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Low Greenhouse Gas Emissions from Oyster Aquaculture

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Supporting Information

ABSTRACT: Production of animal protein is associated with high greenhouse gas (GHG) emissions. Globally, oyster aquaculture is increasing as a way to meet growing demands for protein, yet its associated GHG-emissions are largely unknown. We quantified oyster aquaculture GHG-emissions from the three main constituents of GHG-release associated with terrestrial livestock production: fermentation in the animal gut, manure management, and fodder production. We found that oysters release no methane (CH_4) and only negligible amounts of nitrous oxide (0.00012 ± 0.00004 μmol N_2O gDW^{-1} hr^{-1}) and carbon dioxide (3.556 ± 0.471 μmol CO_2 gDW^{-1} hr^{-1}). Further, sediment fluxes of N_2O and CH_4 were unchanged in the presence of oyster aquaculture, regardless of the length of time it had been in place. Sediment CO_2 release was slightly stimulated between 4 and 6 years of aquaculture presence and then returned to baseline levels but was not significantly different between aquaculture and a control site when all ages of culture were pooled. There is no GHG-release from oyster fodder production. Considering the main drivers of GHG-release in terrestrial livestock systems, oyster aquaculture has less than 0.5% of the GHG-cost of beef, small ruminants, pork, and poultry in terms of CO_2-equivalents per kg protein, suggesting that shellfish aquaculture may provide a a low GHG alternative for future animal protein production compared to land based sources. We estimate that if 10% of the protein from beef consumption in the United States was replaced with protein from oysters, the GHG savings would be equivalent to 10.8 million fewer cars on the road.

INTRODUCTION

The production of animal protein for human consumption has associated greenhouse gas (GHG) costs, which contribute to global climate change. Land-based livestock production releases 7.1 Gt CO_2-equivalent (CO_2-eq) each year, accounting for almost 15% of annual global anthropogenic GHG-emissions.[1] As human population increases and countries gain more wealth, the demand for animal-based protein rises.[2,3] In addition to the high GHG-cost of land-based livestock production, large tracts of arable land are required,[4] with limited land remaining that can be used to increase yield. These two problems demonstrate a critical need for alternate animal protein sources. Shellfish aquaculture has the potential to help fill this void.

In contrast to land-based livestock production, shellfish aquaculture species do not require arable land or the crops grown on it, and recent estimates suggest that only a small portion of the ocean is necessary to produce large quantities of food.[5,6] Global aquaculture production is increasing rapidly, with harvest of fish for human consumption from aquaculture surpassing that from wild harvest in 2014.[7] While finfish aquaculture has been associated with negative environmental impacts and high feed costs,[8−13] shellfish aquaculture might provide a more environmentally friendly option. The shellfish aquaculture industry is particularly well suited to be a major source of protein for human consumption as shellfish can be grown to market size in a relatively short time period, are naturally evolved to live in dense populations, and do not require the addition of cultivated food for growth. The GHG-emissions from shellfish aquaculture production systems, however, remain largely unquantified. Current estimates of shellfish aquaculture GHG release are from modeling studies,[14] back of the envelope calculations,[15] and Life Cycle Assessments (LCA),[16,17] with only a few studies reporting actually measured rates.[18−20]

The majority (94%) of GHGs released from terrestrial livestock farming come from the clearing of land and application of fertilizer to produce fodder (45%), enteric fermentation in the animal's gut (39%), and manure management (10%).[1] From

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these processes, carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) make up 27%, 44%, and 53% of GHG-emissions, respectively.[1] Each kg of CH_4 contributes 25 times the global warming equivalent of one kg of CO_2, and each kg of N_2O contributes 298 times the global warming equivalent of one kg of CO_2. A majority of the CO_2 emissions associated with livestock production are generated from land use change and the combustion of fossil fuels used to grow and transport feed crops and to cool, heat, and ventilate animal production facilities. CO_2 respired by livestock and aquaculture species is often not considered in GHG-assessments as it is considered as a return of carbon (C) fixed during photosynthesis to the atmosphere.[21,22] The majority of CH_4 is produced during enteric fermentation by ruminants, and most N_2O is released during the application of fertilizer for fodder production and livestock manure management.

