Environmental Science & Technology

pubs.acs.org/est

Article

Negligible Greenhouse Gas Release from Sediments in Oyster Habitats

Nicholas E. Ray* and Robinson W. Fulweiler

Cite This: *Environ. Sci. Technol.* 2021, 55, 14225−14233

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

Downloaded via CORNELL UNIV on October 19, 2021 at 23:40:27 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

ABSTRACT: After centuries of decline, oyster populations are now on the rise in coastal systems globally following aquaculture development and restoration efforts. Oysters regulate the biogeochemistry of coastal systems in part by promoting sediment nutrient recycling and removing excess nitrogen via denitrification. Less clear is how oysters alter sediment greenhouse gas (GHG) fluxes—an important consideration as oyster populations grow. Here, we show that sediments in oyster habitats produce carbon dioxide (CO_2), with highest rates in spring (2396.91 ± 381.98 μmol CO_2 m^{-2} h^{-1}) following deposition of seasonal diatom blooms and in summer (2795.20 ± 307.55 μmol CO_2 m^{-2} h^{-1}) when temperatures are high. Sediments in oyster habitats also consistently released methane to the water column (725.94 ± 150.34 nmol CH_4 m^{-2} h^{-1}) with no seasonal pattern. Generally, oyster habitat sediments were a sink for nitrous oxide (N_2O; −36.11 ± 7.24 nmol N_2O m^{-2} h^{-1}), only occasionally releasing N_2O in spring. N_2O release corresponded to high organic matter and dissolved nitrogen availability, suggesting denitrification as the production pathway. Despite potential CO_2 production increases under aquaculture in some locations, we conclude that in temperate regions oysters have an overall negligible impact on sediment GHG cycling.

KEYWORDS: *oyster, nitrous oxide, methane, carbon dioxide, greenhouse gas, aquaculture*

INTRODUCTION

Following centuries of decline,[1,2] today there is substantial effort to rebuild oyster populations in coastal systems around the world. At least 1500 oyster reef restoration projects have been completed since 1990, and 5815 thousand tonnes of oysters raised in aquaculture were harvested in 2018—a 38% increase relative to 2010 harvest.[3,4] These efforts to increase oyster populations aim both to restore ecosystem services and to provide a valuable economic resource.[5,6] Oysters exert a strong influence on biogeochemical cycling in coastal ecosystems, and it is now clear that rates of sediment nutrient recycling and removal of excess nitrogen (N) via denitrification are higher for sediments in oyster habitats than nearby bare sediments.[7] Oysters may enhance sediment greenhouse gas (GHG) production, leading to greater release of GHGs from the water column to the atmosphere, yet the effect of oysters on sediment GHG production remains poorly constrained. As oyster populations continue to grow, it is important to quantify the GHG footprint of reef habitats and aquaculture operations so we may better understand their environmental impact.[8]

Estuaries are a globally important source of carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) to the atmosphere.[9−11] Some coastal systems are a net sink of these GHGs, some act as a source, and this source/sink behavior within estuaries can vary by season. Of particular importance is the role oysters may play in promoting sediment GHG production by loading organic matter-rich biodeposits to the sediments and excreting nutrients directly to the water column,[12,13] as these processes may promote sediment GHG production. We know little about how sediment GHG fluxes may vary in different oyster habitats, and direct measurements of sediment GHG production in oyster habitats have yielded mixed results, even within studies. For example, installation of oyster aquaculture drove a temporary, multiyear (~1 to 3 year) increase in CO_2 and CH_4 release from sediments, before a return to baseline levels,[13] and a similar pattern has been observed for N_2O following construction of restored oyster reefs.[14] In another instance, installation of oyster aquaculture switched sediments from an N_2O source to a sink.[15] Oyster density, and thus the amount of organic matter loaded to sediments, may also alter sediment GHG production,[16] with

Received: August 5, 2021
Revised: September 21, 2021
Accepted: September 22, 2021
Published: October 6, 2021

ACS Publications

© 2021 American Chemical Society

decreased GHG release at medium oyster density and increased GHG release at high density relative to adjacent bare (i.e., oyster-free) sediments.[17]

A recent study of the GHG footprint of oyster aquaculture estimated that oyster aquaculture has less than 0.5% of the GHG cost of terrestrial livestock production on a kg CO_2−eq kg protein^{-1} basis.[13] The total GHG release measured at the oyster farm in that study was substantially lower than previous modeling estimates,[18] as no difference was found in GHG flux from bare sediments or those beneath aquaculture gear when all ages of aquaculture were pooled. However, that estimate only investigated a single farm during the summer. A more accurate estimate requires measurements of the effect of oysters on sediment GHG production across different systems and seasons, which we have conducted here. Sediment respiration and production of CO_2 and CH_4 typically follow seasonal patterns of organic matter deposition and temperature, with the highest rates of production within a month or two of organic matter deposition and higher rates of respiration as temperature increases.[19,20] NO_x availability is an important control on aquatic N_2O production, so water-column N_2O concentrations and sediment N_2O production in temperate estuaries are typically highest in spring when NO_x availability is high.[21−25] We expect that sediment GHG cycling in oyster habitats follows these seasonal patterns. Similarly, direct GHG release from oysters varies across seasons and appears to be regulated by temperature and nutrient availability.[26,27] Thus quantifying seasonal changes in sediment GHG flux across oyster habitats may provide a more robust estimate of their GHG footprint.

To clarify uncertainties in the GHG footprint of oyster habitats, we conducted sediment core incubations to: (1) quantify seasonal patterns of sediment GHG production in oyster habitats (four oyster habitats in one system), (2) test the effect of oyster aquaculture on sediment GHG fluxes across systems (four oyster farms and adjacent bare sediments in three systems), and (3) determine whether sediment organic matter content and initial concentrations of dissolved NH_4^+ and NO_x might predict rates of sediment GHG flux in oyster habitats. We analyzed CH_4 and N_2O fluxes directly using gas chromatography (GC) and determined sediment CO_2 release by measuring sediment O_2 consumption and then applying a 1:1 respiratory quotient.[13,28,29] We used our results to update the GHG footprint of oysters, by converting CH_4 and N_2O to kg CO_2-equivalents using conversion factors of 25 and 298, respectively. While results of this study are specific to the northeastern Atlantic coast of the United States, the general trends and controls on sediment GHG fluxes revealed are likely transferable across temperate systems.

METHODS

Sampling Sites and Scheme. We used two sets of sediment flux incubations in this study. The first set of incubations aimed to quantify sediment GHG production in oyster habitats across seasons, and the second compared how sediment GHG production in aquaculture differed from nearby sediments with no oysters present. In the first set, we collected and incubated sediment cores from four oyster habitats in Narragansett Bay to determine seasonal patterns of sediment GHG production in oyster habitats (Figure S1). Narragansett Bay is a large (~380 km^2), shallow (8.6 m mean depth), temperate estuary, with numerous small embayments and shallow areas that can be used for oyster aquaculture and reef restoration. The four oyster habitats from which we collected sediment cores were from these shallow areas (<2 m depth at high tide). Allen Harbor is a sandy site on the western shore of Narragansett Bay that employs rack-and-bag oyster aquaculture. Bissel Cove is a small embayment less than 10 km south of Allen Harbor and contains a restored oyster habitat. Mill Cove is located between Allen Harbor and Bissel Cove and has the remnants of a formerly large oyster reef. There is a large amount of shell scattered across the sediments at Mill Cove, but few live oysters, likely because of burial under fine, silty sediment. Town Pond also contains a restored oyster habitat in a small embayment connected to Mt. Hope Bay in the northeast of Narragansett Bay. The oyster habitats at Bissel Cove, Mill Cove, and Town Pond may be best described as oyster beds and not reefs—there is little vertical structure, large patches of sediments between individual oysters (between 0.5 and 1 m), or oyster clumps of two to three oysters. We collected sediment cores from Allen Harbor, Bissel Cove, and Town Pond in Summer and Fall 2016 and Spring and Summer 2017. Additional samples from Town Pond were collected in Spring, Summer, and Fall 2018. We sampled Mill Cove in Summer and Fall 2016 (Table S1).

