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EARTH AND ENVIRONMENTAL SCIENCE

SUPPLEMENTARY-RESULT

An assessment of hydrogen sulfide intrusion in the seagrass *Halodule wrightii*

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Abstract

Hydrogen sulfide (H_2S, "sulfide") is a naturally occurring component of the marine sediment. Eutrophication of coastal waters, however, can lead to an excess of sulfide production that can prove toxic to seagrasses. We used stable sulfur isotope ratio ($\delta^{34}S$) measurements to assess sulfide intrusion in the seagrass *Halodule wrightii*, a semi-tropical species found throughout the Gulf of Mexico, Caribbean Sea, and both western and eastern Atlantic coasts. We found a gradient in $\delta^{34}S$ values ($-5.58 \pm 0.54‰+ 13.58 \pm 0.30‰$) from roots to leaves, in accordance with prior observations and those from other species. The results may also represent the first values reported for *H. wrightii* rhizome tissue. The presence of sulfide-derived sulfur in varying proportions (15–55%) among leaf, rhizome, and root tissues suggests *H. wrightii* is able to assimilate sedimentary H_2S into non-toxic forms that constitute a significant portion of the plant's total sulfur content.

Key words: intrusion; seagrass; sulfide; sulfur

Introduction

Seagrasses are marine angiosperms that provide key ecological services to coastal ecosystems. Unfortunately, seagrasses are experiencing a global crisis in terms of habitat decline (Waycott et al., 2009). Excess nutrients in coastal waters can lead to an increase in sulfide levels in seagrass beds (Ruiz-Halpern et al., 2008). H_2S is a potent toxin that can easily cross cell membranes and enter the plant (intrusion), potentially inducing seagrass mortality (Koch & Erskine, 2001). Sulfide intrusion can be assessed using stable sulfur isotope (^{32}S, ^{34}S) measurements. Sulfate-reducing bacteria discriminate against the heavier ^{34}S isotope, preferring the lighter ^{32}S form. This yields sedimentary H_2S with a lower ^{34}S isotopic "signal" (Canfield, 2001). This signal can be quantified in plants, providing an estimate of the proportion of tissue sulfur derived from sedimentary sulfide (Frederiksen et al., 2006).

Thus far, the literature on sulfide intrusion in seagrasses shows relatively few measurements for species besides *Zostera marina*, *Thalassia testudinum*, and *Posidonia oceanica* (Holmer & Hasler-Sheetal, 2014). The objective of this study was to use stable isotope analysis to examine H_2S intrusion in the seagrass *Halodule wrightii*, a semi-tropical species found throughout the Gulf of Mexico, Caribbean, and parts of both the eastern and western Atlantic coasts (Green & Short, 2003). We also estimated the proportion of total sulfur derived from sedimentary sulfide in the root, rhizome, and leaf tissues. Our goal was to obtain a more complete picture of sulfide uptake and distribution in this species, including its influence on the sulfur content in major plant organs.

Figure 1. Sampling site in Oso Bay, Corpus Christi, TX.

Methods

Forty-eight *H. wrightii*, 10 sediment, and five seawater samples were collected from Oso Bay, Corpus Christi, TX, near the campus of Texas A&M University-Corpus Christi (Figure 1). Leaf, rhizome, and root tissues were separated from each plant sample, oven-dried and ground to a fine powder using a ball mill. Sediment sulfide was extracted and precipitated as Ag_2S using a modified total reduced inorganic sulfur (TRIS) distillation method based on Backlund et al. (2005) and Fossing and Jørgensen (1989). A detailed protocol is available at https://doi.org/10.17504/protocols.io.b2b8qarw. Seawater sulfate was precipitated as barium sulfate ($BaSO_4$) under acidic conditions (Grasshoff et al., 1999). Tissue, Ag_2S, and $BaSO_4$ samples were sent to the Stable Isotopes for Biosphere Science (SIBS) Laboratory at Texas A&M University (College Station) for analysis of stable sulfur isotope ratios ($\delta^{34}S$; per mil (‰) units) and total sulfur content (TS; % dw) using elemental analyzer combustion continuous flow isotope ratio mass spectroscopy. $\delta^{34}S$ represents the deviation in the ratio of $^{34}S/^{32}S$ from a particular sample relative to an international standard, and is defined as

$$\delta^{34}S_{\text{sample}} = \left(\frac{(R)_{\text{Sample}}}{(R)_{\text{VCDT}}} - 1\right) \times 1{,}000 \tag{1}$$

where R represents the $^{34}S/^{32}S$ ratio and VCDT corresponds to the Vienna-Canyon Diablo Troilite international standard. $\delta^{34}S$ values can be negative or positive depending on whether a sample is depleted or enriched, respectively, for the S^{34} isotope compared to the standard.