These same processes are likely the major contributors to GHG-emissions from aquaculture systems, though there are fewer studies of GHG-release from aquaculture production systems, and most are LCA. While aquaculture LCA includes the GHG-impact of fodder production, farm equipment, and post farm-gate emissions, it typically does not include the GHG release from enteric fermentation and manure management, despite evidence that they are significant.[14] In this study, we directly measure the CO_2, CH_4, and N_2O released from fodder production, enteric fermentation, and manure management associated with aquaculture production of the Eastern oyster (*Crassostrea virginica*). We omit emissions associated with farm equipment and postfarm gate, as they are likely similar to those from other animal protein sources.

Bivalve aquaculture harvest made up almost 60% of total global marine and coastal aquaculture harvest in 2014, with China as the main producer.[23] While the harvest of oysters raised in culture in the United States is less than 0.3% of the global total, this harvest has increased by 37% between 2011 and 2016 and is the most rapidly growing marine aquaculture sector.[24] Culture practices are relatively similar across continents, with oysters held in cages suspended above the sediment or placed directly on the sediment.

Unlike livestock production systems, there is no GHG-release from growing or transporting fodder for oyster aquaculture as oysters do not rely on feed inputs from the farmer, instead filtering their food directly from the water. Thus, GHG-emissions from oyster aquaculture are driven by the oysters themselves and from microbial metabolism in sediment influenced by deposition of oyster feces and pseudofeces or "manure".

Oysters release CO_2 during respiration and as a product of shell calcification. Similar to other aquatic macrofauna,[25] N_2O can be produced in oyster guts, which harbor denitrifying microbes,[20,26] and in the oyster shell biofilm as a byproduct of nitrification of ammonium excreted by the oyster.[27−29] GHG-emissions from oyster aquaculture may also be associated with organic matter deposition to sediments via the production of feces and pseudofeces. Deposition of organic matter to sediments is well-known for stimulating decomposition, including rates of denitrification,[30−32] thereby likely increasing sediment CO_2 and N_2O release. Whether oysters and the influence of oysters on sediment biogeochemical processes are net sources or sinks of CH_4, until now, remains unknown.

To determine the amount of GHG release associated with oyster aquaculture, we paired field and laboratory incubations to quantify fluxes of CH_4 and N_2O from the sediments beneath oyster aquaculture gear and from market sized oysters raised in culture. We estimated CO_2 production from these two sources by measuring the oxygen flux of the incubation and applying an CO_2/O_2 respiratory quotient of 1:1 for sediment and 0.833:1 for oysters.[33] We quantified GHG-fluxes in the laboratory from the oyster itself. In the field, we measured in situ fluxes to assess the impact of oyster "manure" on sediment GHG-production. Additionally, we tested how sediment GHG-fluxes changed over the course of seven years following installation of the farm using a chronosequence approach. We then compared the results generated in this study with estimates of GHG-release from published land-based livestock production on a kg CO_2-eq kg protein^{-1} basis. We did not quantify GHG-release from the production of supplies used on the oyster farm, or product storage and distribution, and excluded these variables in any of our comparisons with other animal protein sources.

MATERIALS AND METHODS

Field Site Description. We collected oysters for laboratory incubation and determined sediment GHG-flux in situ at a 0.016 km^2 commercial oyster farm in Ninigret Pond, Rhode Island, U.S.A. (41.3576° N, −71.6534° E). Ninigret Pond is one of several shallow, microtidal coastal lagoons on the Atlantic Coast of Rhode Island. These coastal lagoons, formed by eroded glacial deposits in a barrier-beach system, extend from Narragansett Bay (Rhode Island) to Block Island Sound (New York) through tidal inlets.[34] Water residence time for Ninigret Pond is approximately 10 days.[35] Nitrogen (N) from groundwater makes up 6% of the daily N load[36] and has declined since the 1980s, when it was estimated to make up ∼80% of the total N load to the lagoon.[37] The oyster farm is located on the south side of the lagoon adjacent to the barrier spit that separates the lagoon from Block Island Sound. Mean water depth at the oyster farm is ∼1m. The farm uses rack and bag aquaculture, a culture method in which oysters are held in plastic mesh bags attached to PVC racks 10−20 cm above the sediment surface.

