

Supplementary material

Blue carbon sequestration dynamics within a tropical seagrass sediment: long term incubations for changes over climatic scales

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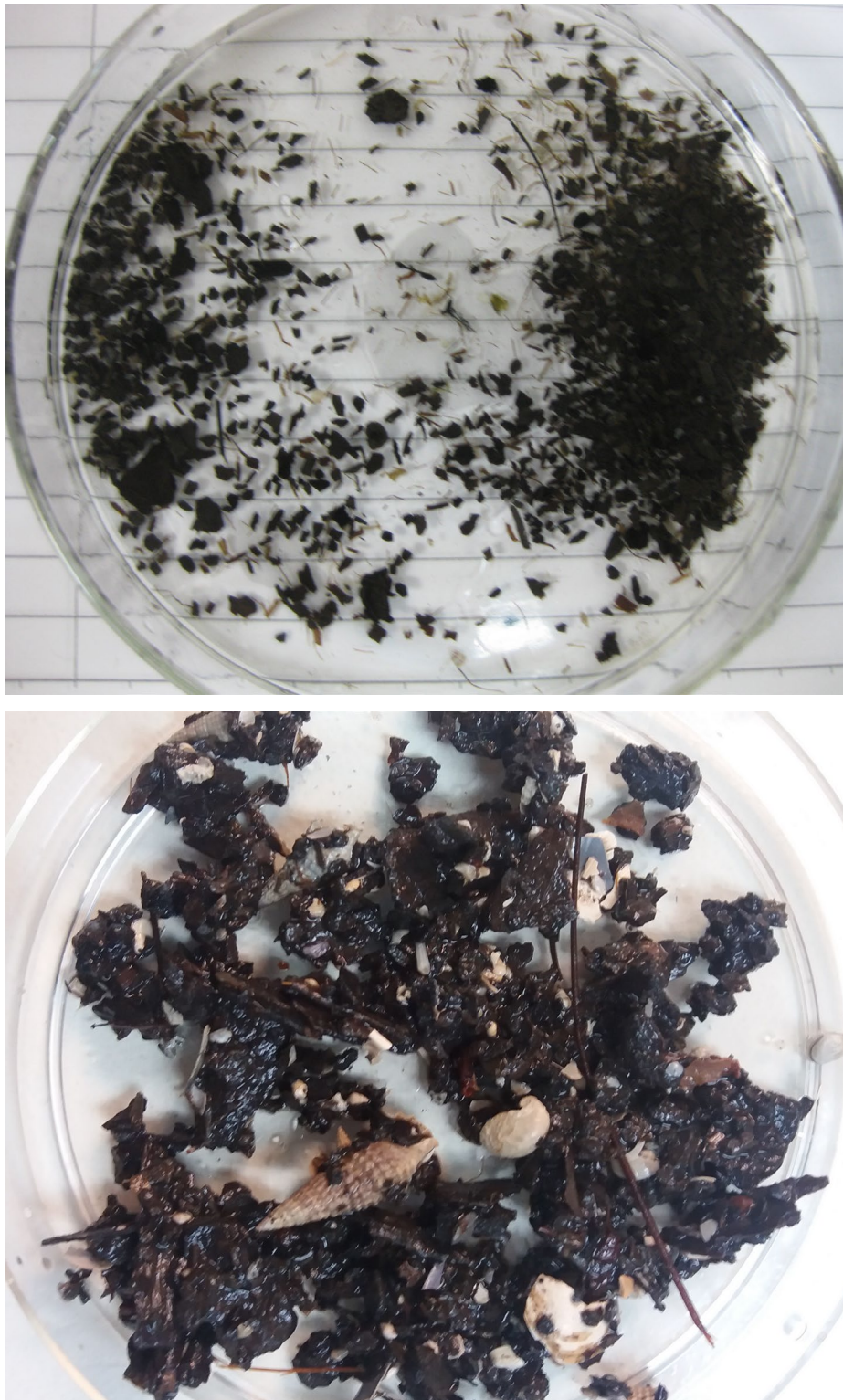
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Collection and processing of sediments used in incubation experiment

