The following supplement accompanies the article

Sources of sediment carbon sequestered in restored seagrass meadows

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Introduction

The dominant grass, *Spartina alterniflora*, in marshes surrounding the restored seagrass beds is another potential source of organic matter accumulating in restored seagrass meadows. In order to include an additional carbon source in the Bayesian mixing model, we had to use an additional tracer, the molar C:N ratio. The C:N ratio can be used as a tracer as the ratio value can be organic matter source specific (Middelburg and Nieuwenhuize 1998; Gonneea et al. 2004), although it can be a less precise tracer than $\delta^{13}C$ and $\delta^{15}N$. In this case, the C:N of *Spartina* is much higher than the aquatic carbon sources considered in the main text and can therefore be used to determine if *Spartina* contributes substantially (>10%) to the sediment carbon pool in the seagrass beds.

Methods

The C:N ratio for sediment samples was measured as described in the main text along with the $\delta^{13}C$ and $^{\delta 5}N$ isotope measurement. Molar C:N values for benthic diatoms and *Spartina alterniflora* (SP) were not available for the bays used in this study at the Virginia Coast Reserve Long Term Ecological Research Site (VCR-LTER). Therefore, we used values from the literature, some of which include measurements from the VCR-LTER system but not the specific study bays, to define all of the end member values (Table S1).

A 4-source, 3-tracer Bayesian mixing model was developed to determine the potential contribution of SP as an additional source of carbon to the sediments (sed; Table S2). The other sources were seagrass, *Zostera marina* (Z), benthic microalgae/diatoms and seston (BD/S), and various samples of macroaglae (MA), as outlined in the main text. The three tracers for this model run were $\delta^{13}C$, $\delta^{15}N$, and C:N ratio which were used in the following system of equations to define the carbon source contributions to the sediment.

$$\delta^{13}C_{sed} = \left((\delta^{13}C_Z \times \phi_Z) + (\delta^{13}C_{MA} \times \phi_{MA}) + (\delta^{13}C_{BD/S} \times \phi_{BD/S}) + (\delta^{13}C_{SP} \times \phi_{SP})\right) \times (1-\phi_{IC}) + (\delta^{13}C_{IC} \times \phi_{IC})$$

$$\delta^{15}N_{sed} = (\delta^{15}N_Z \times \phi_Z) + (\delta^{15}N_{MA} \times \phi_{MA}) + (\delta^{15}N_{BD/S} \times \phi_{BD/S}) + (\delta^{15}N_{SP} \times \phi_{SP})$$

$$C:N_{sed} = (C:N_Z \times \phi_Z) + (C:N_{MA} \times \phi_{MA}) + (C:N_{BD/S} \times \phi_{BD/S}) + (C:N_{SP} \times \phi_{SP})$$

$$1 = \phi_Z + \phi_{MA} + \phi_{BD/S} + \phi_{SP}$$

Results were summarized using the same methods as the previous 3-source, 2-tracer model presented in the main text. The prior distributions and initialization of the Bayesian mixing model were the same as the model presented in the main text.

Results

The $\delta^{13}C$ and $\delta^{15}N$ values of $C:N_{SP}$ overlap with many of the other end member values. The molar C:N value of SP is much higher than the other end members, separating SP in $\delta^{13}C$, $\delta^{15}N$, C:N mixing space (Figure S1). The $C:N_{sed}$ is much lower than $C:N_{SP}$, but still within range of the other C:N values of the end members.

The median of the posterior distribution for the contribution of SP to the whole core carbon sediment pool was less than 2% and the mean was less than 3% (Figure S2).Similar to the 3-source, 2-tracer model presented in the main text, Z and BD/S were the two main sources of carbon to the sediment with the patterns among sites remaining the same. The contribution of MA remained low. Splitting the cores at each site into sections, the greatest mean contribution of SP (3.8%) was in the HI unveg site from 6-10 cm (Figure S3). All other mean SP contributions at all depths and sites were less than 3.7%.

Discussion

Based on this analysis, the contribution of SP to the sediment carbon pool in the restored seagrass beds was minimal (less than 4%). Without the addition of molar C:N as a tracer, it would have been difficult to tease apart the contributions of SP to the sediment carbon pool given that it overlapped with other end member isotope values. The high and clearly separated $C:N_{SP}$ values provided the necessary information for the model to discern that SP was not a large contributor to the sediment organic carbon pool. Similarly low marsh grass contributions have been observed in other systems due to sampling locations being far away from marshes, or hydrology of the lagoon (Gonneea et al. 2004; Hemminga et al. 1994).

