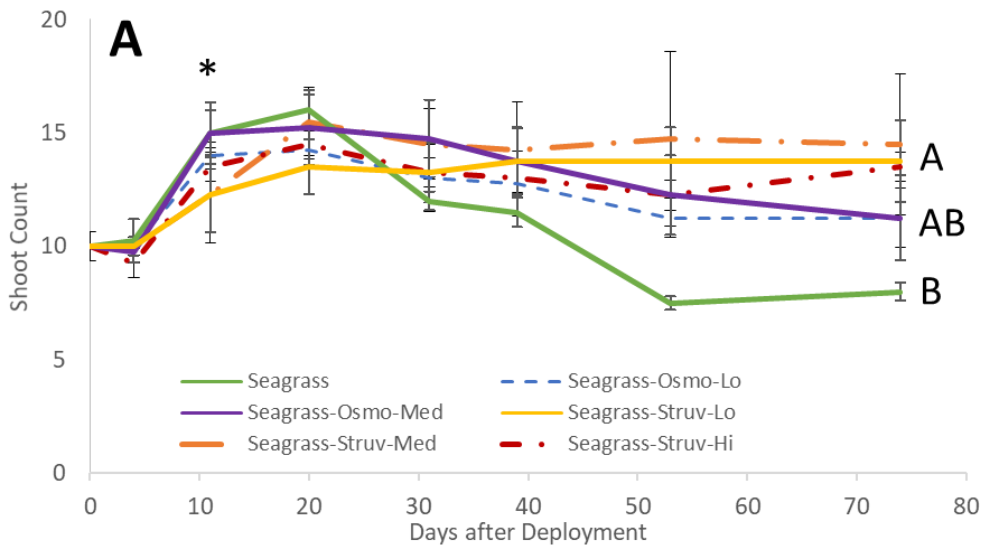
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 768 Figure 1. Porewater total dissolved phosphorus (TDP) (A), and seagrass shoot count  
 769 (B) taken during the first mesocosm experiment. The treatments were labelled  
 770 Seagrass (for the unfertilized seagrass plots), Seagrass-Osmocote™ (for planted plots  
 771 fertilized with Osmocote™), and Seagrass-Struv (planted plots fertilized with struvite).  
 772 The asterisks designate significant differences between treatments for the same sample  
 773 dates. Points represent the mean of six replicates ( $\pm$  SE).



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775 Figure 2. Shoot count (A) and blade length (B) from the second mesocosm experiment.