The sediments were taken from 22 cores from 5 different sites within the northern part of the Salut estuarine lagoon, spaced around 30-150 metres apart. Samples were collected with an acrylic corer with core lengths from 37 cm to 47 cm cores. The sediment cores were taken by the boatman, Mr. Awang Azmee, by hand, and capped with rubber bungs. The core was placed in a bucket vertically and the sediment that was resuspended by the coring action in the water column of the corer was allowed to be deposited back onto the surface of the sediments before extrusion. An extruder bar with 0.5 cm graduations was used to push in one of the smaller bungs from the bottom into the tube, thus pushing out the sediment column from the corer tube. The top 2 cm of the sediment column was first cut off from the sediment column with a plastic cake knife then immediately bagged in sealable Ziploc bags and placed into an icebox (ambient temperature in icebox = 10.2°C). This process was also repeated for the 20-22 cm section of the cores, being stored in a separate Ziploc bag. The rest of the sediment column was discarded, with the exception of the 2-20 cm section of 5 cores, which were also kept and taken as an in-house standard to ensure accuracy during the loss on ignition phase of the experiment between different batches. Sediments from the same sections of different cores were placed into the same Ziploc bags until they were half full, after which the bags were replaced. The Ziploc bags were squeezed around and mixed in order to homogenize the sediments from the different cores. Once the sediments arrived back at the laboratory, the bags were placed into a fridge at 7°C and stored overnight. The sediments were then sieved while wet using a sediment shaker with a mesh size of 1mm, in order to remove any large particles and organisms which may cause biases due to more organisms living at certain depths. Any benthic organisms growing in situ would also not constitute the total organic matter protected from remineralisation as well, and thus their removal is justified. The sediments were mixed into a slurry and placed into the glass mason jars, ensuring that the same amount of water content was present in each of the jars. The total volume of the slurry in each jar was 400 ml, with the sediment from 20-22cm having a 1:1 ratio of sediment to seawater, and the sediment from 0-2 cm having its sediment:seawater ratio adjusted accordingly to match, giving both slurries a water content of 81.9%. The seawater used was obtained during high tide at the UMS Jetty on the 19th of October 2016 at 15.37, filtered through Whatman GF/B filter paper and boiled to remove any microorganisms and dissolved gases.



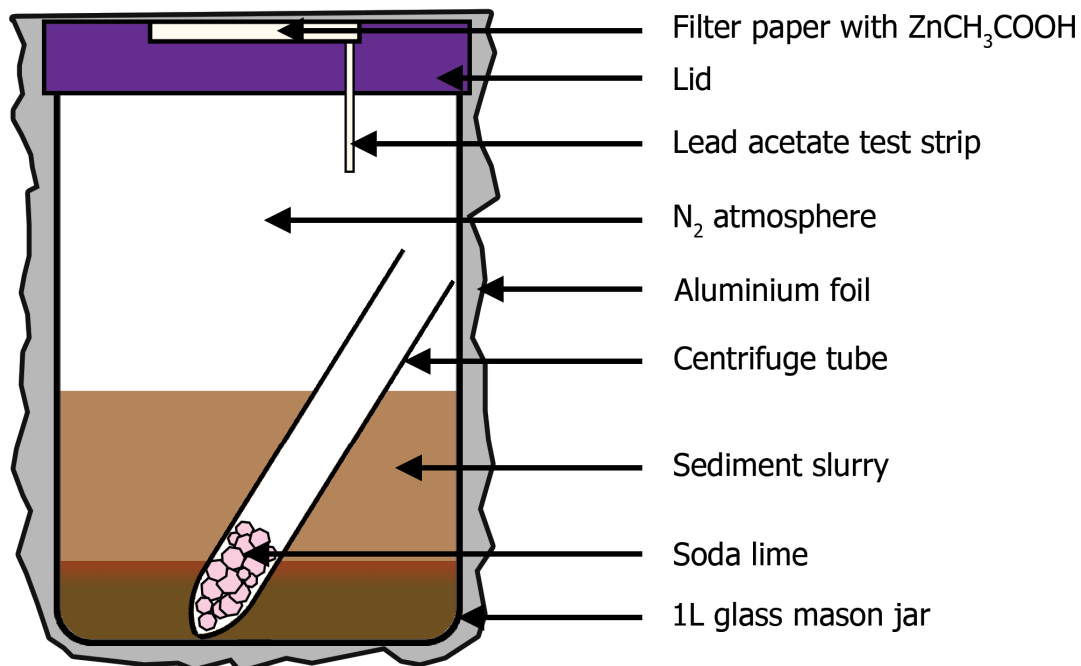
Supplemental Figure S1. Sample of one of the sediment cores taken for the incubation study at Salut Lagoon. Note the transition in the colour of the sediment at ~24 cm, indicated by the arrow.