In the second set of sediment flux incubations, we collected cores from beneath four oyster farms and adjacent bare sediments (~10 m away from aquaculture gear) to determine if oysters have the same effect on sediment GHG production across sites with varying environmental conditions (Figure S2). The first site was located in Ninigret Pond, a shallow, microtidal lagoon with a water residence time of approximately 10 days.[30] The oyster farm in Ninigret Pond is located on the backside of a sandy barrier spit that separates the lagoon from Block Island Sound. Sediment GHG fluxes were previously measured at this oyster farm in 2014 and 2015.[13] In Narragansett Bay, we sampled from two sites. Roger Williams University (RWU) maintains a small oyster farm on a sandy/gravelly site adjacent to a pier near to where Mt. Hope Bay enters Narragansett Bay proper. Saltbox oyster farm is located nearby in an embayment with fine, silty/mucky sediments and less wave energy than RWU. The fourth farm is located in Duxbury Bay on fine, silt/clay sediments. Duxbury Bay has strong tides (~3 m depth) with approximately 66% of water exchanged with the Atlantic Ocean each tidal cycle.[31] We sampled each site twice between June and October 2018 (Table S1). The farm in Ninigret Pond employs rack-and-bag culture, and the other farms employ cages, though in all cases, oysters were suspended approximately 10−20 cm from the sediment surface.

Sediment Core Collection and Incubation. We collected sediment cores by hand or with a pole corer. At oyster beds, we collected cores from spaces in between oysters, and at aquaculture facilities, we collected cores from directly underneath aquaculture gear. Cores were made of clear PVC tubing 28 cm in length with an internal cross-sectional area of 0.00785 m^2. During sediment collection, we aimed to collect between 10 and 12 cm of sediment. Upon collection, we checked each core to ensure that the sediment−water interface was intact and that there was sufficient overlying water before capping each core and moving them to a cooler filled with site water to maintain constant temperature until return to the lab. We also collected unfiltered site water in acid-washed plastic carboys. Upon return to the lab (within 6 h of core collection), cores were moved to a water bath in an environmental chamber set to the same temperature as the field site. We

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

uncapped the cores and carboys and bubbled them gently overnight with aquarium pumps to maintain oxygenation in the overlying water.[32,33]

Immediately prior to beginning flux incubations the following day, we siphoned off the overlying water and replaced it with site water from the carboys.[32,33] We also filled an empty core tube with site water to serve as a control to account for any gas production or consumption in the water column. Before capping each core, we collected 60 mL of water in an acid-washed syringe and filtered it through a GF/F filter into a polyethylene bottle which was frozen until the analysis of dissolved nutrient concentrations (NH_4^+ and NO_x).

After sampling for initial nutrient concentrations, we carefully capped each core with acrylic lids equipped with inflow and outflow ports, ensuring that there was no air headspace or bubbles in any core. We then collected the first of five sample time points for dissolved gas analysis. At each sample time point, we filled four 12 mL exetainers with water from the core, allowing the exetainers to overflow several times, before adding 25 μL of saturated zinc chloride solution to stop any biological activity and capping the exetainers with gas-tight septa. Two exetainers were reserved for the analysis of CH_4 and N_2O concentrations and flux, and the other two were reserved for the analysis of O_2 concentration (and estimation of CO_2 flux). The five sample time points were spaced to ensure the O_2 concentration in each core (except the water control) dropped at least 2.0 mg L^{-1} without becoming hypoxic (O_2 concentration $\leq$ 2.0 mg L^{-1}).[13,32] The incubation chamber was kept dark throughout the incubation, except for brief periods to collect samples and check dissolved oxygen concentration. Temperature in the chamber remained constant from the time cores were returned to the lab until incubations had ended. Incubations typically lasted between 4 and 10 h.

Immediately following collection of the last dissolved gas samples, we removed the core lids and siphoned out the water. For cores collected in 2016 and 2017, we used an acid-washed 60 mL syringe with the tip cut off to collect a sample of the top 1 cm of sediment for later analysis of % organic matter. For the cores collected in 2018, we collected the top 2 cm of sediment from all three cores and mixed it. These sediments were used in another experiment, but we reserved a subsample for the analysis of % organic matter. All sediment samples were stored at −20 °C until analysis.

One core from Town Pond in summer 2017 had a positive O_2 flux, indicating a likely leak in the chamber. We excluded all fluxes measured using this core from our analysis.

Sample Analysis and Flux Calculations. N_2O and CH_4 concentrations were determined using GC following a headspace equilibration technique.[13,33] First, a gas headspace was made in each sample exetainer by simultaneously removing 5 mL of sample and injecting 5 mL of ultrahigh purity helium using gas-tight glass syringes inserted through the exetainer septa.[33] Each sample exetainer was then shaken for 30 s and allowed to equilibrate for at least 1 h prior to the analysis of the headspace on the GC (Shimadzu 2014 GC; Shimadzu Corporation, Kyoto, Japan), which was equipped with an electron capture detector with a ^{63}Ni source for the analysis of N_2O and a flame ionization detector for the analysis of CH_4. The sample headspace (4 mL) was analyzed on the GC, and N_2O and CH_4 concentrations were estimated by comparing the area under the generated peak against a standard curve of peak areas made from different concentrations of an external standard made up of 4977 ppb CH_4 and 495 ppb N_2O in N_2. Each standard curve had six time points and an $R^2 \geq 0.995$. Minimum detection limits were 83.21 ppb for CH_4 and 16.83 ppb for N_2O ppb during sample analysis.

O_2 concentrations were determined using a membrane inlet mass spectrometer (Bay Instruments, Cambridge, MD) to measure O_2:Ar and then back calculating O_2 concentration based on the theoretical Ar concentration at the given temperature and salinity of the water in each incubation core.[34−36]

To calculate fluxes of each gas, we used a linear regression approach. When the change in concentration over time was linear with an $R^2 \geq 0.65$ and a significant slope defined as $p \leq 0.10$, we prorated this flux to the cross-sectional area of the sediment surface and volume of overlying water.[13,33,37] If the calculated $R^2 < 0.65$ or $p > 0.10$, we considered there to be no flux and assigned a value of zero.[13] To convert from an O_2 flux to a CO_2 flux, we applied a 1:1 respiratory quotient.[13,28,29] The RQ approach is only a rough estimate of CO_2 production as it does not capture CO_2 produced during anaerobic decomposition processes (such as denitrification or sulfate reduction). However, it is unlikely to result in fundamentally biased data and likely provides a reasonable estimate of the impact of oysters on sediment CO_2 production.[38]