$\delta^{34}S$ values from seagrass tissues (root, rhizome, leaf), sediment sulfide, and seawater sulfate were used to calculate the F_{sulfide} parameter, an estimate of the percentage of the total sulfur content within a tissue that is derived from sedimentary sulfide (Frederiksen et al., 2006):

$$F_{\text{sulfide}} = \frac{\delta^{34}S_{\text{tissue}} - \delta^{34}S_{\text{sulfate}}}{\delta^{34}S_{\text{sulfide}} - \delta^{34}S_{\text{sulfate}}} \times 100 \tag{2}$$

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Table 1. $\delta^{34}S$ values of sulfur sources (sediment TRIS or seawater sulfate) from *H. wrightii* bed in Oso Bay, Corpus Christi, TX

Species	Sediment (TRIS)		Seawater sulfate	
Location	*N*	$\delta^{34}S$ (‰)	*N*	$\delta^{34}S$ (‰)
H. wrightii Corpus, Christi, TX	10	-27.38 ± 1.41	4	$+21.11 \pm 0.76$

Note. Values are given as sample mean ± SD. N = number of observations.
Abbreviation: TRIS, total reduced inorganic sulfur.

Results

The mean $\delta^{34}S$ value from 10 sediment samples was -27.38 ± 1.41‰, while that of the seawater sulfate samples was $+21.11 \pm 0.76$‰ (Table 1). Mean $\delta^{34}S$ values for seagrass tissues ranged from -5.58 ± 3.73‰ for roots to $+13.58 \pm 2.04$‰ for leaves (Table 2). $F_{sulfide}$ values ranged from $15.51 \pm 4.2\%$ of the total sulfur content in leaves to $55.02 \pm 7.68\%$ in roots (Table 2).

While the proportion of sulfur derived from sediment sulfide varied, the total sulfur content (%TS) across tissues was similar (Table 2). Mean TS values ranged from $0.49 \pm 0.18\%$ in rhizomes to $0.55 \pm 0.23\%$ in roots. Variation in mean $\delta^{34}S$, $F_{sulfide}$, and TS values among tissues was assessed with a one-way analysis of variance (ANOVA) test. Results showed significant differences for $F_{sulfide}$ and $\delta^{34}S$ across seagrass tissues, but no statistically significant difference for TS (Figure 2).

Discussion

The mean $\delta^{34}S$ values for *H. wrightii* leaf and root tissues were higher than those previously reported for this species (+9.3‰ and −7.4‰, respectively, Holmer & Hasler-Sheetal, 2014) but the high level of variation for these measurements across studies suggests the differences may not be significant. Although we could find no previous report for *H. wrightii* rhizomes, the mean $\delta^{34}S$ value for this tissue was similar to one calculated across a number of seagrass species (+5.1‰, Holmer & Hasler-Sheetal, 2014).

We found a gradient in $\delta^{34}S$ values from roots to leaves, suggesting that H_2S enters the roots and then passes up to the rhizome and leaf tissue, either as sulfide itself or in an oxidized or metabolized form. This was quantified as $F_{sulfide}$, which estimated that approximately 55, 32, and 15% of the total sulfur content in roots, rhizomes, and leaves, respectively, came from sediment-derived H_2S. The range of values suggests a mixing of the sulfur pools (seawater sulfate and sedimentary sulfide) in the various tissues, similar to other species. A comparable gradient, however, was not observed for total sulfur content, which remained similar across tissue types. This could suggest that, while H_2S can intrude and become distributed throughout the plant, the level does not exceed *H. wrightii*'s normal metabolic requirements for sulfur.

Conclusion

Our findings suggest that significant sedimentary H_2S intrusion can occur in *H. wrightii,* entering through the roots and then becoming distributed throughout the plant. The results verified a trend previously observed for *H. wrightii,* and seagrasses in general. They also represent the first report we are aware of for rhizome tissue from this species. The relatively high proportions of total sulfur content derived from sedimentary sulfide in root, rhizome, and leaf tissue suggest *H. wrightii* is able to convert H_2S into non-toxic forms that can accumulate and mix with other sulfur-containing compounds derived from seawater sulfate, as demonstrated in *Z. marina* (Hasler-Sheetal & Holmer, 2015). Diverse levels of intrusion, however, did not translate into differences in total sulfur content among tissues, suggesting that H_2S-derived products may constitute a normal part of their sulfur budget.