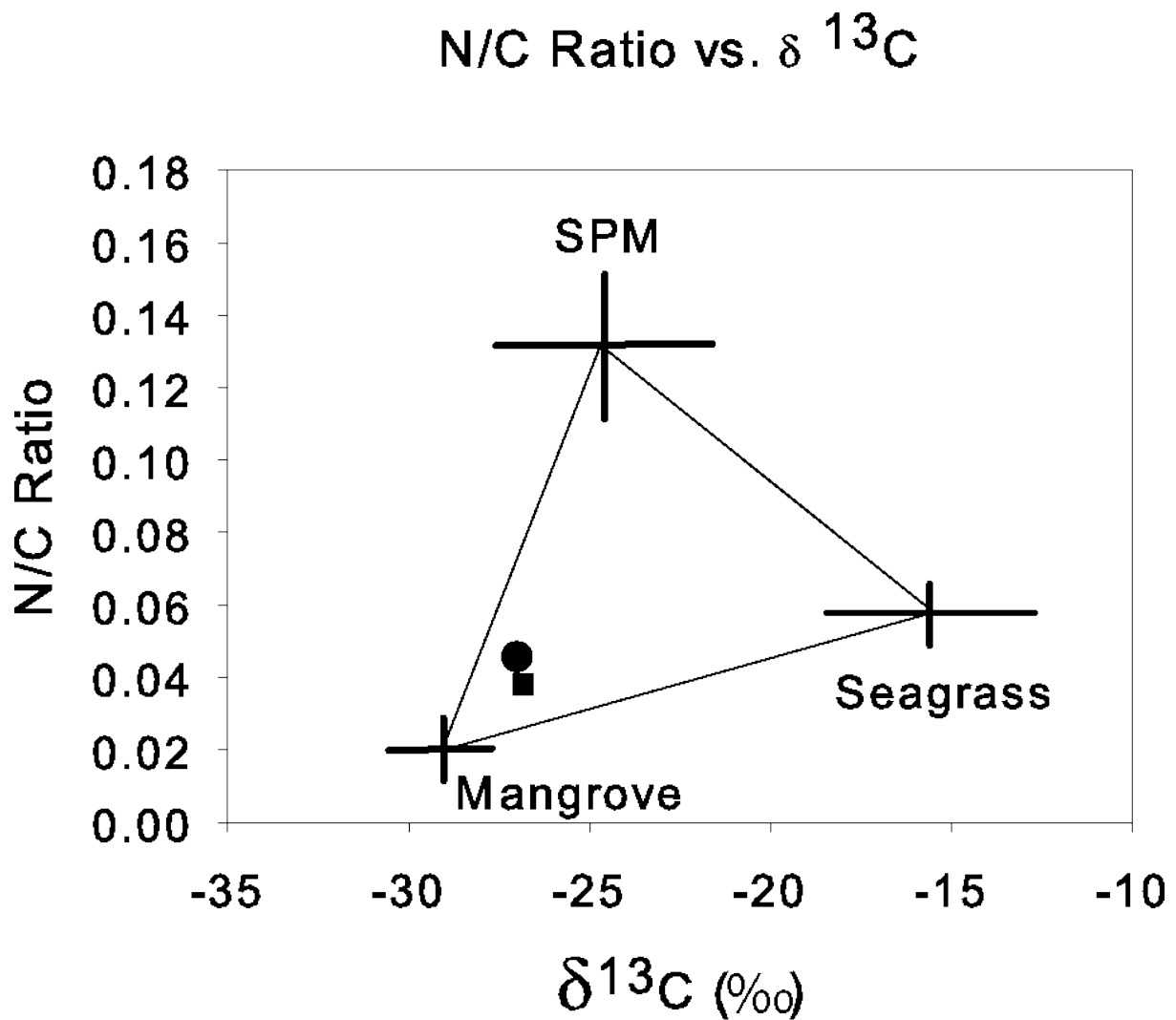
Supplemental Figure S2. The remnants of detritus larger than 1mm in size retained on the sieve after wet sieving. Top: Surface 2 cm sediment detritus, Bottom: Detritus taken from below 26 cm depth, within the lower, more fibrous brown facies. Both sediments were taken from the seagrass beds within the northern Salut estuary.