Sediment sampling sites were selected based on age and location within the farm after discussion with the farm owner. We sampled three sites within the farm that had been in use for 2, 4, and 6 yrs as of summer 2014 (in summer 2015, these sites became 3, 5, and 7 yrs old respectively). A control site was selected ∼10 m upstream of the farm. Sampling various ages of aquaculture within a farm allowed us to isolate the effect of farm age. Aquaculture activities continued unobstructed during data collection.

A HOBO Dissolved Oxygen Data logger (Onset Computer Corporation, Bourne, MA) was deployed adjacent to the study site to record dissolved oxygen (DO) every 15 min, and HOBO Pendant data loggers were deployed to record ambient air and water temperature every 15 min. Salinity was recorded during in situ incubations using a Hach HQd equipped with a CDC401 probe (Table S1).

Oyster GHG-Fluxes: Laboratory Incubations. Three laboratory incubations measured oyster GHG-fluxes on three separate occasions in the summer of 2015 (July 23, August 3, and August 12). Market size oysters (∼7.62 cm length)[38] were immediately transported from the farm to an environmentally controlled room at Boston University and kept in the dark at 24 °C. Site water was collected in 20 L carboys and transported to the lab for incubations. Within 24−48 h of oyster collection, each oyster was weighed and their length, width, and depth of cup was recorded. Following incubation, oysters were shucked, and the shell and tissue were separated. We recorded the wet

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weight of the shell and tissue and then placed both in a 60 °C drying oven until there was less than a 0.1% reduction in mass over a 24 h period, at which point dry shell and tissue mass were determined.

For each laboratory incubation, we used three replicate incubation chambers containing four oysters (n = 9 chambers and 36 oysters across the three incubations). We also incubated three chambers containing only site water to account for any production or consumption of GHGs in the water column and any methodological artifacts. The chambers were constructed of clear PVC tubes (28 cm height, 2.15 L volume). The bottom of each chamber was sealed using a PVC base with a rubber O-ring. Incubations began by filling six incubation chambers with site water and haphazardly distributing 12 oysters between three chambers. We attempted to minimize variation in oyster size between incubation chambers by selecting for similar total oyster mass per chamber. The chambers were then placed in a 24 °C water bath and capped with gastight acrylic lids fitted with inflow and outflow ports. Magnetic stir bars attached to the lid kept the water evenly mixed (~40 revolutions min^{-1}).

During incubation chamber sampling, site-water was gravity-fed from a carboy through the inflow tube into the incubation chamber, allowing sample water to be pushed from the chamber through the outflow tube. Water samples were collected in duplicate in 12 mL Labco Limited Exetainer vials with gastight septa over five time points and analyzed for N_2O and CH_4 in order to calculate gas flux values as change in concentration over time. Vials were filled from the bottom up and allowed to overflow before 25 μL of saturated zinc chloride solution (ZnCl) was added as a preservative. Samples were capped immediately following preservation to prevent atmospheric contamination and stored at 4 °C until analysis.

We measured the dissolved oxygen (DO) concentration of the chamber water immediately before and after the incubation using a Hach LDO101 DO sensor. Incubations were timed to allow for the longest length of time between sampling points while also allowing the dissolved oxygen (DO) level of the water inside the incubation chamber to fall at least 2 mg L^{-1} from the start of the incubation but not below 2.0 mg L^{-1}.[30,36] If the incubation chambers become hypoxic (DO < 2.0 mg L^{-1}), dominant microbial processes change, impacting the observed gas fluxes.