776 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-

777 Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-

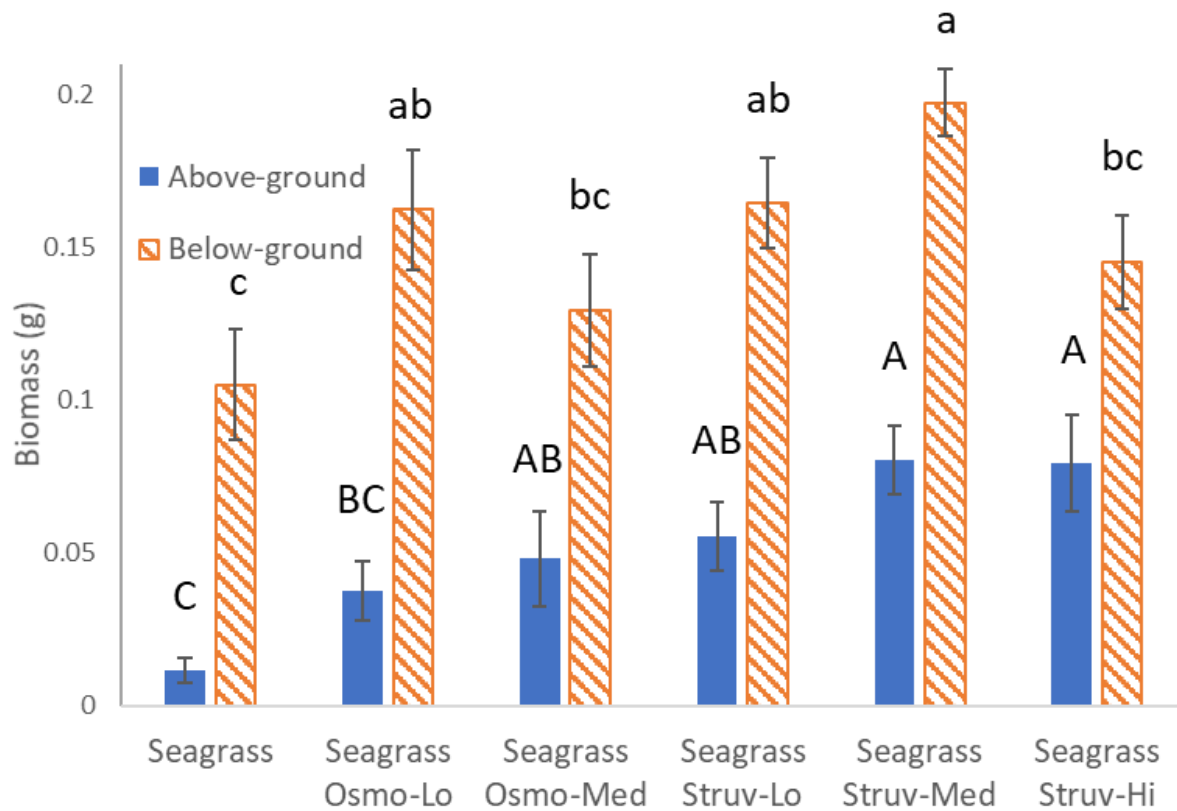
778 Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with

779 struvite). \*: Five shoots were added to each plot to match the first/single dose

780 experiment. Letters designate significant differences between treatments for the same

781 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-

782 ground biomass, which had two to four replicates.



783 Figure 3. Above and belowground biomass from the second mesocosm experiment.  
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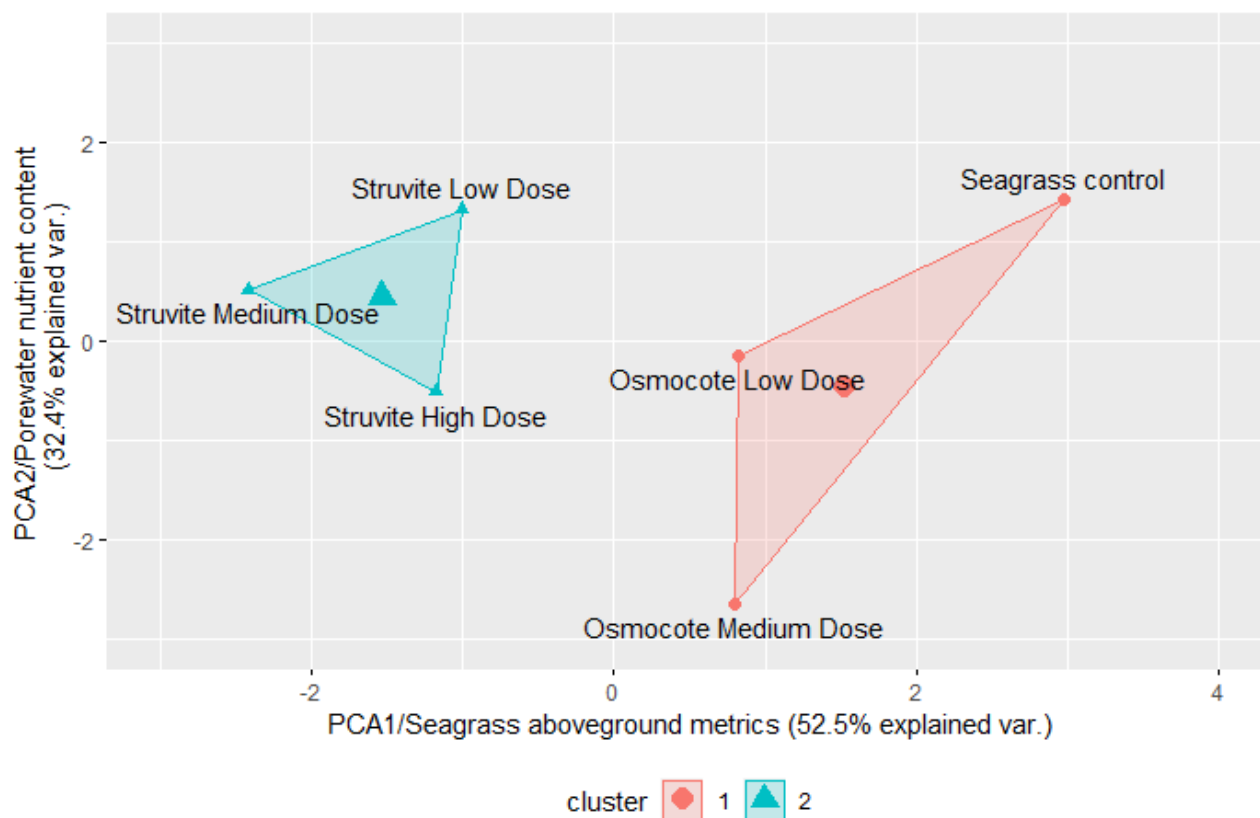
785 The treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-  
786 Lo and Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-  
787 Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with  
788 struvite). Letters designate significant differences between treatments for the same  
789 sample dates. Points represent the mean of four replicates ( $\pm$  SE), except for above-  
790 ground biomass, which had two to four replicates.