Incubation experiment design and methods

The experiment makes use of 1 litre glass mason jars, in which the sediment slurries were housed and incubated in. These jars have a piece of cellulose filter paper affixed to the lid with epoxy, which was saturated with 1 ppt of zinc acetate solution in order to absorb any toxic hydrogen sulphide metabolite which was released during the incubation which may affect the results of the experiment. A strip of lead acetate paper was also affixed to the lid in order to visualise any H_2S production. The structure of the jars used is shown in the Supplementary Figure S3.



Supplementary Figure S3. The jars used in the incubation. The soda lime and zinc acetate impregnated filter paper serve to absorb toxic metabolites, while the N_2 atmosphere within the jars ensures anoxic conditions are maintained.



Supplementary Figure S4. N/C ratios of surface 2 cm sediments, denoted by a circle, and sediments from the 20-22 cm horizon, denoted by a square, against their ^{13}C values. The crosses show the values of similar sources in a tropical Mexican mangrove (Eagle *et al.* 2004).

Table S1. Coordinates of the sites where the cores used in the study were taken.

Sites starting with ICB denote the coordinates of the 5 sites where the cores for the incubation experiment were taken from.

Site	Longitude (° N)	Latitude (° E)
Salut SIT	6.10728	116.15278
Mengkabong SIT	6.13083	116.16228
ICB 1	6.10745	116.15231
ICB 2	6.10727	116.15382
ICB 3	6.10670	116.15473
ICB 4	6.10667	116.15525
ICB 5	6.10688	116.15517

Table S2. POC content of the sediments throughout the anoxic incubation period and subsequent aeration incubation.

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. All units are in mol 100 g⁻¹.

Time (Days)	S1	S2	S3	S4	B1	B2	B3	B4
0	0.6124	0.6124	0.5612	0.5684	0.5321	0.5321	0.5532	0.5532
7	0.5869	0.5614	0.5535	0.5691	0.5375	0.5391	0.5169	0.5182
21	0.5613	0.5646	0.5726	0.5626	0.5275	0.5433	0.5158	0.5389
42	0.5701	0.5756	0.5524	0.5683	0.5283	0.5270	0.5203	0.5465
63	0.5233	0.5246	0.5300	0.5456	0.4684	0.4562	0.4857	0.4854
105	0.5292	0.5246	0.5287	0.5230	0.5055	0.5003	0.4870	0.5228
140	0.5543	0.5569	0.5396	0.5204	0.4975	0.5070	0.4338	0.4698
175	0.4857	0.4878	0.4859	0.4946	0.4638	0.4727	0.4639	0.4666
210	0.4668	0.4640	0.4680	0.4745	0.4269	0.4220	0.4220	0.4364
308	0.4738	--	0.4860	0.5025	0.4603	0.4629	0.4433	0.4619
365	0.4737	--	0.5017	0.4653	0.4433	0.4513	0.4421	0.4467
400	0.4725	--	0.4679	0.4655	0.4279	0.4381	0.4274	0.4185
470	0.4700	--	0.4655	0.4592	0.4251	0.4587	0.4450	0.4509
500	0.4651	--	0.4493	0.4688	0.4117	0.4021	0.4254	0.4152
531	0.4135	--	0.3820	0.3706	0.3692	0.3346	0.3283	0.3892

Table S3. BOC content of the sediments throughout the anoxic incubation period and subsequent aeration incubation.