Unfortunately, we could not locate any published values of sediment RQ in oyster habitats. There is, however, a recently published study that reports simultaneous O_2 and dissolved inorganic carbon (DIC) fluxes from sediments beneath a mussel farm and nearby control sediment in a temperate estuary.[39] The data set presented by Hylén et al. (2021) lends credibility to using an RQ approach to compare CO_2 from sediments in oyster habitats and nearby reference sites. To calculate RQ beneath mussels and the reference site using the data from Hylén et al. (2021), we included all chamber incubations that had both O_2 and DIC measurements when mussels were actively filtering at the farm site (July and October) and excluded incubations that only reported fluxes for either O_2 or DIC, incubations the authors indicated were "outliers," and months when no mussels were present at the farm site (June and February). While there was variance in RQ between months, there was no difference in RQ between sediments beneath mussel aquaculture (1.23 ± 0.38 and $n = 10$) and nearby reference sediments (1.23 ± 0.30 and $n = 9$). Thus, we can conclude that using an RQ value rather than direct measures of DIC is unlikely to lead to changes in our comparison of CO_2 flux between bare sediments and those beneath bivalve aquaculture. It might, however, lead to a slight misestimation of the actual rate of CO_2 production and may not always be accurate across seasons.[40]

We determined concentrations of NH_4^+ and NO_x using high-resolution digital colorimetry on a Seal AutoAnalyzer 3, following SEAL method G171-96 for NH_4^+ and G172-96 for NO_x. Minimum detection limits during analyses were 0.080 μmol L^{-1} for NH_4^+ and 0.013 μmol L^{-1} for NO_x. Spring 2018 Town Pond nutrient samples were not analyzed as they were left out overnight and not immediately frozen for preservation until analysis.

Sediment % organic matter was calculated as the change in the mass of a sediment sample before and after combustion in a 500 °C muffle furnace for 4 h.

Statistical Analysis. All statistical analyses were conducted using R Statistical Software Version 4.0.0.[41] We used the *lme4* package to create mixed models for comparing sediment fluxes from oyster habitats between seasons and whether fluxes

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

differed between bare sediments and sediments beneath oysters.[42] We kept the data in two separate datasets for our analyses (fluxes measured in 2016 and 2017, and Town Pond 2018 samples were used for seasonal comparisons, and oyster farms sampled in 2018 were used for comparison with bare sediments). First, we determined the data distribution for each flux type across seasons and in the presence and absence of oysters using the *fitdistrplus* package (Table S2).[43] We then created models using season as a fixed effect and site as a random effect to compare sediment fluxes across seasons and models using the presence/absence of oysters as a fixed effect and site as a random effect to compare fluxes from bare sediments and those beneath oysters (Table S2). We compared between seasons and the presence/absence of oysters using least-squares means tests via the *emmeans* package.[44] All figures were obtained using the *ggplot2* package.[45]

To test whether concentrations of dissolved NH_4^+, NO_x, or sediment % organic matter could predict sediment GHG fluxes in oyster habitats, we pooled flux measurements from sediments in oyster habitats from both sediment flux experiments (excluding bare sediments) and used a series of linear regressions.

Data Availability. The data set generated during this study and used in this manuscript can be accessed via the Figshare Repository at: http://10.6084/m9.figshare.12943109.

RESULTS AND DISCUSSION

Seasonal Patterns of GHG Cycling in Oyster Habitats.

Sediments in oyster habitats were always a source of CO_2 to the water column (2276.68 ± 202.53 μmol CO_2 m^{-2} h^{-1}) and typically a sink for N_2O (−36.11 ± 7.24 nmol N_2O m^{-2} h^{-1}), and the magnitude of CO_2 and N_2O fluxes varied seasonally (Figure 1). There was little variance in sediment CH_4 flux across seasons, and sediments were almost always a net source of CH_4 to the water column (725.94 ± 150.34 nmol CH_4 m^{-2} h^{-1}). CO_2 release was significantly higher in the spring (2396.91 ± 381.98 μmol CO_2 m^{-2} h^{-1}) and summer (2795.20 ± 307.55 μmol CO_2 m^{-2} h^{-1}), compared to the fall (1385.42 ± 268.19 μmol CO_2 m^{-2} h^{-1}; Table S3). The largest N_2O sink was in the summer (−56.36 ± 7.65 nmol N_2O m^{-2} h^{-1}), with less uptake observed in the fall (−21.57 ± 9.09 nmol N_2O m^{-2} h^{-1}) and spring (−15.46 ± 21.96 nmol N_2O m^{-2} h^{-1}). Spring N_2O fluxes were highly variable, including an instance of net production.

The seasonal patterns of CO_2 and N_2O fluxes we observed are consistent within the context of seasonal patterns of phytoplankton productivity in Narragansett Bay, which has historically been characterized by a large winter−spring diatom bloom and lower productivity during the summer.[46,47] If oysters drive sediment GHG production by increasing organic matter deposition to the sediments during filter-feeding, it might be expected that the highest GHG fluxes will occur following large phytoplankton blooms. We were able to test this hypothesis in our seasonal incubations as the spring sediment core incubation was conducted shortly after the winter−spring bloom, summer core incubations were conducted following a period of low productivity, and fall core incubations were conducted following a smaller fall bloom.[32] This water-column production impacted the sediments as we measured the highest sediment % organic matter in the spring (3.97 ± 1.00%) followed by the fall (3.65 ± 1.22%) and lowest % organic matter in the summer (2.74 ± 0.57%). Mean N_2O fluxes followed this seasonal pattern of sediment organic matter content, and we recorded the most—and highest—net positive N_2O fluxes from sediment cores in the spring, no net production events in the summer, and a single positive N_2O flux in the fall. A simple linear regression of N_2O flux as a function of organic matter using just the seasonal flux data demonstrates a significant relationship (p = 0.002, R^2 = 0.21, and df = 41; Figure S3), albeit one that only describes a fifth of the variance in the data. The concentration of NO_x at the start of the incubation is a better predictor of N_2O flux (p < 0.001, R^2 = 0.49, and df = 44; Figure S3), suggesting that N_2O fluxes in oyster habitats are tightly linked to organic matter availability and NO_x concentration. A multiple linear regression using both sediment organic matter and the concentration of NO_x at the start of the incubation as predictive variables slightly improves the predictive power of the model (p < 0.001, R^2 = 0.54, and df = 36). Organic matter and NO_x availability are both factors known to control denitrification,[48] one of the two key microbial metabolic pathways responsible for N_2O production in marine systems. NO_x is used to oxidize organic matter, releasing energy for the organism. Indeed, measured net sediment di-nitrogen fluxes at the same sites demonstrated net denitrification in the spring when sediment organic matter and water-column NO_x concentration were high, a net zero flux in the summer, and net nitrogen fixation in the fall.[32] We propose that inefficient

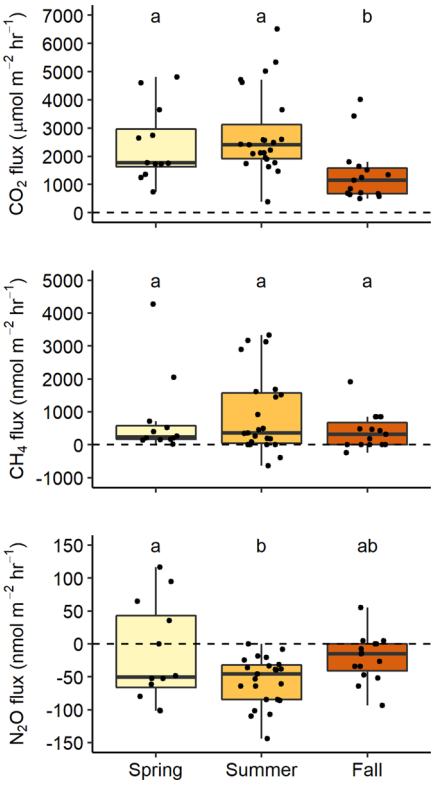

Figure 1. Seasonal patterns of sediment carbon dioxide (CO_2) methane (CH_4) and nitrous oxide (N_2O) fluxes at three oyster reefs and one farm (Figure S1). Each point represents a single flux measurement, with points above zero (dashed line) indicative of net production, and those below indicative of net consumption. Seasons with the same letter are not statistically different from each other (p > 0.05; Table S3) following least-squares mean tests. Note that CO_2 fluxes are reported in μmol m^{-2} h^{-1} and CH_4 and N_2O are reported in nmol m^{-2} h^{-1}.