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794 Figure 4. Clustering results of treatment methods from the second experiment. No  
 795 overlapping clusters were formed, indicating a significantly different global effect of  
 796 struvite onto the water chemistry and plant growth characteristics compared to  
 797 Osmocote™ treatment or control plot. Seagrass aboveground metrics (shoot count, blade  
 798 length and aboveground biomass) were heavily correlated ( $r > 95\%$ ,  $p < 0.001$ ) with first  
 799 principal component which explained 52.5% of the variance in the dataset. Porewater  
 800 nutrient dynamics such as TDN and TDP were most correlated ( $p < 0.05$ ) and contributing  
 801 to second principal component which explained 32.4% of the variance in the dataset. The  
 802 treatments were labelled Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and  
 803 Seagrass-Osmo-Med (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo,  
 804 Seagrass-Struv-Med, and Seagrass-Struv-Hi (planted plots fertilized with struvite).

805 Table S-1. Two-way linear mixed effects test results for shoot count and TDP from the  
 806 single dose experiment/Experiment #1. Factors include treatment, date sampled, and  
 807 the interaction between these factors.

Source	Variable					
	Shoot			TDP		
	-----count-----			-----mg L <sup>-1</sup> -----		
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	2	35.91	< 0.0001	5	246.7	< 0.0001
Date	7	10.07	< 0.0001	2	19.50	< 0.0001
Treatment x Date	14	27.95	< 0.0001	10	2.201	0.0328

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822 Table S-2. Two-way linear mixed effects test results for seagrass metrics and  
 823 porewater nutrients from the multi-dose experiment/Experiment #2. Factors include  
 824 treatment, date sampled, and the interaction between these factors.

Source	Variable											
	Shoot			Length			TDN			TDP		
	-----count-----			-----cm-----			-----mg L <sup>-1</sup> -----					
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	5	0.5703	0.7219	5	13.57	< 0.0001	8	22.37	< 0.0001	8	42.30	< 0.0001
Date	6	18.83	< 0.0001	3	83.75	< 0.0001	2	1.820	0.1686	3	128.2	< 0.0001
Type x Date	30	1.683	0.0289	15	2.118	0.0265	16	1.106	0.3636	24	6.341	< 0.0001

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826 Table S-3. Linear mixed effects test table for biomass taken at the end of the multi-  
 827 dose experiment/Experiment #2.