We were unable to restrict the parameterization of the end members for this analysis to VCR-LTER data collected in or near the same restored seagrass meadows studied here as there was minimal $C:N_{SP}$ and no C:N values for benthic diatoms in this system. As such, the results should be more cautiously interpreted as some of the end member values are more generic and not place specific. However, it should be noted that all of the $C:N_{SP}$ values, though not specific to Hog Island or South Bay (the systems in this study) did come from measurements of *S. alterniflora* within the VCR system. The necessity of non-lagoon specific literature values for end member parameterization combined with the minimal contribution of *Spartina alterniflora* in this analysis was the basis for excluding marsh grass from the analysis presented in the main text. However, using molar C:N as a tracer of source, *S. alterniflora* is clearly a minor constituent of the OM pool in the restored seagrass sediments.

References:

Cloern JE, Canuel EA, Harris D (2002) Stable carbon and nitrogen isotope composition of aquatic and terrestrial plants of the San Francisco Bay estuarine system. Limnol Oceanogr 47: 713 – 729.

Gonneea ME, Paytan A, Herrera-Silveira JA (2004) Tracing organic matter sources and carbon burial in mangrove sediments over the past 160 years. Estuar Coast Shelf Sci 61: 211 – 227.

Hemming MA, Slim FJ, Kazungu J, Ganssen GM, Nieuwenhuize J, Kruyt NM (1994) Carbon outwelling from a mangrove forest with adjacent seagrass beds and coral reefs (Gazi Bay, Kenya). Mar Ecol Prog Ser 106: 291 – 301.

Hondula KL, Pace ML (2012) Using multiple stable isotopes including deuterium (δ2H) to trace organic matter in complex near-shore lagoon. M.S. thesis. University of Virginia.

Middelburg JJ, Nieuwenhuize J (1998) Carbon and nitrogen stable isotopes in suspended matter and sediments from the Schelde Estuary. Marine Chemistry 60: 217 – 225.

Mozdzer TJ (2009) Variation in the availability and utilization of dissolved organic nitrogen by the smooth cordgrass, *Spartina alterniflora*. Ph.D. thesis, University of Virginia.

Olcott CA (2011) Impacts of nitrogen addition on the monthly above- and belowground production of *Spartina alterniflora* in a Virginia marsh. Undergraduate thesis, University of Virginia.
Tyler C (1997) Geomorphological and hydrological controls on pattern and process in a developing barrier island salt marsh. M.S. thesis. University of Virginia.

Table S1: End member tracer values for the 4-source, 3-tracer mixing model. All values are from Hondula (2012) unless otherwise noted.

End Member	$\delta^{13}C$ (±s.d.)	$\delta^{15}N$ (±s.d.)	Molar C:N (±s.d.)
Z. marina (Z)	-10.1 (±0.4)	6.6 (±0.68)	23.0 (±4.2)
S. alterniflora (SP)	-13.7 (±0.6)	6.8 (±3.2)	47.1 (±9.9)[a,b,c]
Benthic diatoms/seston (BD/S)	-23.3 (±2.2)	5.6 (±1.2)	9.0 (±2.4)
Macroalgae (MA)	-17.4 (±3.3)	9.5 (±1.3)	19.3 (±7.3)[d]

[a]Tyler (1997)

[b]Mozdzer (2009)

[c]Olcott (2011)

[d]Cloern et al. (2002)

Table S2: Means, standard deviations (±SD), and sample size (*n*) for $\delta^{13}C$, $\delta^{15}N$, and C:N of the sediment

	$\delta^{13}C$ (‰)			$\delta^{15}N$ (‰)			Molar C:N		
0 – 10 cm Sediment	Mean	±SD	*n*	Mean	±SD	*n*	Mean	±SD	*n*
HI Unveg	-17.7	0.8	10	6.0	0.3	10	12.1	0.4	10
4-year	-15.4	0.6	10	5.9	0.2	10	13.2	0.4	10
SB Unveg	-14.4	1.7	10	5.5	0.2	10	12.4	0.5	10
10-year	-16.5	0.6	10	5.6	0.1	10	12.2	0.4	10
0 – 3 cm Sediment									
HI Unveg	-16.8	0.6	3	5.8	0.1	3	12.3	0.2	3
4-year	-15.1	0.2	3	6.1	0.3	3	13.1	0.3	3
SB Unveg	-12.2	0.8	3	5.5	0.2	3	11.8	0.4	3
10-year	-16.0	0.9	3	5.6	0.2	3	12.3	0.4	3
3 – 6 cm Sediment									
HI Unveg	-17.8	0.2	3	5.8	0.1	3	12.2	0.0	3
4-year	-15.1	0.4	3	5.7	0.0	3	13.4	0.4	3
SB Unveg	-14.8	0.5	3	5.5	0.2	3	12.6	0.4	3
10-year	-17.0	0.4	3	5.6	0.1	3	12.0	0.4	3
6 – 10 cm Sediment									
HI Unveg	-18.4	0.2	4	6.3	0.3	4	11.8	0.4	4
4-year	-16.0	0.6	4	5.9	0.2	4	13.1	0.3	4
SB Unveg	-15.8	0.5	4	5.4	0.2	4	12.6	0.3	4
10-year	-16.5	0.3	4	5.5	0.2	4	12.2	0.5	4