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Table 2. Total sulfur (TS) and δ^{34}S values for *H. wrightii* leaf, rhizome, and root samples from Oso Bay, Corpus Christi, TX

Species	Leaf			Rhizome			Root		
Location	TS (% dw)	δ^{34}S (‰)	$F_{sulfide}$ (%)	TS (% dw)	δ^{34}S (‰)	$F_{sulfide}$ (%)	TS (% dw)	δ^{34}S (‰)	$F_{sulfide}$ (%)
H. wrightii Corpus Christi, TX	0.54 ± 0.12	+13.58 ± 2.04	15.51 ± 4.2	0.49 ± 0.18	+5.72 ± 3.37	31.72 ± 2.04	0.55 ± 0.23	−5.58 ± 3.73	55.02 ± 7.68

Note. Values are given as mean ± SD. Sample sizes for each tissue were $N_{leaf} = 47$, $N_{rhizome} = 48$, and $N_{root} = 48$.

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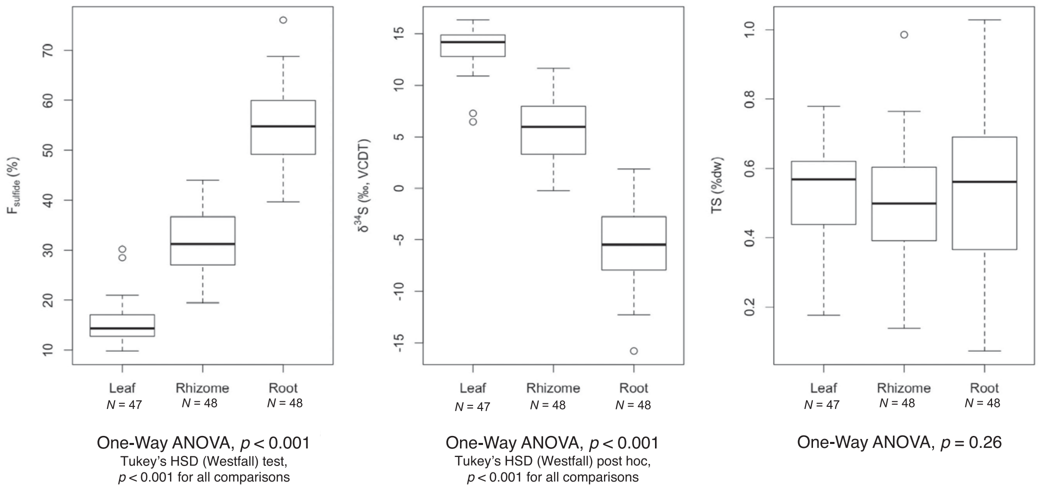

Figure 2. Boxplots representing variation among $F_{sulfide}$ (a), δ^{34}S (b), and total sulfur (c) values for *H. wrightii* tissues. *p*-values from one-way ANOVAs are included below each graph. Individual dots represent outliers, as defined by any number larger than 3rd Quantile (Q_3) + 1.5 interquartile range (IQR) or smaller than 1st Quantile (Q_1) – 1.5 IQR. Lines outside the box (whiskers) extend to the smallest and largest non-outliers.

Acknowledgments. We thank Richard Coffin, Paula Rose, and Hao Yu for assistance with preparation of samples for isotopic analysis.

Supplementary Materials. To view supplementary material for this article, please visit http://doi.org/10.1017/exp.2022.15.

Data availability statement. The isotope results used in this study are available in the Supplementary Materials (Supplementary Table S1).

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Conflict of interest. The authors have no conflicts of interest to declare.

Authorship contributions. S.R.-R. and P.D.L. designed the study and collected the samples for analysis. S.R.-R. conducted the data collection and statistical analyses. S.R.-R. and P.D.L. wrote the article.

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Peer Reviews

Reviewing editor: Dr. Il-Nam Kim
Department of Physical and Environmental Sciences, Texas A&M University-Corpus Christi, Corpus Christi, Texas 78412, USA

Minor revisions requested.