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

denitrification is the main driver of N_2O release from sediments in oyster habitats and hypothesize that oyster habitats in systems with relatively low dissolved nutrient concentrations and organic matter loading will release less N_2O than oyster habitats in systems with high dissolved nutrient availability and organic matter loading. Nitrification is an autotrophic process, requiring NH_4^+ but no organic matter. If nitrification was important for producing N_2O in oyster habitats, we would expect to find a relationship between NH_4^+ concentration and N_2O flux in oyster habitats across seasons, which we did not.

CO_2 fluxes did not track the seasonal pattern of productivity as closely, as they were higher in summer than spring and lowest in fall. CO_2 release was likely driven by organic matter deposition in the spring (3.97 ± 1.00%) and then remained high during the summer when temperatures warmed (24.1 ± 0.2 °C; 13.1 ± 0.1 °C in spring) despite decreased organic matter availability (2.74 ± 0.57%). CO_2 release was unexpectedly low in the fall when sediment organic matter was again high (3.65 ± 1.22%) and temperature was higher than that in the spring (17.9 ± 0.6 °C). This observation may possibly be due to organic matter of a relatively lower quality in fall than that in the spring.[32] Neither sediment organic matter ($p = 0.756$, $R^2 = 0.002$, and df = 41) nor temperature ($p = 0.319$, $R^2 = 0.021$, and df = 48) was a good predictor of sediment CO_2 release in the seasonal study.

We did not record seasonal differences in sediment CH_4 production, though CH_4 fluxes did have a similar seasonal pattern to CO_2 fluxes, with some of the highest instances of sediment CH_4 release in the summer and lowest rates generally in the fall. When considering just the fluxes in the seasonal data set, neither temperature ($p = 0.513$, $R^2 = 0.01$, and df = 48), salinity ($p = 0.849$, $R^2 = 0.00$, and df = 48), nor sediment organic matter ($p = 0.067$, $R^2 = 0.08$, and df = 41) had significant predictive power for CH_4 fluxes. There is evidence that rates of methanotrophy in Narragansett Bay scale with temperature,[49] and this may provide a possible explanation for the seasonal patterns we observed: as temperatures increased in the summer so did methanotrophy, offsetting potential increases in CH_4 release via methanogenesis. Then as temperatures decreased in the fall, methanotrophy also declined, and net CH_4 fluxes remained relatively the same.

The high variability within seasons appears to be driven by differences in water quality and sediment conditions. Water quality (i.e., dissolved nutrient concentrations) varied daily and between sites. In one instance in the spring, starting NO_x at Town Pond was high (Table S1), likely because of the site being adjacent to a golf course, and our sampling taking place the day following a precipitation event, which could have led to runoff of fertilizer applied in the golf course. On this date at Town Pond, we recorded the three highest rates of sediment N_2O release in the seasonal study. Sediment organic matter also varied between sites, but followed a similar seasonal pattern across all sites. Thus, both short-term pulse events that may be small in the spatial scale and larger scale, system wide patterns of productivity contribute to between and within site variability.

Influence of Oysters on GHG Fluxes across Systems. Sediment CO_2 fluxes were higher beneath aquaculture (1662.01 ± 217.11 μmol CO_2 m^{-2} h^{-1}) than from bare sediments (1274.77 ± 195.36 μmol CO_2 m^{-2} h^{-1}), though this difference did not meet our criteria for statistical significance ($p = 0.053$; Figure 2). There was high variability in the effect of

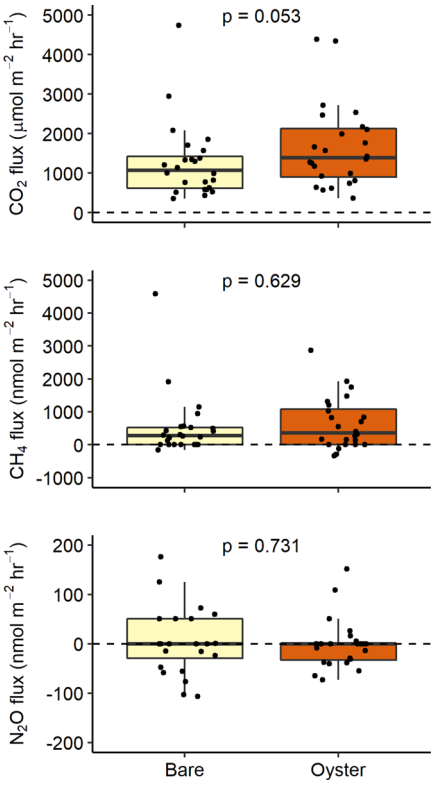

Figure 2. Fluxes from bare sediments and sediment beneath oyster aquaculture across systems in the summer (Figure S2). Each point represents an individual flux measurement, with those above the zero-line indicating sediment release to the water column, and those below indicating a sink. *p*-values indicate the results of a least-squares means test comparing the two treatments. Note that CO_2 fluxes are reported in μmol m^{-2} h^{-1} and CH_4 and N_2O are reported in nmol m^{-2} h^{-1}.

aquaculture on sediment CO_2 production both within and between sites (Table 1), suggesting that site-specific conditions may determine whether oysters alter rates of sediment CO_2 production. For example, at Duxbury Bay and Ninigret Pond sediment CO_2 release was nearly twice as high beneath aquaculture compared to bare sediments, while at RWU and Saltbox, sediment CO_2 fluxes were similar regardless of the presence of oysters (Table 1). Duxbury Bay and RWU have relatively high tidal energy and daily water exchange, while Ninigret Pond and Saltbox do not, suggesting that these factors may not be important when considering oyster impact on sediment CO_2 production.

The site with the greatest enhancement in sediment CO_2 release was Ninigret Pond (Table 1), which we previously sampled in 2014 and 2015.[13] In that study, we sampled along a chronosequence of sediments beneath aquaculture that had been in place for varying lengths of time (0−7 year). We observed no difference in CO_2 flux between bare sediments and those beneath aquaculture gear, aside from enhanced CO_2 release from sediments beneath aquaculture that had been in place between 4 and 6 years.[13] In this study, we resampled from the oldest corner of the farm during the first sampling (June; the aquaculture gear would have been in place for 10 years in 2018) and from the newest corner of the farm in the second round of sampling (August; the aquaculture gear would have been in place here for 6 years). Both sites showed similar enhancement in CO_2 production beneath aquaculture relative to bare sediments. Our 2018 measurements from the new 6