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. All units are in mol 100 g⁻¹.

Time (Days)	S1	S2	S3	S4	B1	B2	B3	B4
0	0.0692	0.0692	0.0692	0.0692	0.0589	0.0589	0.0589	0.0589
7	0.0748	0.0750	0.0777	0.0848	0.0771	0.0770	0.0644	0.0729
21	0.0883	0.0849	0.0849	0.0939	0.0891	0.1052	0.0721	0.0882
42	0.1018	0.1126	0.1221	0.0986	0.0905	0.1122	0.0982	0.1010
63	0.0825	0.0909	0.0972	0.1084	0.0761	0.0762	0.0830	0.0931
105	0.0993	0.1118	0.1029	0.0982	0.1191	0.0909	0.1014	0.1042
140	0.1254	0.1302	0.1103	0.1055	0.0895	0.0910	0.0686	0.0523
175	0.0682	0.0637	0.0674	0.0693	0.0491	0.0701	0.0788	0.0529
210	0.0503	0.0485	0.0599	0.0467	0.0446	0.0428	0.0385	0.0358
308	0.0464	--	0.0453	0.0496	0.0400	0.0393	0.0292	0.0407
365	0.0619	--	0.0559	0.0578	0.0585	0.0551	0.0538	0.0613
400	0.0783	--	0.0845	0.0898	0.0769	0.0839	0.0817	0.0899
470	0.0541	--	0.0632	0.0578	0.0297	0.0334	0.0422	0.0415
500	0.0793	--	0.0748	0.0719	0.0555	0.0048	0.0474	0.0465
531	0.1015	--	0.0281	0.1049	0.0074	0.0260	0.0150	0.0322

Table S4. PIC content of the sediments throughout the anoxic incubation period and subsequent aeration incubation

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. All units are in mol 100 g⁻¹.

Time (Days)	S1	S2	S3	S4	B1	B2	B3	B4
0	0.1117	0.1117	0.1117	0.1117	0.1121	0.1121	0.1121	0.1121
7	0.1389	0.1460	0.1438	0.1404	0.1371	0.1349	0.1364	0.146
21	0.1425	0.1426	0.1412	0.1397	0.1391	0.1335	0.1459	0.1376
42	0.1419	0.1373	0.1425	0.1608	0.1474	0.1417	0.1611	0.1377
63	0.1540	0.1533	0.1505	0.1436	0.1511	0.1444	0.1559	0.1507
105	0.1532	0.1483	0.1416	0.1501	0.1403	0.1395	0.1512	0.143
140	0.1437	0.1430	0.1730	0.1662	0.1230	0.1217	0.1616	0.1713
175	0.1272	0.1259	0.1302	0.1227	0.1291	0.1030	0.1252	0.1178
210	0.1307	0.1384	0.1303	0.1446	0.1302	0.1309	0.1292	0.1390
308	0.1376	--	0.1334	0.1269	0.1466	0.1445	0.1425	0.1309
365	0.1292	--	0.1820	0.2043	0.2063	0.2149	0.2104	0.2085
400	0.2164	--	0.2152	0.2130	0.2310	0.228	0.2238	0.2171
470	0.2422	--	0.2330	0.2362	0.2787	0.2392	0.2347	0.2416
500	0.2265	--	0.2818	0.2418	0.2908	0.2841	0.2778	0.2899
531	0.1727	--	0.1951	0.2507	0.1935	0.1669	0.1674	0.1520

Table S5. CO₂ absorbed by the soda lime placed within the headspace of the sediment slurry incubation bottles throughout the anoxic incubation period.

S1, S3 and S4 are the identifiers of the 3 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. All units are in mol 100 g dry weight of sediment⁻¹.