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Review 1: An assessment of sulfide (H2S) intrusion in the seagrass Halodule wrightii

Reviewer: Diana Chin
University of Florida, Engineering School of Sustainable

Date of review: 13 December 2021

Conflict of interest statement. Reviewer declares none.

Comment

Comments to the Author: Nice short paper. Minor comments. L.9-10,L.22: These are natural processes that can be intensified by human influence. Sulfide intrusion is not anthropogenic per se and not unusual in (most) seagrass species. Coastal sediments are generally anoxic below a couple cm regardless of eutrophication status. L.21-22: Citation(s). L. 31-32: Perhaps more accurate to say that relatively few measurements are available except in those species. Holmer&Hasler-Sheetal2014 (HHS) compiles TS/d34S data from many species, including 4 pubs with d34S data in Halodule wrightii (Hw). True, there are no d34S data in Hw rhizomes or TS/Fsulfide for any tissue for Hw. L.31-37, discussion/ conclusion: Consider elaborating slightly on motivation and contributions of study. L.13-14,L.82-87: Could use context – as implied in L.94 but not cited, this pattern/process is expected in many seagrass species from data in HHS and elsewhere. L.80-81: Why not compare to the Hw-specific data in HHS? Also not sure I agree with "most". Per HHS and Holmer&Kendrick2013, smaller species often exhibit greater belowground sulfide intrusion. Discussion: Any ideas why d34S but not TS can proxy for Fsulfide here, in contrast to HHS, Holmeretal.2009 (Thalassia, but see Syringodium), etc.? L.15-17,L.92: Perhaps "suggests" rather than "indicates". Form and complete conversion cannot be determined from TS/d34S/ Fsulfide. Figure2,text: Consider post-hoc tests; consider giving p-value as e.g.p<0.001 (2.2E-16 is the low default in base R); consider giving sample sizes on figure in addition to tables. General: Very minor proofs, e.g. BaSO4, Fsulfide formatting; define all scientific names and acronyms e.g. VCDT, IQR, Thalassia, Zostera.

Score Card

Presentation

4.3 /5

Is the article written in clear and proper English? (30%)	5/5
Is the data presented in the most useful manner? (40%)	4/5
Does the paper cite relevant and related articles appropriately? (30%)	4/5

Context

4.2 /5

Does the title suitably represent the article? (25%)	5/5
Does the abstract correctly embody the content of the article? (25%)	4/5
Does the introduction give appropriate context? (25%)	4/5
Is the objective of the experiment clearly defined? (25%)	4/5

Analysis

3.6 /5

Does the discussion adequately interpret the results presented? (40%)	3/5
Is the conclusion consistent with the results and discussion? (40%)	4/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	4/5

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Review 2: An assessment of sulfide (H2S) intrusion in the seagrass Halodule wrightii

Reviewer: Dr. Mariane Holmer

Date of review: 06 August 2022

Conflict of interest statement. Reviewer declares none

Comment

Comments to the Author: This is a short paper providing the results of a field sampling of Halodule wrightii documenting intrusion of sedimentary sulfides into this seagrass. The results follow observations in other seagrasses and confirms intrusion of sulfides and to a large extent given the low 34S values in roots and rhizomes. The study provides the foundation for further studies of sulfur dynamics in H. wrightii and is despite the relative small dataset important for the field.

There are few minor comments:

L. 70 - change rhizomes to roots

L. 73 - add Fsulfide in the table (N can be moved to the table text)

L. 94 - suggest to add reference Sulfide Intrusion and Detoxification in the Seagrass Zostera marina Hasler-Sheetal, H. & Holmer, M., 1. Jun 2015, In: PLoS ONE. 10, 6, 19 p. This paper shows possible detoxification mechanisms in seagrasses supporting the conclusion of this paper.

Score Card

Presentation

3.6 /5

Is the article written in clear and proper English? (30%)	5/5
Is the data presented in the most useful manner? (40%)	3/5
Does the paper cite relevant and related articles appropriately? (30%)	3/5

Context

5.0 /5

Does the title suitably represent the article? (25%)	5/5
Does the abstract correctly embody the content of the article? (25%)	5/5
Does the introduction give appropriate context? (25%)	5/5
Is the objective of the experiment clearly defined? (25%)	5/5

Analysis

4.6 /5

Does the discussion adequately interpret the results presented? (40%)	4/5
Is the conclusion consistent with the results and discussion? (40%)	5/5
Are the limitations of the experiment as well as the contributions of the experiment clearly outlined? (20%)	5/5