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

Table 1. Greenhouse Gas Fluxes from Sediments beneath Oyster Aquaculture and Nearby Bare Sediments at Four Oyster Aquaculture Facilities and Sediment Organic Matter Content (0−2 cm Depth) at Each Site[a]

location	latitude	longitude	sediment treatment	CO_2 Flux (μmol m^{-2} h^{-1})	CH_4 Flux (nmol m^{-2} h^{-1})	N_2O Flux (nmol m^{-2} h^{-1})	sediment organic matter (%LOI)
Duxbury Bay	42.02252	−70.64671	bare	598.93 ± 74.31	464.50 ± 164.78	12.25 ± 18.69	1.26 ± 0.07
			oyster	1127.85 ± 293.99	714.33 ± 289.03	11.85 ± 9.96	3.28 ± 0.64
Ninigret Pond	41.62993	−71.23055	bare	1198.12 ± 215.75	204.17 ± 101.98	−50.50 ± 16.05	0.80 ± 0.06
			oyster	2013.28 ± 210.75	575.00 ± 273.37	−42.67 ± 5.75	0.85 ± 0.01
RWU	41.64982	−71.25644	bare	1029.44 ± 168.32	179.83 ± 90.75	−2.53 ± 2.53	1.14 ± 0.07
			oyster	935.57 ± 196.63	43.17 ± 79.91	−12.33 ± 12.33	1.32 ± 0.24
Saltbox	41.35740	−71.65565	bare	2272.58 ± 564.07	1289.33 ± 722.23	55.38 ± 40.06	1.69 ± 0.33
			oyster	2571.35 ± 587.50	1191 ± 432.88	37.88 ± 30.42	5.83 ± 0.74
all			bare	1274.77 ± 195.36	534.46 ± 199.09	3.65 ± 13.52	1.22 ± 0.14
			oyster	1662.01 ± 217.11	630.88 ± 162.50	−1.27 ± 10.20	2.82 ± 0.77

[a]Results are presented as mean ± standard error. $N = 6$ per treatment, per site except for organic matter $N = 2$.

Table 2. Results of Linear Regressions Investigating whether Carbon Dioxide (CO_2), Methane (CH_4), and Nitrous Oxide (N_2O) Fluxes from Sediments beneath Oysters Can Be Predicted by Sediment Organic Matter Content, Ammonium (NH_4^+), and Nitrate+Nitrite (NO_x) Concentrations in the Overlying Water Column, Temperature, or Salinity with Significant Relationships ($p \leq 0.05$) in Bold and Italicized (Figures S4−S8)

	CO_2 Flux (μmol m^{-2} h^{-1})			CH_4 Flux (nmol m^{-2} h^{-1})			N_2O Flux (nmol m^{-2} h^{-1})		
sediment property	p-value	R^2	df	p-value	R^2	df	p-value	R^2	df
% organic matter	0.161	0.030	65	***0.007***	***0.106***	***65***	***<0.001***	***0.199***	***65***
$[NO_x]$ (μmol L^{-1})	0.219	0.022	68	0.170	0.027	68	***<0.001***	***0.240***	***68***
$[NH_4^+]$ (μmol L^{-1})	0.688	0.002	68	***0.045***	***0.058***	***68***	***0.001***	***0.151***	***68***
temperature (°C)	0.903	0.000	72	0.565	0.005	72	0.208	0.022	72
salinity	0.182	0.025	72	0.942	0.000	72	0.141	0.030	72

year old site matched the pattern previously observed for enhanced CO_2 efflux from the 6 year old site in the previous study, but the high CO_2 release from the 10 year site suggests a possible second peak of enhanced CO_2 production. One potential explanation for these observations is that the sediment response to the pressure of oyster aquaculture may have continued along a nonlinear path with a second peak in CO_2 production.[13,50] Similar nonlinear patterns occurring over decadal time scales have been observed for soil CO_2 release under warming pressure[51] and sediment O_2 demand following reductions in nutrient and organic matter loading.[52] Alternatively, we may have sampled shortly after pulses of biodeposit loading to the sediments by oysters. We did not record a significant relationship between sediment organic matter and CO_2 production in this study (Table 2), though it is possible that because sediment organic matter content in Ninigret Pond is relatively low (<1%: Table 1) any labile organic matter in oyster biodeposits decomposes upon reaching the sediment. Previous measurements indicate that oyster biodeposits have an organic matter content of 15% and rapidly decompose to DIC at a rate of 1.6 μmol g dry weight^{-1} h.[53] Because sediment organic matter beneath aquaculture was similar to adjacent bare sediments in Ninigret Pond, it is likely that any biodeposits were rapidly decomposed.

There were no differences ($p = 0.629$) in CH_4 fluxes between bare sediments (534.46 ± 199.09 nmol CH_4 m^{-2} h^{-1}) and sediment beneath aquaculture (630.88 ± 162.50 nmol CH_4 m^{-2} h^{-1}). Bare sediments were a small N_2O source (3.65 ± 13.52 nmol N_2O m^{-2} h^{-1}) while those beneath aquaculture were a small N_2O sink (−1.27 ± 10.19 nmol N_2O m^{-2} h^{-1}), but this difference was not significantly different ($p = 0.731$). The reduction in N_2O release under oyster aquaculture relative to bare sediment was consistent across sites, while two sites demonstrated slightly higher CH_4 fluxes beneath aquaculture and two sites slightly lower (Table 1).

Factors Regulating Sediment GHG Fluxes in Oyster Habitats. When we pooled all fluxes from sediments in oyster habitats (both the seasonal incubations and incubations from oyster farms across systems), none of the variables we considered were good predictors of the magnitude of CO_2 release (Table 2). However, sediment organic matter was a significant predictor of both N_2O and CH_4 fluxes, as was NH_4^+ concentration, though to a lesser degree (Table 2). NO_x concentration was also a good predictor of N_2O flux. These results suggest that sediment GHG production in oyster habitats will be higher in systems and seasons with greater nutrient and organic matter loading.[10,24,54] When all fluxes from sediments in oyster habitats were pooled, NH_4^+ concentration had some predictive power for N_2O fluxes. While this may be due to N_2O release during nitrification, this relationship might also hint at the importance of coupled nitrification−denitrification, where NH_4^+ is used to create the NO_x needed for denitrification, and N_2O is then released during denitrification rather than nitrification. With this data set, we cannot make a definitive conclusion, though the evidence from just the incubations used to determine sediment GHG dynamics in oyster habitats across seasons (i.e., frequent N_2O consumption and the greater strength of the relationship between NO_x concentration and N_2O flux) suggests denitrification as the likely driver of sediment N_2O fluxes in oyster habitats.

In a broader management context, locating oyster restoration projects and oyster farms in areas with low dissolved nutrient concentrations may limit the total GHG production. Furthermore, there is substantial evidence for direct release of N_2O from oysters[26,27,55] and that rates of

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

oyster N_2O release can be predicted by the amount of dissolved inorganic nitrogen (DIN) in the water column, with N_2O consumption by oysters when no DIN is present.[26] Locating new oyster habitats in areas of low DIN may help to reduce total N_2O production and emissions from the system. Whether oysters release CH_4 is less clear,[13,27] and quantifying the controls on CH_4 fluxes associated with oysters is an important next step.

Updated Estimate of the Oyster Aquaculture GHG Footprint. Ray et al.[13] estimated that the GHG footprint of oyster aquaculture is 0.13 kg CO_2-eq kg protein^{-1}, with the major GHG emission (excluding equipment and post farm processing) being N_2O from the oyster itself, and no emissions from fodder production (as oysters feed on naturally occurring plankton and detritus), or sediment release. CO_2 respired by oysters was excluded from their estimate as this respired CO_2 is considered as a return of CO_2 fixed by phytoplankton during photosynthesis to the atmosphere.[56,57] Here, our results support previous findings that oysters have a negligible effect on sediment N_2O and CH_4 fluxes, but may lead to greater sediment CO_2 release depending on the oyster farm. It also appears that any change in sediment CO_2 flux is much more important than changes to CH_4 or N_2O flux in the context of CO_2-equivalents (Figure 3; we converted CH_4 and N_2O into CO_2-eq using global warming potentials of 25 and 298, respectively).