Sediment GHG-Fluxes: In situ Incubations. Sediment GHG-fluxes were determined in the summers of 2014 and 2015 using an in situ approach,[39] similar to the laboratory incubations described previously. Our in situ method involved the installation of permanent bases in the sediment to which we attached incubation chambers for sampling events.[39] We installed three chamber bases at a control site and nine chamber bases beneath oyster aquaculture gear (three rings at each of three locations within the farm) throughout the farm on June 5, 2014. We installed bases by removing sediment with a shovel, placing the base in the space created, and then filling the base with the removed sediment. Sampling began on June 30, 2014, more than 3 weeks after installation, allowing for the disturbed sediment to return to equilibrium conditions.[40] Chamber bases at the control site were initially installed in water too shallow to allow for a watertight seal when the chamber was attached, so they were moved to deeper water. This led to no samples being collected from the control site in July 2014.

During sampling events, HOBO Pendant light and temperature loggers set to record every minute were installed in each chamber immediately prior to attaching the shaded chamber lid to ensure that light and temperature within the chamber remained constant throughout the incubation. Cordless drills were used to spin stirrer bars within the chambers at ~40 rpm to maintain even mixing. Site-water was gravity-fed from an insulated carboy hung from a tripod through an inflow tube into the incubation chamber, and sample collection for each N_2O and CH_4 proceeded in the same way as described in the laboratory incubations.

GHG-Sample Analysis. We determined N_2O and CH_4 fluxes using a headspace equilibration technique, followed by analysis of the headspace using a gas chromatograph (GC).[41] Headspace equilibration began by injecting 5 mL of high purity helium (UHP He) into each sample exetainer vial while simultaneously removing 5 mL of liquid sample. Vials were then shaken and allowed to equilibrate for at least 1 h. After equilibration, 4 mL of headspace was removed from the sample vial and injected into a Shimadzu GC-2014 gas chromatograph equipped with a flame ionization detector for CH_4 and an electron capture detector (ECD) with a ^{63}Ni source for N_2O. The columns contained HayeSep and Shimalite. N_2 gas was used as the carrier gas, and p5 (5% CH_4, 95% Ar) was used as the ECD makeup gas.

We determined concentrations of N_2O and CH_4 by comparing sample peak area against a standard curve of the peak areas of different concentrations of an externally mixed standard (Airgas, Billerica MA). The externally mixed standard consisted of 5000 ppb CH_4 and 500 ppb N_2O in N_2. The standard curve was calculated using the following ratios of standard to UHP He: 5:0, 4:1, 3:2, 2:3, 1:4, and 0:5. N_2O and CH_4 standard curves all had $R^2 > 0.995$.

Flux Calculations. A linear regression of GHG-concentration over time was used to calculate flux rate of N_2O and CH_4. The slope of the calculated regression line (μM hr^{-1}) was then converted to a sediment flux rate (μmol m^{-2} hr^{-1}) or g dry oyster weight (μmol g^{-1} hr^{-1}) flux rate by equations 1 or 2

$$\text{Flux Rate}\left(\frac{\mu\text{mol}}{\text{m}^2 \times \text{hr}}\right) = \frac{\text{Slope of Regression}\left(\frac{\mu\text{ mol}}{\text{L} \times \text{hr}}\right) \times \text{Chamber Vol(L)}}{\text{Sediment Area}(\text{m}^2)} \quad (1)$$

$$\text{Flux Rate}\left(\frac{\mu\text{mol}}{\text{g} \times \text{hr}}\right) = \frac{\text{Slope of Regression}\left(\frac{\mu\text{ mol}}{\text{L} \times \text{hr}}\right) \times \text{Chamber Vol(L)}}{\text{Oyster Dry Tissue Mass}(\text{g})} \quad (2)$$

Flux rates were only considered significant when $R^2 \geq 0.65$ and $p \leq 0.10$.[39,41,42] Regressions with $R^2 < 0.65$ were considered to exhibit no flux and were assigned a value of 0 if the GHG-concentration did not change.[41] All flux measurements made in this study exhibited a linear change in GHG-concentration over time.

O_2 flux was estimated as the difference in O_2 concentrations between the beginning and end of the incubation. We converted measured O_2 fluxes to CO_2 flux assuming a respiratory quotient of $1CO_2/0.833{:}1O_2$ for oysters[33] and a respiratory quotient of $1CO_2/1O_2$ for the sediment.[43]

Statistical Analysis. All statistical analysis was conducted in R version 3.3.2. We considered all the results of all statistical tests to be significant when $p < 0.05$. We tested for significant release

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of N_2O, CO_2, and CH_4 from oysters by comparing whether the mean oyster flux of each GHG was different from zero using two-tailed, one-sample Wilcoxon signed rank tests. For CO_2 fluxes, we converted the observed O_2 flux to a CO_2 flux prior to analysis.