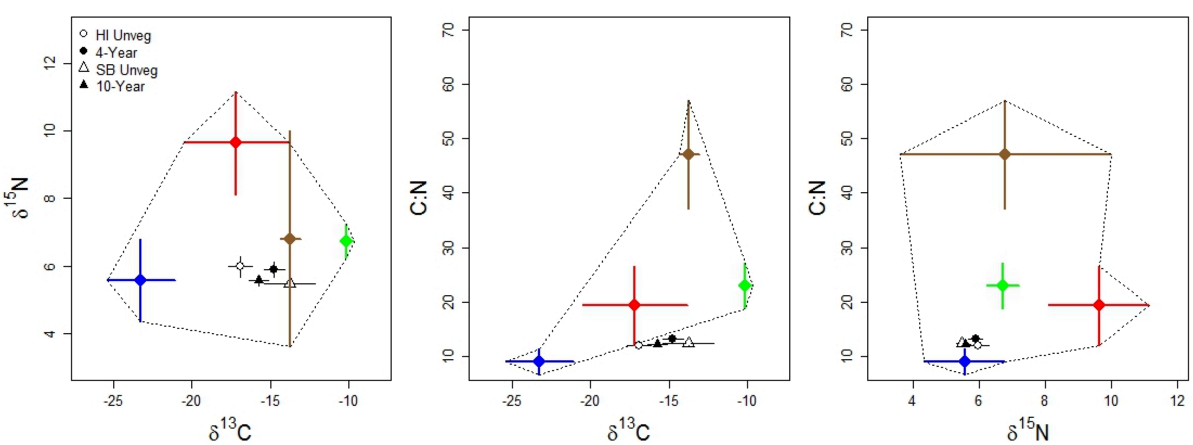

Figure S1: Isotope and C:N biplots of the four end members (Z=green, BD/S=blue, MA=red, SP=brown) and sediment values (error bars are standard deviation). The sediment values are the average of the whole core. The dashed gray lines bound the average source value ± standard deviation with the expectation that sediment values will lie within the grey lines.