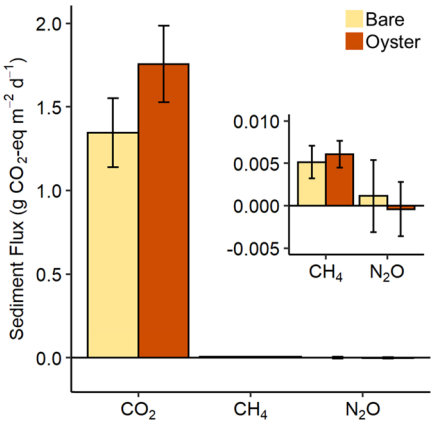

Figure 3. Relative contribution of carbon dioxide, methane, and nitrous oxide to the total greenhouse gas footprint (in terms of CO_2-equivalents) from bare sediments and those beneath oyster aquaculture in the summer. Error bars represent standard error.

There is still debate whether enhancement of sediment CO_2 release and respired CO_2 in shellfish culture systems should be considered as simply returning CO_2 fixed during photosynthesis to the atmosphere or included in the GHG footprint.[7,58−61] Furthermore, there is evidence that oyster reefs may create a C sink through burial and assimilation[60] and that the oyster shell may be used as a low GHG limestone substitute;[62,63] thus the role of shellfish in coastal C cycling must be considered in an ecosystem and full life-cycle context.[61]

Regardless of whether sediment CO_2 emissions should be included when calculating the GHG footprint of oyster aquaculture when sediment CO_2 release is stimulated, we can use our measured rates of sediment CO_2 flux to estimate what is likely an upper boundary for the GHG footprint of oyster aquaculture. The CO_2 fluxes from Ninigret Pond showed the greatest difference in mean CO_2 flux between bare sediments and that beneath aquaculture gear (Table 1), so we elected to use the difference in the mean flux between bare sediments and those beneath aquaculture gear for the scaling exercise. We base our calculation off that employed by Ray et al.[13] and make the following assumptions: oyster density at the farm is the same as that in the previous study (approximately 732.5 g dry tissue m^{-2}), the difference in sediment CO_2 release between bare sediment and that beneath aquaculture gear is constant throughout the year (815.16 μmol m^{-2} h^{-1}; Table 1), it takes 2 years for oysters to reach market size, oysters have a wet to dry tissue mass ratio of 4:1,[13] and oysters have 5.22 g protein per 100 g wet tissue mass.[64] When sediment CO_2 flux enhancement is included in this estimate, an additional 8.15 kg CO_2-eq kg protein^{-1} must be added to the previously estimated 0.13 kg CO_2-eq g protein^{-1}, yielding a new GHG footprint of oysters raised in an aquaculture of 8.28 kg CO_2-eq g protein^{-1}. While this is a substantial increase, it still leaves the GHG footprint of oyster aquaculture much smaller than similarly calculated GHG footprints for beef (465.5 kg CO_2-eq g protein^{-1}), pork (51.8 kg CO_2-eq g protein^{-1}), or poultry (39.5 kg CO_2-eq g protein^{-1}; excluding equipment and postfarm processing for all terrestrial livestock sources).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c05253.

Maps of sampling locations, linear regressions of sediment GHG fluxes and environmental variables, conditions at the beginning of incubations, data distributions and model parameters, and results of statistical tests comparing fluxes between seasons (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Nicholas E. Ray − *Department of Biology, Boston University, Boston, Massachusetts 02215, United States; Department of Environmental Science, Stockholm University, Stockholm 106 91, Sweden;* Present Address: Department of Ecology & Evolutionary Biology, Cornell University, Ithaca, New York, United States (N.E.R); orcid.org/0000-0002-1959-3120; Email: ner35@cornell.edu

Author

Robinson W. Fulweiler − *Department of Biology and Department of Earth and Environment, Boston University, Boston, Massachusetts 02215, United States;* orcid.org/0000-0003-0871-4246

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.est.1c05253

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by Rhode Island Sea Grant award to R.W.F. and Warren McLeod Research Fellowships from the Boston University Marine Program to N.E.R. We thank Jim Arnoux, Matt Griffin, and Christian Horn for letting us sample at their oyster farms. We are thankful to Alia Al-Haj, Claudia

Mazur, Emily Chua, Johnny Elguero, James Garner, Lucy Zipf, Isabel Sanchez-Viruet, Brendan Kelly, Melissa Hagy, Gretchen McCarthy, Julia Masterman, Claire MacGill, Victoria Momyer, and Emily Scott for assistance with field and laboratory work.

REFERENCES

(1) Beck, M. W.; Brumbaugh, R. D.; Airoldi, L.; Carranza, A.; Coen, L. D.; Crawford, C.; Defeo, O.; Edgar, G. J.; Hancock, B.; Kay, M. C.; Lenihan, H. S.; Luckenbach, M. W.; Toropova, C. L.; Zhang, G.; Guo, X. Oyster Reefs at Risk and Recommendations for Conservation, Restoration, and Management. *Bioscience* **2011**, *61*, 107−116.

(2) Zu Ermgassen, P. S. E.; Spalding, M. D.; Blake, B.; Coen, L. D.; Dumbauld, B.; Geiger, S.; Grabowski, J. H.; Grizzle, R.; Luckenbach, M.; McGraw, K.; Rodney, W.; Ruesink, J. L.; Powers, S. P.; Brumbaugh, R. Historical Ecology with Real Numbers: Past and Present Extent and Biomass of an Imperilled Estuarine Habitat. *Proc. R. Soc. B Biol. Sci.* **2012**, *279*, 3393−3400.

(3) FAO. The State of World Fisheries and Aquaculture 2020. *Sustainability in Action*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2020.

(4) Duarte, C. M.; Agusti, S.; Barbier, E.; Britten, G. L.; Castilla, J. C.; Gattuso, J.-P.; Fulweiler, R. W.; Hughes, T. P.; Knowlton, N.; Lovelock, C. E.; Lotze, H. K.; Predragovic, M.; Poloczanska, E.; Roberts, C.; Worm, B. Rebuilding Marine Life. *Nature* **2020**, *580*, 39−51.

(5) Coen, L. D.; Brumbaugh, R. D.; Bushek, D.; Grizzle, R.; Luckenbach, M. W.; Posey, M. H.; Powers, S. P.; Tolley, S. G. Ecosystem Services Related to Oyster Restoration. *Mar. Ecol. Prog. Ser.* **2007**, *341*, 303−307.

(6) National Marine Fisheries Service. *Fisheries of the United States, 2018*; 2020.

(7) Ray, N. E.; Fulweiler, R. W. Meta-Analysis of Oyster Impacts on Coastal Biogeochemistry. *Nat. Sustain.* **2021**, *4*, 261−269.

(8) Naylor, R. L.; Hardy, R. W.; Buschmann, A. H.; Bush, S. R.; Cao, L.; Klinger, D. H.; Little, D. C.; Lubchenco, J.; Shumway, S. E.; Troell, M. A 20-Year Retrospective Review of Global Aquaculture. *Nature* **2021**, *591*, 551−563.

(9) Abril, G.; Borges, A. V. Carbon Dioxide and Methane Emissions from Estuaries. In *Greenhouse Gas Emissions: Fluxes and Processes, Hydroelectric Reservoirs and Natural Environments*; Tremblay, A., Varfalvy, L., Roehm, C., Garneau, M., Eds.; Environmental Science Series, Springer: Berlin, Heidelberg, New York, 2004; pp. 187−207.