Variable						
Aboveground Biomass			Belowground Biomass			
Source	-----g-----					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Type	4	5.231	0.0077	4	4.560	0.0131

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845 Table S-4. Linear mixed effects test table for tissue nutrients (percent weight) taken at the end of the multi-dose  
 846 experiment/Experiment #2.

Variable												
Aboveground %TN				Aboveground %TP			Belowground %TN			Belowground %TP		
Source	-----Percent-----											
Parameter	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
Type	4	0.784	0.560	3	0.583	0.639	4	3.072	0.049	4	0.539	0.709

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848 Table S-5. Linear mixed effects test table for tissue nutrient mass (biomass x %  
 849 nutrients) taken at the end of the multi-dose experiment/Experiment #2. Combined  
 850 weights present the aboveground TN and TP weights were removed for the analysis (n=  
 851 2 for Osmocote™ treatments, unfertilized seagrass removed). Belowground weights for  
 852 both TN and TP did not have combined samples.

Variable												
Source												
-----g-----												
Parameter	Aboveground TN Mass			Aboveground TP Mass			Belowground TN Mass			Belowground TP Mass		
Type	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value	DF	F statistic	P value
	3	1.619	0.2370	3	2.524	0.1070	4	3.276	0.0406	4	3.705	0.0273

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880 Table S-6. Mean sediment TC/TN values taken at the end of the multi-dose  
 881 experiment/Experiment #2. The treatments were labelled control (unfertilized,  
 882 unplanted plots), Osmo (unplanted fertilized with Osmocote), Struv (unplanted fertilized  
 883 with struvite), S-Control (unfertilized seagrass plots), S-Osmo-Lo and S-Osmo-Med  
 884 (planted plots fertilized with Osmocote), S-Struv-Lo, S-Struv-Med, and S-Struv-Hi  
 885 (planted plots fertilized with struvite).

Treatment	Variable			
	TC		TN	
	Mean	SE	Mean	SE
	-----mg/kg-----			
Control	52.55 <sup>NS</sup>	2.22	2.058 <sup>NS</sup>	0.009
Control-Osmo	57.55 <sup>NS</sup>	2.24	2.073 <sup>NS</sup>	0.015
Control-Struv	53.53 <sup>NS</sup>	6.73	2.036 <sup>NS</sup>	0.016
Seagrass	52.09 <sup>NS</sup>	4.33	2.020 <sup>NS</sup>	0.032
Seagrass-Osmo-Lo	50.56 <sup>NS</sup>	3.28	2.0259 <sup>NS</sup>	0.005
Seagrass-Osmo-Med	49.46 <sup>NS</sup>	3.56	2.053 <sup>NS</sup>	0.027
Seagrass-Struv-Lo	52.02 <sup>NS</sup>	6.17	2.057 <sup>NS</sup>	0.027
Seagrass-Struv-Med	48.70 <sup>NS</sup>	5.02	2.023 <sup>NS</sup>	0.026
Seagrass-Struv-Hi	58.16 <sup>NS</sup>	5.63	2.096 <sup>NS</sup>	0.02

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903 Table S-7. Mixed effects test table for sediment TC/TN taken at the end of the multi-  
 904 dose experiment/Experiment #2.

Source	Variable					
	TC			TN		
	---mg/kg---					
Parameter	DF	F statistic	P value	DF	F statistic	P value
Treatment	8	0.5028	0.8435	8	1.376	0.2514

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924 Table S-8. Mean porewater nutrient measurements from the second experiment. The  
 925 treatments were labelled Control (unfertilized, unplanted plots), Control-Osmo  
 926 (unplanted fertilized with Osmocote™), Control-Struv (unplanted fertilized with struvite),  
 927 Seagrass (unfertilized seagrass plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med  
 928 (planted plots fertilized with Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med,  
 929 and Seagrass-Struv-Hi (planted plots fertilized with struvite). Only TDP was analyzed  
 930 for Day 31, therefore TDN values at that date are designated “NA.” Letters within  
 931 biomass type represent a significantly different mean based on linear mixed model  
 932 analyses (NS= not significant).