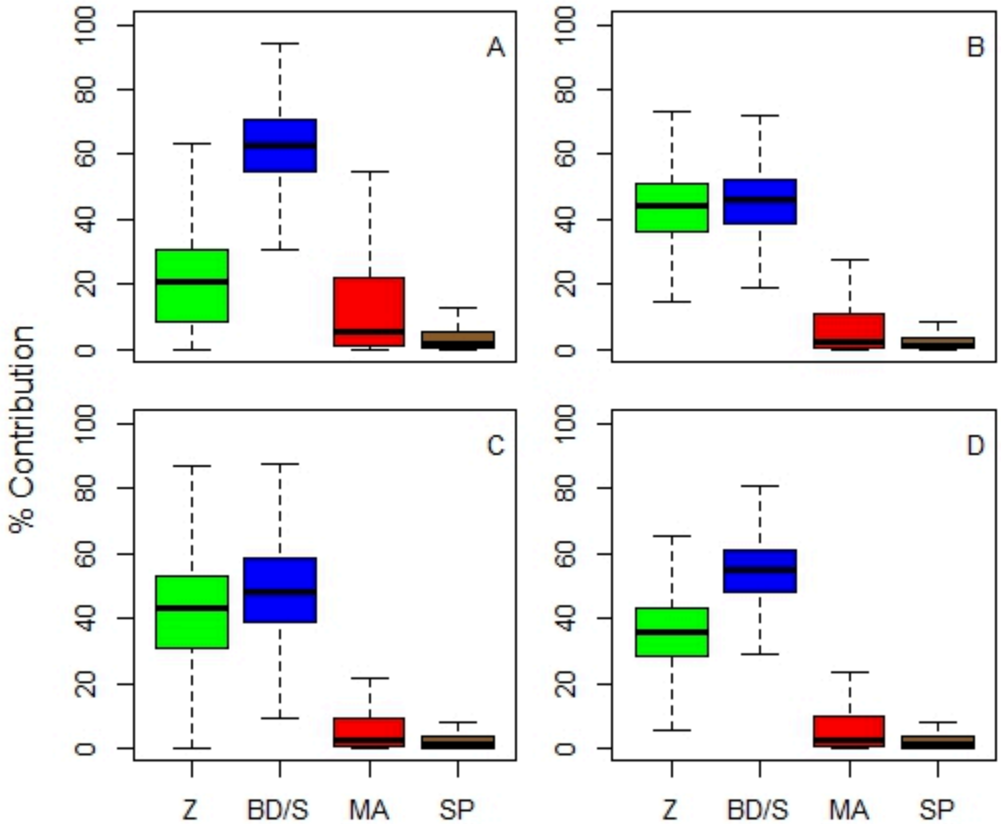

Figure S2: Boxplots of the posterior distributions of the source contribution to the top 10 cm of the sediment carbon pool. **A)** unvegetated sediment in Hog Island Bay (HI Unveg), **B)** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C)** unvegetated sediment in South Bay (SB Unveg), **D)** 10-year restored seagrass treatment in South Bay (10 year).

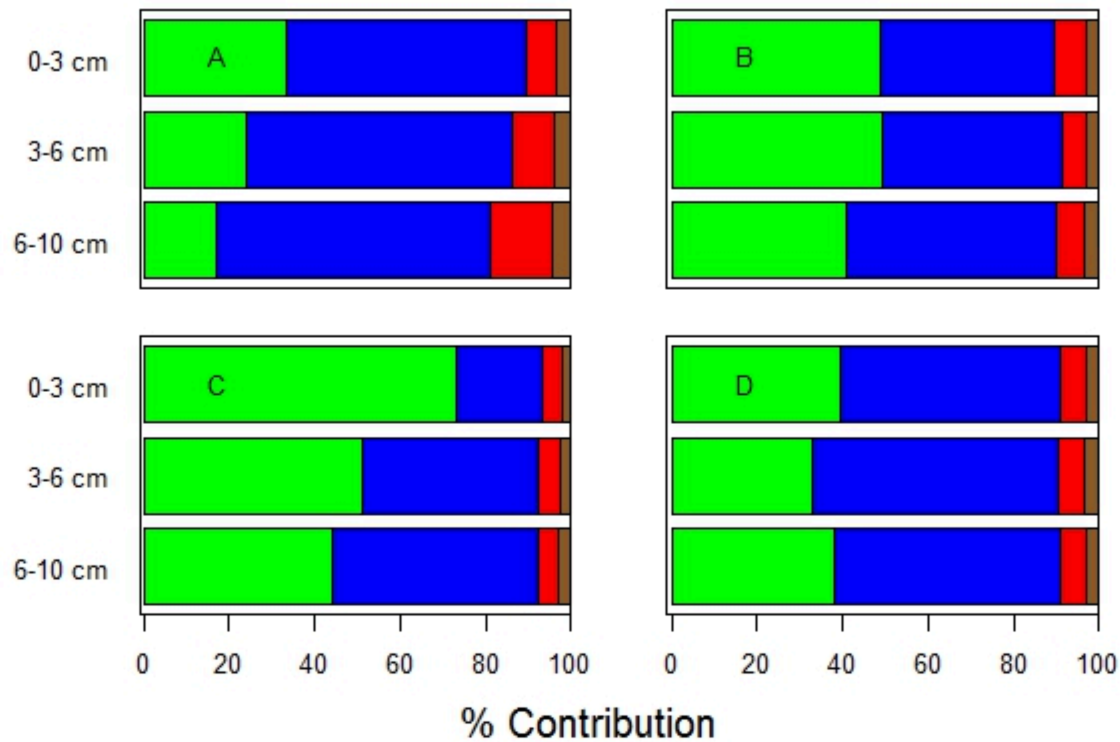

Figure S3: Mean source contribution (Z=green, BD/S=blue, MA=red, SP=brown) in intervals down the sediment profile. **A)** unvegetated sediment in Hog Island Bay (HI Unveg), **B)** 4-year restored seagrass treatment in Hog Island Bay (4 year), **C)** unvegetated sediment in South Bay (SB Unveg), **D)** 10-year restored seagrass treatment in South Bay (10 year).