(10) Murray, R. H.; Erler, D. V.; Eyre, B. D. Nitrous Oxide Fluxes in Estuarine Environments: Response to Global Change. *Glob. Chang. Biol.* **2015**, *21*, 3219−3245.

(11) Ciais, P.; Sabine, C.; Bala, G.; Bopp, L.; Brovkin, V.; Canadell, J.; Chhabra, A.; DeFries, R.; Galloway, J.; Heimann, M.; Jones, C.; Le Quéré, C.; Myneni, R. B.; Piao, S.; Thornton, P. Carbon and Other Biogeochemical Cycles. In *Climate Change 2013: The Physical Science Basis. Contribution of Working Group 1 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*; Stocker, T., Qin, D., Plattner, G.-K., Tignor, M., Allen, S., Boschung, J., Nauels, A., Xia, Y., Bex, V., Midgley, P., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, 2013.

(12) Newell, R.; Fisher, T.; Holyoke, R.; Cornwell, J. Influence of Eastern Oysters on Nitrogen and Phosphorus Regeneration in Chesapeake Bay, USA. In *The Comparative Roles of Suspension-Feeders in Ecosystems*; Springer: Dordrecht, Netherlands, 2005; Vol. *47*, pp. 93−120.

(13) Ray, N. E.; Maguire, T. J.; al-Haj, A. N.; Henning, M.; Fulweiler, R. W. Low Greenhouse Gas Emissions from Oyster Aquaculture. *Environ. Sci. Technol.* **2019**, *53*, 9118−9127.

(14) Onorevole, K. M.; Thompson, S. P.; Piehler, M. F. Living Shorelines Enhance Nitrogen Removal Capacity over Time. *Ecol. Eng.* **2018**, *120*, 238−248.

(15) Erler, D. V.; Welsh, D. T.; Bennet, W. W.; Meziane, T.; Hubas, C.; Nizzoli, D.; Ferguson, A. J. P. The Impact of Suspended Oyster Farming on Nitrogen Cycling and Nitrous Oxide Production in a Sub-Tropical Australian Estuary. *Estuar. Coast. Shelf Sci.* **2017**, *192*, 117−127.

(16) Green, D. S.; Boots, B.; Crowe, T. P. Effects of Non-Indigenous Oysters on Microbial Diversity and Ecosystem Functioning. *PLoS One* **2012**, *7*, e48410.

(17) Green, D. S.; Rocha, C.; Crowe, T. P. Effects of Non-Indigenous Oysters on Ecosystem Processes Vary with Abundance and Context. *Ecosystems* **2013**, *16*, 881−893.

(18) Hu, Z.; Lee, J. W.; Chandran, K.; Kim, S.; Khanal, S. K. Nitrous Oxide (N_2O) Emission from Aquaculture: A Review. *Environ. Sci. Technol.* **2012**, *46*, 6470−6480.

(19) Therkildsen, M. S.; Lomstein, B. A. Seasonal Variation in Net Benthic C-Mineralization in a Shallow Estuary. *FEMS Microbiol. Ecol.* **1993**, *12*, 131−142.

(20) Middelburg, J. J.; Klaver, G.; Nieuwenhuize, J.; Wielemaker, A.; de Haas, W.; Vlug, T.; van der Nat, J. Organic Matter Mineralization in Intertidal Sediments along an Estuarine Gradient. *Mar. Ecol. Prog. Ser.* **1996**, *132*, 157−168.

(21) Bange, H. W.; Dahlke, S.; Ramesh, R.; Meyer-Reil, L.-A.; Rapsomanikis, S.; Andreae, M. O. Seasonal Study of Methane and Nitrous Oxide in the Coastal Waters of the Southern Baltic Sea. *Estuar. Coast. Shelf Sci* **1998**, *47*, 807−817.

(22) Kieskamp, W. M.; Lohse, L.; Epping, E.; Helder, W. Seasonal Variation in Denitrification Rates and Nitrous Oxide Fluxes in Intertidal Sediments of the Western Wadden Sea. *Mar. Ecol. Prog. Ser.* **1991**, *72*, 145−151.

(23) Jørgensen, B.; Sørensen, J. Seasonal Cycles of O_2, NO_3^- and SO_4^{2-} Reduction in Estuarine Sediments: The Significance of an NO_3^- Reduction Maximum in the Spring. *Mar. Ecol. Prog. Ser.* **1985**, *24*, 65−74.

(24) Seitzinger, S. P.; Nixon, S. W. Eutrophication and the Rate of Denitrification and N2O Production in Coastal Marine Sediments. *Limnol. Oceanogr.* **1985**, *30*, 1332−1339.

(25) Middelburg, J. J.; Klaver, G.; Nieuwenhuize, J.; Markusse, R. M.; Vlug, T.; van der Nat, F. J. W. A. Nitrous Oxide Emissions from Estuarine Intertidal Sediments. *Hydrobiologia* **1995**, *311*, 43−55.

(26) Gárate, M.; Moseman-Valtierra, S.; Moen, A. Potential Nitrous Oxide Production by Marine Shellfish in Response to Warming and Nutrient Enrichment. *Mar. Pollut. Bull.* **2019**, *146*, 236−246.

(27) McCarthy, G.; Ray, N. E.; Fulweiler, R. W. Greenhouse Gas Emissions from Native and Non-Native Oysters. *Front. Environ. Sci.* **2019**, *7*, 194.

(28) Jackson, M.; Owens, M. S.; Cornwell, J. C.; Kellogg, M. L. Comparison of Methods for Determining Biogeochemical Fluxes from a Restored Oyster Reef. *PLoS One* **2018**, *13*, No. e0209799.

(29) Nixon, S. W.; Kelly, J. R.; Furnas, B. N.; Oviatt, C. A.; Hale, S. S. Phosphorus Regeneration and the Metabolism of Coastal Bottom Communities. In *Marine benthic dynamics*; Tenore, K. R., Coull, B. C., Eds.; University of South Carolina Press: Columbia, South Carolina, 1980; pp. 219−242.

(30) Hougham, A. L.; Moran, S. B. Water Mass Ages of Coastal Ponds Estimated Using ^{223}Ra and ^{224}Ra as Tracers. *Mar. Chem.* **2007**, *105*, 194−207.

(31) Davis, J. Western Cape Cod Bay: Hydrographic, Geological, Ecological, and Meteorological Backgrounds for Environmental Studies. In *Observations on the ecology and biology of western Cape Cod Bay, Massachusetts*; Davis, J., Merriman, D., Eds.; Springer-Verlag: New York, 1984; pp. 1−18.

(32) Ray, N. E.; Fulweiler, R. W. Seasonal Patterns of Benthic-Pelagic Coupling in Oyster Habitats. *Mar. Ecol. Prog. Ser.* **2020**, *652*, 95−109.

(33) Foster, S. Q.; Fulweiler, R. W. Sediment Nitrous Oxide Fluxes Are Dominated by Uptake in a Temperate Estuary. *Front. Mar. Sci.* **2016**, *3*, 40.

(34) Kana, T. M.; Darkangelo, C.; Hunt, M. D.; Oldham, J. B.; Bennett, G. E.; Cornwell, J. C. Membrane Inlet Mass Spectrometer for Rapid High-Precision Determination of N_2, O_2, and Ar in Environmental Water Samples. *Anal. Chem.* **1994**, *66*, 4166−4170.