Time (Days)	S1	S3	S4	B1	B2	B3	B4
0	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
7	0.0170	0.0115	0.0104	0.0074	0.0071	0.0067	0.0068
21	0.0345	0.0277	0.0278	0.0173	0.0170	0.0165	0.0177
42	0.0544	0.0471	0.0498	0.0310	0.0307	0.0307	0.0330
63	0.0753	0.0667	0.0658	0.0436	0.0421	0.0427	0.0444
105	0.1032	0.1004	0.0964	0.0682	0.0654	0.0674	0.0689
140	0.1343	0.1349	0.1252	0.0887	0.0874	0.0901	0.1704
175	0.1599	0.1658	0.1531	0.1115	0.3222	0.2182	0.1951
210	0.1836	0.1903	0.1551	0.1303	0.3141	0.2359	0.1944
308	0.2347	0.2509	0.2569	0.1677	0.3819	0.2805	0.2707
365	0.2804	0.2464	0.2170	0.1427	0.3414	0.5471	0.2308
400	0.2575	0.2971	0.2645	0.1794	0.3541	0.5869	0.3038
470	0.2817	0.3271	0.2924	0.1783	0.3906	0.6026	0.3266
500	0.3246	0.3029	0.3133	0.1907	0.3704	0.5842	0.3026

Table S6. Ammonia content of the porewater of the sediment slurry throughout the anoxic incubation period and subsequent aeration incubation.

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. All units are in mmol 100 g dry weight of sediment⁻¹.

Time (Days)	S1	S2	S3	S4	B1	B2	B3	B4
0	0.0877	0.0877	0.0877	0.0877	0.1391	0.1391	0.1391	0.1391
7	0.0970	0.0939	0.0927	0.1038	0.0749	0.0636	0.0647	0.0740
21	0.1066	0.1031	0.1019	0.1141	0.0821	0.0697	0.0709	0.0812
42	0.0208	0.0332	0.0290	0.0667	0.0093	0.0061	0.0072	0.0069
63	0.0223	0.0220	0.0207	0.0274	0.0090	0.0072	0.0066	0.0113
105	0.0960	0.0371	0.0772	0.0316	0.0138	0.0105	0.0176	0.0101
140	0.0215	0.0707	0.0231	0.0327	0.0092	0.0128	-	0.0081
175	0.0565	0.0404	0.0207	0.0262	0.0091	0.0095	0.0117	0.0120
210	0.0627	0.0680	0.0663	0.0669	0.0099	0.0092	0.0066	0.0069
308	0.1958	-	0.2479	0.1682	0.0711	0.1130	0.0874	0.0813
365	0.0607	-	0.0746	0.0749	0.0228	0.0165	0.0178	0.0123
400	0.0924	-	0.1184	0.1073	0.0277	0.0199	0.0352	0.0229
470	0.1340	-	0.0966	0.1268	0.0454	0.0576	0.0700	0.0490
500	0.1657	-	0.1925	0.1782	0.0710	0.0689	0.0786	0.0431
531	0.0249	-	0.0429	0.0578	0.0579	0.0317	0.0092	0.0254

Table S7. The 355 nm, 400 nm, 440 nm and 550 nm absorbance values of the porewater of the surface sediment slurry throughout the anoxic incubation period and subsequent aeration incubation.

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. Blank spaces represent samples which did not provide a reliable reading.