Variable		TDN		TDP	
Days after Deployment	Treatment	Mean	SE	Mean	SE
		-----mg/L-----			
6	Control	1.51 <sup>NS</sup>	0.38	0.128 <sup>D</sup>	0.004
	Control-Osmo	12.03 <sup>NS</sup>	5.16	9.640 <sup>AB</sup>	3.685
	Control-Struv	4.35 <sup>NS</sup>	1.52	2.093 <sup>C</sup>	0.276
	Seagrass	1.41 <sup>NS</sup>	0.45	0.182 <sup>D</sup>	0.021
	Seagrass-Osmo-Lo	7.89 <sup>NS</sup>	4.34	4.750 <sup>BC</sup>	1.169
	Seagrass-Osmo-Med	26.8 <sup>NS</sup>	7.53	17.68 <sup>A</sup>	6.738
	Seagrass-Struv-Lo	4.33 <sup>NS</sup>	1.60	2.795 <sup>C</sup>	0.962
	Seagrass-Struv-Med	3.32 <sup>NS</sup>	0.88	1.943 <sup>C</sup>	0.211
	Seagrass-Struv-Hi	4.79 <sup>NS</sup>	0.86	2.620 <sup>C</sup>	0.351
20	Control	1.10 <sup>NS</sup>	0.16	0.133 <sup>C</sup>	0.010
	Control-Osmo	19.8 <sup>NS</sup>	2.14	0.508 <sup>A</sup>	0.102
	Control-Struv	5.59 <sup>NS</sup>	1.91	0.303 <sup>ABC</sup>	0.138
	Seagrass	0.78 <sup>NS</sup>	0.14	0.134 <sup>C</sup>	0.016
	Seagrass-Osmo-Lo	6.20 <sup>NS</sup>	2.33	0.289 <sup>ABC</sup>	0.094
	Seagrass-Osmo-Med	12.02 <sup>NS</sup>	0.82	0.472 <sup>AB</sup>	0.279
	Seagrass-Struv-Lo	2.47 <sup>NS</sup>	0.42	0.272 <sup>ABC</sup>	0.107
	Seagrass-Struv-Med	2.58 <sup>NS</sup>	0.80	0.187 <sup>BC</sup>	0.021
	Seagrass-Struv-Hi	6.81 <sup>NS</sup>	1.73	0.322 <sup>ABC</sup>	0.113

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934 Table S-8. Continued.

		Variable			
Days after Deployment	Treatment	TDN		TDP	
		Mean	SE	Mean	SE
-----mg/L-----					
31	Control	NA	NA	0.110 <sup>D</sup>	0.009
	Control-Osmo	NA	NA	2.455 <sup>BC</sup>	0.560
	Control-Struv	NA	NA	2.923 <sup>ABC</sup>	0.966
	Seagrass	NA	NA	0.114 <sup>D</sup>	0.016
	Seagrass-Osmo-Lo	NA	NA	1.47 <sup>C</sup>	0.375
	Seagrass-Osmo-Med	NA	NA	9.733 <sup>AB</sup>	6.667
	Seagrass-Struv-Lo	NA	NA	0.163 <sup>D</sup>	0.038
	Seagrass-Struv-Med	NA	NA	1.353 <sup>C</sup>	0.166
74	Control	1.97 <sup>NS</sup>	0.19	0.085 <sup>C</sup>	0.007
	Control-Osmo	12.2 <sup>NS</sup>	7.54	0.488 <sup>A</sup>	0.135
	Control-Struv	7.75 <sup>NS</sup>	2.00	0.159 <sup>BC</sup>	0.054
	Seagrass	1.68 <sup>NS</sup>	0.15	0.084 <sup>C</sup>	0.021
	Seagrass-Osmo-Lo	5.39 <sup>NS</sup>	0.54	0.261 <sup>AB</sup>	0.067
	Seagrass-Osmo-Med	17.3 <sup>NS</sup>	4.98	0.551 <sup>A</sup>	0.105
	Seagrass-Struv-Lo	4.04 <sup>NS</sup>	1.23	0.162 <sup>BC</sup>	0.064
	Seagrass-Struv-Med	4.74 <sup>NS</sup>	1.03	0.143 <sup>BC</sup>	0.023
	Seagrass-Struv-Hi	9.32 <sup>NS</sup>	1.62	0.156 <sup>BC</sup>	0.017