To test whether sediment beneath oysters exchanges GHGs with the water column at rates different than from bare sediment, we used a mixed model approach. To begin, we transformed our data in order to best meet the assumptions of mixed models.[44] N_2O flux data was first mirrored around zero, by multiplying flux values by −1, and then shifted so all values were positive by adding one plus the value of the most negative N_2O flux. We then applied a square root transformation to the mirrored and shifted N_2O data. The CH_4 flux data were shifted and transformed in the same way. CO_2 fluxes were log normally distributed, so we applied a log transformation. We then tested whether the transformed flux data best fit a normal, log-normal, or gamma distribution using the *fitdistrplus* package[45] and found that transformed N_2O and CO_2 fluxes best fit a normal distribution, while transformed CH_4 fluxes best fit a log-normal distribution.

We generated multiple generalized linear models (GLMs) and generalized linear mixed models (GLMMs) using the *lme4* package.[46] We treated the presence or absence of aquaculture as a fixed effect in all models and sequentially added all possible combinations of temperature and salinity as fixed effects and incubation chamber ID as a random effect. Eight total models were constructed for fluxes of each GHG. We compared and selected the model that best represented the data via lowest Akaike information criterion (AIC; Table S2)[47] and then compared the best model with the second best using likelihood ratio tests. In cases where the best model was not significantly different from the second best, we elected to use the model with the fewest variables.

The best model to describe sediment fluxes of N_2O and CH_4 was the presence or absence of oyster aquaculture alone (Table S2). For N_2O, the model with the lowest AIC score was significantly better than the next lowest score (likelihood ratio test $\chi^2 = 5.619$, $p = 0.018$). The second best CH_4 model added salinity as a fixed effect and was not significantly better or worse than the model with just presence or absence of aquaculture (likelihood ratio test $\chi^2 = 0.819$, $p = 0.366$). AIC indicated that sediment CO_2 fluxes were best described by the presence or absence of oyster aquaculture and temperature as fixed effects, though this model was not significantly different from the model with just the presence or absence of culture as a fixed effect (likelihood ratio test $\chi^2 = 3.73$, $p = 0.053$), thus we elected to use the simplest model. We then compared for significance between fluxes when oyster aquaculture was present or absent using pairwise least-squares means tests using the *emmeans* package.[48]

To determine whether fluxes varied with the length of time aquaculture gear had been in place, we repeated the process described for comparing sites with and without culture, substituting site age for the presence or absence of culture in the models. When comparing fluxes based on the length of time aquaculture gear had been in place, the simplest model was the best for describing fluxes of N_2O (likelihood ratio test $\chi^2 = 0.209$, $p = 0.648$) and CH_4 (likelihood ratio test $\chi^2 = 0.072$, $p = 0.788$; Table S3). Sediment CO_2 release was best described using the temperature and salinity of the overlying water in addition to the length of time aquaculture gear had been in place and was significantly better than the next best model via AIC (likelihood ratio test $\chi^2 = 35.203$, $p < 0.001$).

Comparison of Oyster GHG-Emissions to Livestock GHG-Emissions. We compared the measurements of oyster GHG-release and sediment GHG-release from this study to terrestrial livestock using values from two reports published by the Food and Agriculture Organization of the United Nations.[49,50] We elected to use these reports as they provided global average GHG-emissions values for terrestrial livestock, and both used the same methods. We converted the reported total $kgCO_2$-eq into N_2O, CH_4, and CO_2 components of the livestock studies and excluded all emissions from direct and indirect energy use, postfarm emissions, and those emissions labeled as "other" as we did not estimate these emissions from oyster culture in our study (SI Text 1). The total emissions for beef and small ruminants (sheep and goats) from the previous study were reported as kg CO_2-eq kg carcass^{-1}. We converted these values to kg CO_2-eq kg protein^{-1} by first converting the carcass weight to meat using a conversion factor of 0.75 for beef and 0.70 for small ruminants[49] and then dividing this value by the percent protein of the product using values from the USDA Food Composition Database (basic Report 13047 for beef, 17224 for lamb, and 15245 for oysters).[51] For beef, we used the USDA reported 19.42 g protein in every 100 g of beef product and 16.56 g protein per 100 g lamb for the small ruminants. GHG-emissions for pork and poultry were reported in kg CO_2-eq kg protein^{-1}.[50]