Time (days)	355 nm absorbance				400 nm absorbance				440 nm absorbance				550 nm absorbance			
	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4	S1	S2	S3	S4
0	0.057	0.057	0.057	0.057	0.039	0.039	0.039	0.039	0.022	0.022	0.022	0.022	0.008	0.008	0.008	0.008
7	0.068	0.082	--	0.071	0.050	0.060	--	0.051	0.029	0.035	--	0.028	0.013	0.016	--	0.011
21	0.070	0.075	--	0.072	0.049	0.051	--	0.052	0.027	0.028	--	0.030	0.011	0.011	--	0.013
42	--	0.087	0.082	0.081	0.048	0.043	0.055	0.058	--	0.038	0.030	0.035	--	0.018	0.011	0.017
63	0.084	0.063	0.059	0.072	--	0.061	0.045	0.056	0.028	0.033	0.035	0.033	0.015	0.015	0.009	0.011
105	0.057	0.060	0.033	0.076	0.064	0.049	0.036	0.048	--	0.017	0.035	0.023	--	0.006	0.007	0.026
140	0.057	0.058	--	0.044	0.036	0.033	--	0.027	0.027	0.022	0.039	0.017	0.015	0.024	0.012	0.008
175	0.067	0.055	0.065	0.062	0.047	0.034	0.042	0.039	0.036	0.023	0.028	0.028	0.022	0.014	0.015	0.016
210	0.077	0.073	0.080	0.084	0.051	0.059	0.057	0.069	0.040	0.037	0.037	0.033	0.025	0.017	0.017	0.022
308	0.082	--	0.084	0.076	0.048	--	0.046	0.039	0.032	--	0.029	0.025	0.014	--	0.011	0.013
365	0.085	--	0.057	0.065	0.051	--	0.029	0.034	0.037	--	0.018	0.023	0.021	--	0.008	0.011
400	0.069	--	0.071	0.073	0.049	--	0.037	0.033	0.008	--	0.018	0.018	0.012	--	0.008	0.007
470	0.062	--	0.063	0.073	0.031	--	0.029	0.033	0.010	--	0.010	0.019	0.007	--	0.005	0.011
500	0.080	--	0.074	0.076	0.041	--	0.035	0.039	0.007	--	0.009	0.007	0.007	--	0.005	0.011

531	0.104	--	0.076	0.134	0.078	--	0.056	0.099	0.569	--	0.726	0.840	0.034	--	0.025	0.033
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Table S8. The 355 nm, 400 nm, 440 nm and 550 nm absorbance values of the porewater of the surface sediment slurry throughout the anoxic incubation period and subsequent aeration incubation.

B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm. Blank spaces represent samples which did not provide a reliable reading.

Time (days)	355 nm absorbance				400 nm absorbance				440 nm absorbance				550 nm absorbance			
	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4	B1	B2	B3	B4
0	0.052	0.052	0.052	0.052	0.031	0.031	0.031	0.031	0.021	0.021	0.021	0.021	0.012	0.012	0.012	0.012
7	--	0.071	0.076	0.067	--	0.049	0.052	0.043	--	0.036	0.036	0.029	--	0.020	0.019	0.015
21	0.044	--	0.064	0.068	0.026	--	0.045	0.062	0.015	--	0.032	0.026	0.006	--	0.019	0.019
42	0.070	--	0.074	0.083	0.048	--	0.051	0.059	0.034	--	0.036	0.043	0.009	--	0.009	0.015
63	--	0.084	--	0.081	--	0.064	--	0.059	--	0.036	--	0.029	--	0.020	--	0.015
105	0.068	0.083	0.074	0.072	0.049	0.052	0.055	0.053	0.039	0.049	0.045	0.042	0.018	0.019	0.021	0.020
140	0.078	0.062	--	0.064	0.058	0.042	--	0.045	0.047	0.031	--	0.036	0.024	0.019	--	0.024
175	0.079	0.043	0.082	0.075	0.059	0.025	0.058	0.043	0.048	0.017	0.047	0.045	0.025	0.010	0.023	0.019
210	0.069	0.073	0.052	0.061	0.054	0.059	0.064	0.046	0.039	0.041	0.033	0.032	0.016	0.028	0.016	0.009
308	0.074	0.06	0.072	0.064	0.043	0.031	0.039	0.036	0.029	0.02	0.026	0.025	0.014	0.01	0.012	0.013
365	0.084	0.083	0.061	0.048	0.051	0.038	0.029	0.037	0.042	0.024	0.016	0.014	0.028	0.017	0.009	0.007
400	0.087	0.072	0.075	0.087	0.048	0.036	0.049	0.035	0.019	0.017	0.021	0.019	0.014	0.012	0.012	0.008
470	0.042	0.052	0.047	0.041	0.029	0.024	0.021	0.016	0.012	0.015	0.01	0.007	0.011	0.009	0.005	0.003

500	0.082	0.083	0.084	0.080	0.033	0.035	0.029	0.049	0.014	0.013	0.015	0.013	0.009	0.007	0.007	0.008
531	0.104	0.166	0.162	0.125	0.092	0.138	0.098	1.562	0.501	0.845	1.419	0.387	0.066	0.107	0.043	0.021

Table S9. pH of the porewater of the sediment slurry throughout the anoxic incubation period and subsequent aeration incubation.