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233

(35) Weiss, R. F. The Solubility of Nitrogen, Oxygen and Argon in Water and Seawater. *Deep Sea Res. Oceanogr. Abstr.* **1970**, *17*, 721−735.
(36) Colt, J. *Computation of Dissolved Gas Concentrations in Water as Functions of Temperature, Salinity, and Pressure*; American Fisheries Society Special Publication: Bethesda, MD, 1984; Vol. *14*.
(37) Prairie, Y. T. Evaluating the Predictive Power of Regression Models. *Can. J. Fish. Aquat. Sci.* **1996**, *53*, 490−492.
(38) Williams, P. J.; del Giorgio, P. A. Respiration in Aquatic Systems: History and Background. In *Respiration in Aquatic Ecosystems*; del Giorgio, P. A., Williams, P. J., Eds.; Oxford University Press: New York, 2005; pp. 1−17.
(39) Hylén, A.; Taylor, D.; Kononets, M.; Lindegarth, M.; Stedt, A.; Bonaglia, S.; Bergström, P. In Situ Characterization of Benthic Fluxes and Denitrification Efficiency in a Newly Re-Established Mussel Farm. *Sci. Total Environ.* **2021**, *782*, No. 146853.
(40) Hopkinson, C. S.; Smith, E. Estuarine Respiration: An Overview of Benthic, Pelagic, and Whole System Respiration. In *Respiration in Aquatic Ecosystems*; del Giorgio, P. A., Williams, P. J., Eds.; Oxford University Press: New York, 2005.
(41) R Core Team. *R: A Language and Environment for Statistical Computing*; Vienna, Austria, 2014.
(42) Bates, D.; Maechler, M.; Bolker, B.; Walker, S. Fitting Linear Mixed-Effects Models Using *Lme4*. arXiv:1406.5823v1, 2014.
(43) Delignette-Muller, L. M.; Dutang, C. Fitdistrplus: An R Package for Fitting Fistributions. *J. Stat. Softw.* **2015**, *64*, 1−34.
(44) Lenth, R. *Emmeans: Estimated Marginal Means, Aka Least-Squares Means*. R Package Version 1.2.3, 2018.
(45) Wickham, H. *Ggplot2: Elegant Graphics for Data Analysis*; Springer-Verlag: New York, 2016.
(46) Smayda, T. J.; Borkman, D. Nutrient and Plankton Dynamics in Narragansett Bay. In *Science for Ecosystem Based Management*; Desbonnet, A., Costa-Pierce, B., Eds.; Springer: New York, 2008.
(47) Nixon, S. W.; Fulweiler, R. W.; Buckley, B. A.; Granger, S. L.; Nowicki, B. L.; Henry, K. M. The Impact of Changing Climate on Phenology, Productivity, and Benthic-Pelagic Coupling in Narragansett Bay. *Estuar. Coast. Shelf Sci.* **2009**, *82*, 1−18.
(48) Cornwell, J. C.; Kemp, W. M.; Kana, T. M. Denitrification in Coastal Ecosystems: Methods, Environmental Controls, and Ecosystem Level Controls, a Review. *Aquat. Ecol.* **1999**, *33*, 41−54.
(49) McAleer, C. *Environmental Factors Affecting Methane Cycling in Narragansett Bay*. University of Rhode Island, 2018.
(50) Ray, N. E.; Al-Haj, A.; Fulweiler, R. W. Sediment Biogeochemistry along an Oyster Aquaculture Chronosequence. *Mar. Ecol. Prog. Ser.* **2020**, *646*, 13−27.
(51) Melillo, J. M.; Frey, S. D.; DeAngelis, K. M.; Werner, W. J.; Bernard, M. J.; Bowles, F. P.; Pold, G.; Knorr, M. A.; Grandy, A. S. Long-Term Pattern and Magnitude of Soil Carbon Feedback to the Climate System in a Warming World. *Science* **2017**, *358*, 101−105.
(52) Tucker, J.; Giblin, A. E.; Hopkinson, C. S.; Kelsey, S. W.; Howes, B. L. Response of Benthic Metabolism and Nutrient Cycling to Reductions Inwastewater Loading to Boston Harbor, USA. *Estuar. Coast. Shelf Sci.* **2014**, *151*, 54−68.
(53) Murphy, A. E.; Kolkmeyer, R.; Song, B.; Anderson, I. C.; Bowen, J. Bioreactivity and Microbiome of Biodeposits from Filter-Feeding Bivalves. *Microb. Ecol.* **2019**, *77*, 343−357.
(54) Seitzinger, S. P.; Nixon, S. W.; Pilson, M. E. Q. Denitrification and Nitrous Oxide Production in a Coastal Marine Ecosystem. *Limnol. Oceanogr.* **1984**, *29*, 73−83.
(55) Ray, N. E.; Henning, M. C.; Fulweiler, R. W. Nitrogen and Phosphorus Cycling in the Digestive System and Shell Biofilm of the Eastern Oyster (*Crassostrea Virginica*). *Mar. Ecol. Prog. Ser.* **2019**, *621*, 95−105.
(56) *PAS 2050−2:2012 Assessment of Life Cycle Greenhouse Gas Emissions - Supplementary Requirements for the Application of PAS2050:2011 to Seafood and Other Aquatic Products*; 2012.
(57) *PAS 2050:2011 Specification for the Assessment of the Life Cycle Greenhouse Gas Emissions of Goods and Services*; 2011.
(58) Munari, C.; Rossetti, E.; Mistri, M. Shell Formation in Cultivated Bivalves Cannot Be Part of Carbon Trading Systems: A Study Case with *Mytilus Galloprovincialis*. *Mar. Environ. Res.* **2013**, *92*, 264−267.
(59) Ray, N. E.; O'Meara, T.; Wiliamson, T.; Izursa, J.-L. L.; Kangas, P. C. Consideration of Carbon Dioxide Release during Shell Production in LCA of Bivalves. *Int. J. Life Cycle Assess.* **2018**, *23*, 1042−1048.
(60) Fodrie, F. J.; Rodriguez, A. B.; Gittman, R. K.; Grabowski, J. H.; Lindquist, N. L.; Peterson, C. H.; Piehler, M. F.; Ridge, J. T. Oyster Reefs as Carbon Sources and Sinks. *Proc. R. Soc. B Biol. Sci.* **2017**, *284*, No. 20170891.
(61) Filgueira, R.; Byron, C. J.; Comeau, L. A.; Costa-Pierce, B.; Cranford, P. J.; Ferreira, J. G.; Grant, J.; Guyondet, T.; Jansen, H. M.; Landry, T.; McKindsey, C. W.; Petersen, J. K.; Reid, G. K.; Robinson, S. M. C.; Smaal, A.; Sonier, R.; Strand; Strohmeier, T. An Integrated Ecosystem Approach for Assessing the Potential Role of Cultivated Bivalve Shells as Part of the Carbon Trading System. *Mar. Ecol. Prog. Ser.* **2015**, *518*, 281−287.
(62) Her, S.; Park, T.; Zalnezhad, E.; Bae, S. Synthesis and Characterization of Cement Clinker Using Recycled Pulverized Oyster and Scallop Shell as Limestone Substitutes. *J. Cleaner Prod.* **2021**, *278*, No. 123987.
(63) Alvarenga, R. A. F. .; Galindro, B. M.; Helpa, C. . F.; Soares, S. R. The Recycling of Oyster Shells: An Environmental Analysis Using Life Cycle Assessment. *J. Environ. Manage.* **2012**, *106*, 102−109.
(64) ARS. *USDA National Nutrient Database for Standard Reference, Legacy*; Agricultural Research Service (ARS): Beltsville, Maryland, 2018.

https://doi.org/10.1021/acs.est.1c05253
Environ. Sci. Technol. 2021, 55, 14225−14233