To estimate the release of GHGs by oysters, we used the rates measured in this study. We excluded the CO_2 respired from the oysters, as this value is not considered when determining GHG-emissions in livestock production. Oysters did not produce or consume CH_4 and sediment GHG-fluxes were unchanged in the presence of oyster aquaculture and were therefore not included in our estimate. Since we did not assess the relationship between oyster size and N_2O release, we took a conservative approach and assumed that all oysters release N_2O at the same rate (0.00012 μmol N_2O gDW^{-1} hr^{-1}), regardless of size. We also assumed that the rates we measured in summer would hold year round, though they are likely highest in summer during high temperatures when the metabolism of the oyster and associated biofilms is most rapid. We multiplied this emission rate by the length of time it takes to raise an oyster to market size (approximately 2 years, or 17376 h) and divided by a ratio of wet to dry tissue of 4:1 (using data derived from this study). We then divided by an oyster protein content of 5.22 g protein per 100 g wet oyster tissue and multiplied by the atomic mass of N_2O (44.1 g mol^{-1}) and its global warming potential of 298 kg CO_2 kg^{-1} N_2O (eq 3).

$$\frac{\text{kgCO}_2\text{-eq}}{\text{kg protein}} = \frac{1.2 \times 10^{-10}\text{molN}_2\text{O} \times 17376\ \text{hr} \times 1\ \text{gDW} \times 100\ \text{gWW} \times 44.01\ \text{gN}_2\text{O} \times 298\ \text{kgCO}_2\text{-eq}}{\text{gDW} \times \text{hr} \times 4\ \text{gWW} \times 5.22\ \text{gprotein} \times \text{molN}_2\text{O} \times \text{kgN}_2\text{O}} \quad (3)$$

Data Availability. The oyster and sediment flux data that support the findings of this study are available in the figshare repository, with the identifier [https://doi.org/10.6084/m9.figshare.8343851].

RESULTS AND DISCUSSION

Emissions of GHGs from Oysters. We found that oysters release N_2O and CO_2 but not CH_4 (Table 1). N_2O release from oysters (0.00012 μmol N_2O gDW^{-1} hr^{-1} or 0.00035 μmol N_2O

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Table 1. Greenhouse Gas Release from Eastern Oysters (*Crassostrea virginica*, $n = 9$), and Results of Two-Tailed, One-Sample Wilcoxon Signed Rank Tests[a]

Gas	Median Flux (μmol gDW^{-1} hr^{-1})	Median Absolute Deviation	Mean Flux ± Standard Error (μmol gDW^{-1} hr^{-1})	*V*-statistic	*p*-value
CO_2	3.146	1.021	3.556 ± 0.471	45	**0.004**
CH_4	0	0.00032	0.00038 ± 0.00046	9	0.787
N_2O	0.00009	0.00012	0.00012 ± 0.00004	45	**0.004**

[a]Where the *V*-statistic is the sum of all positive ranks and the *p*-value indicates whether release of the gas proceeds at rates significantly different from zero.

indiv^{-1} hr^{-1}) is similar to the rate reported for the Sydney Rock Oyster (*Saccostrea glomerata*; 0.00078 μmol N_2O indiv^{-1} hr^{-1})[20] and considerably less than rates reported for blue mussels (*Mytlius edulis*; 0.012 μmol N_2O gDW^{-1} hr^{-1}),[25] zebra mussels (*Dreissena polymorpha*; 0.015 μmol N_2O gDW^{-1} hr^{-1}),[25] and manila clams (*Ruditapes philippinarum*; 11.5 μmol N_2O gDW^{-1} hr^{-1}).[19] This comparison suggests that N_2O release may be specific to different bivalve genera, possibly driven by differences in gut retention time, shell surface area for biofilms, or the microbial community inhabiting the gut and shell biofilm. In this study, we measured net GHG-fluxes, so it remains unclear whether oysters simply do not produce CH_4 or if any CH_4 they do produce is used by methanotrophs living in the shell biofilm or inhabiting their gills.