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955 Table S-9. Mean percent tissue nutrient content and ratio taken at the end of the  
 956 second experiment. The treatments were labelled Seagrass (unfertilized seagrass  
 957 plots), Seagrass-Osmo-Lo and Seagrass-Osmo-Med (planted plots fertilized with  
 958 Osmocote™), Seagrass-Struv-Lo, Seagrass-Struv-Med, and Seagrass-Struv-Hi  
 959 (planted plots fertilized with struvite). Combined weights present the aboveground TN  
 960 and TP weights were removed for the analysis (n= 2 for Osmocote™ treatments,  
 961 unfertilized seagrass removed). Belowground weights for both TN and TP did not have  
 962 combined samples. Letters within biomass type represent a significantly different mean  
 963 based on linear mixed model analyses (NS= not significant).

Biomass Type	Treatment	TN		TP		TN:TP	
		Mean	SE	Mean	SE	Mean	SE
		-----Percent-----				Wt/Wt Ratio	
Above-ground	Seagrass	1.90 <sup>NS</sup>	NA	NA	NA	NA	NA
	Seagrass-Osmo-Lo	2.14 <sup>NS</sup>	0.11	0.258 <sup>NS</sup>	0.016	8.33 <sup>NS</sup>	0.57
	Seagrass-Osmo-Med	2.08 <sup>NS</sup>	0.11	0.249 <sup>NS</sup>	0.017	8.47 <sup>NS</sup>	0.88
	Seagrass-Struv-Lo	2.34 <sup>NS</sup>	0.23	0.236 <sup>NS</sup>	0.007	10.01 <sup>NS</sup>	1.20
	Seagrass-Struv-Med	2.31 <sup>NS</sup>	0.14	0.246 <sup>NS</sup>	0.010	9.38 <sup>NS</sup>	0.29
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA
Below-ground	Seagrass	0.65 <sup>AB</sup>	0.06	0.166 <sup>NS</sup>	0.018	3.90 <sup>NS</sup>	0.36
	Seagrass-Osmo-Lo	0.53 <sup>B</sup>	0.07	0.154 <sup>NS</sup>	0.009	3.44 <sup>NS</sup>	0.47
	Seagrass-Osmo-Med	0.84 <sup>A</sup>	0.06	0.179 <sup>NS</sup>	0.008	4.71 <sup>NS</sup>	0.36
	Seagrass-Struv-Lo	0.66 <sup>AB</sup>	0.05	0.168 <sup>NS</sup>	0.011	3.94 <sup>NS</sup>	0.29
	Seagrass-Struv-Med	0.71 <sup>AB</sup>	0.07	0.164 <sup>NS</sup>	0.011	4.32 <sup>NS</sup>	0.45
	Seagrass-Struv-Hi	NA	NA	NA	NA	NA	NA

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972 Figure S-1. Image showing an example of the differences in above and belowground  
973 biomass in seagrasses from the first experiment. Observable stunted roots (possibly  
974 root burn) are visible in the Osmocote™ treated plots (SP) versus the control (S) and  
975 struvite treated plots (SS).

**Declaration of interests**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

## **Author Contributions**

MacDonnell, C.: Conceptualization, data curation, formal analysis, methodology, visualization, project administration, investigation, roles/writing- original draft/review and editing.

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Barbour, S.: Investigation, resources, project administration.

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Makinia, J.: Resources, funding acquisition, writing- review and editing.

Inglett, P.W.: Conceptualization, supervision, data curation, project administration, formal analysis, methodology, visualization, roles/writing- original draft/review and editing.