S1, S2, S3 and S4 are the identifiers of the 4 replicates of the surface 2 cm. B1, B2, B3 and B4 are the identifiers of the 4 replicates of the sediments from 20-22 cm.

Time (Days)	S1	S2	S3	S4	B1	B2	B3	B4
105	7.4	7.4	7.7	7.1	6.0	5.7	5.6	5.5
140	7.0	7.4	7.3	7.1	6.5	5.8	5.7	5.8
175	6.7	7.1	6.9	6.1	5.7	6.3	6.2	5.9
210	7.3	6.9	7.3	7.1	6.2	5.9	6.0	5.8
308	5.2	--	5.3	5.6	5.5	5.7	5.8	5.2
365	5.3	--	5.2	5.1	5.2	5.5	4.9	5.3
400	5.5	--	5.7	5.1	5.1	5.1	5.6	5.2
470	5.6	--	5.3	5.7	5.4	5.4	5.0	5.8
500	5.6	--	5.4	5.7	5.3	5.5	5.3	6.0

Table S10. Iron content of the sediments throughout the anoxic incubation period and subsequent aeration incubation.

S1, S3 and S4 are the identifiers of 3 replicates of the surface 2 cm. B1, B2, and B3 are the identifiers of 3 replicates of the sediments from 20-22 cm. All units are in mol 100 g⁻¹.

Time (days)	S1	S3	S4	B1	B2	B3
7	0.0542	0.0562	0.0522	0.0448	0.0448	0.0410
21	0.0591	0.0512	0.0521	0.0504	0.0504	0.0455
42	0.0476	0.0538	0.0523	0.0532	0.0532	0.0436
63	0.0537	0.0554	0.0537	0.0427	0.0427	0.0502
147	0.0535	0.0554	0.0535	0.0457	0.0457	0.0443
231	0.0533	0.0571	0.0528	0.0457	0.0457	0.0458
329	--	0.0513	0.0560	0.0433	0.0433	0.0523
365	0.0565	0.0551	0.0528	0.0472	0.0472	0.0537
400	0.0575	0.0556	0.0526	0.0468	0.0468	0.0482
500	0.0803	0.0558	0.0575	0.0494	0.0494	0.0465

Table S11. Uncorrected stable isotopes data of ^{13}C and ^{15}N of selected sediments used in the incubation experiment.

S 0 and B 0 are the samples of the surface 2 cm and sediments from 20-22 cm at the start of the incubation, while S1 210, S2 210, S3 210 and S4 210 are the identifiers of the 4 replicates of the surface 2 cm after 210 days of anoxic incubation. B1 210, B2 210, B3 210 and B4 210 are the identifiers of the 4 replicates of the sediments from 20-22 cm after 210 days of anoxic incubation.

Sample	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	%C	%N	N/C Ratio
S 0	-26.92	4.18	7.67	0.37	0.066
B 0	-26.70	2.21	7.25	0.28	0.071
S1 210	-27.00	4.33	7.30	0.33	0.066
S1 210	-26.99	4.05	7.44	0.34	0.065
S2 210	-27.02	4.01	7.67	0.35	0.064
S3 210	-27.00	4.02	7.48	0.34	0.065
S4 210	-27.08	4.08	7.53	0.34	0.064
B1 210	-26.86	2.40	7.35	0.28	0.071
B2 210	-26.90	2.26	7.30	0.28	0.071
B3 210	-26.85	2.31	7.55	0.28	0.069
B4 210	-26.84	2.45	7.33	0.28	0.071

References

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