Oysters respired CO_2 at a rate of 3.56 μmol CO_2 gDW^{-1} hr^{-1}, within the range of previously reported rates of respiration.[33,52] This estimate does not include the CO_2 released during oyster shell formation which accounts for an additional 4.26 μmol CO_2 gDW^{-1} hr^{-1} (Supporting Information Text 2), more than doubling the total CO_2 release.

Emissions of GHGs from Sediment Beneath Oyster Aquaculture. Oyster aquaculture did not impact sediment GHG-emissions. Both bare sediments and those beneath oyster aquaculture cages consumed N_2O and released CO_2 and CH_4 (Figure 1). For each GHG, the best model to describe sediment GHG flux only included the presence or absence of oyster aquaculture, yet differences between GHG-fluxes from oyster aquaculture sediment and non-aquaculture (i.e., control or bare) sediments were not significant (N_2O, $t = 0.099$, $p = 0.922$, residual $df = 52$; CO_2, $t = 1.429$, $p = 0.159$, residual $df = 54$; CH_4, $t = 0.961$, $p = 0.341$, residual $df = 52$). N_2O and CH_4 fluxes did not change with the length of time for which the aquaculture gear had been in place (Table S4). We did record a slight, but significant, stimulation of sediment CO_2 release when aquaculture was in place for more than three years and then a return to baseline conditions after six years (Figure 1F; Table S4).

Net uptake of N_2O by sediment at both the control site (−0.288 ± 0.163 μmol m^{-2} hr^{-1}) and beneath oyster aquaculture (−0.229 ± 0.055 μmol m^{-2} hr^{-1}) follows the pattern of sediment N_2O uptake in nearby estuaries.[41,53] The only other study we could locate that measured sediment N_2O flux at an oyster farm demonstrated that sediment switched from being a net source of N_2O to a net sink beneath oyster aquaculture.[20] Additionally, molecular evidence in other systems shows no change in the expression of the genes used in denitrification (*nirS*, *nirK*) or nitrification (*amoA*) in sediment beneath oyster aquaculture relative to bare sediment,[54,55] indicating oyster aquaculture does not promote sediment N_2O release. As sediment N_2O release is often considered a consequence of inefficient nitrification and denitrification processes,[56] it appears as if oyster aquaculture either does not decrease the efficiency of either process or may even increase denitrification efficiency, leading to N_2O scavenging.This hypothesis of N_2O scavenging in sediments beneath oyster aquaculture is supported by Erler et al.[57] who demonstrated sediment production of N_2O using isotope tracers, yet still measured net N_2O uptake by sediment, demonstrating that oyster aquaculture may make the sediment N cycle more tightly coupled.

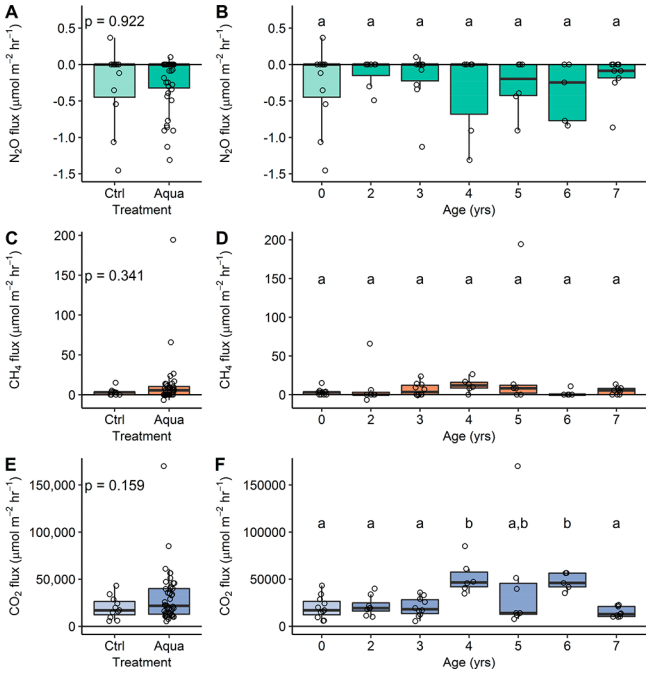

Figure 1. Nitrous oxide (N_2O; A, B), methane (CH_4; C, D), and carbon dioxide (CO_2; E, F) fluxes from sediment beneath oyster aquaculture gear (Aqua, $n = 44$ for N_2O and CH_4, $n = 46$ for CO_2) and control sites (Ctrl, $n = 11$ for all fluxes) and over varying lengths of time (years) aquaculture gear has been in place. The *p*-values indicate the results of least-squares mean tests comparing fluxes from bare sediment and sediment beneath oyster aquaculture (parts A, C, and E), while groups with the same letter (parts B, D, and F) are not statistically different from one another. Individual points represent a single flux measurement.

We observed no significant difference in sediment CO_2 release between bare sediment (19922.33 ± 3536.63 μmol m^{-2} hr^{-1}) and beneath oyster aquaculture (30468.23 ± 4102.60 μmol m^{-2} hr^{-1}). There is disagreement between and within previous studies whether sediment oxygen demand (and thus CO_2 efflux) below oyster aquaculture is affected[20,54,58] or unaffected.[39,40,59,60] Here, we demonstrated that the length of time for which the aquaculture gear has been in place can influence sediment CO_2 release, but this impact is temporary and disappears in time, which could explain the variation in results between past studies. It is likely that the rate of stimulation and return to baseline conditions varies between systems, and the best method to minimize sediment CO_2 release beneath oyster aquaculture is to keep gear in one place following establishment of the oyster farm. While we cannot conclusively explain why sediment CO_2 release was only temporarily stimulated, it is possible that aerobic decomposition dominated in the sediment during the early stages of aquaculture presence,

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and then anaerobic decomposition pathways became more prevalent.

In coastal systems, such as those where oyster aquaculture dominates, it is possible that much of the organic matter moved to the sediment beneath the oyster cages is used in anaerobic decomposition (e.g., denitrification, methanogenesis) instead of aerobic decomposition, limiting the release of CO_2 from aerobic decomposition. Thus, it is likely that we underestimated the total CO_2 production from both the aquaculture and non-aquaculture sediments as we measured sediment O_2 flux and used a respiratory quotient of $1CO_2/1CO_2$. However, we do not expect that the inclusion of CO_2 produced during anaerobic respiration would yield a significant difference in the flux of CO_2 from sediment beneath oyster aquaculture and non-aquaculture sediment, as the anaerobic processes we measured (denitrification via N_2O flux and methanogenesis via CH_4 flux) were not different between the treatments, indicative of similar rates of anaerobic respiration and thus CO_2 production.

To our knowledge, this is the first study to compare CH_4 release from bare sediment and sediment beneath oyster aquaculture gear. The CH_4 fluxes we measured from sediment beneath oyster aquaculture (11.31 ± 4.70 μmol m^{-2} CH_4 hr^{-1}) were higher than those from bare sediment (3.29 ± 1.30 μmol CH_4 m^{-2} hr^{-1}) but not statistically significantly so. Hou et al.[61] suggested that release of CH_4 from the sediment was greater beneath oyster aquaculture than a polyculture of an unspecified bivalve and kelp, but no comparison was made with bare sediment. Bonaglia et al.[62] added Baltic clams (*Limecola balthica*) to sediment and recorded increased sediment CH_4 efflux, though their results cannot be applied to an aquaculture facility as they effectively created a short-term perturbation experiment. In our study, sediment CH_4 release followed a similar pattern over the length of time for which aquaculture gear had been in place, with slight—but not statistically significant—stimulation between years 3 and 5. This pattern suggests an initial increase in the activity of methanogens, leading to CH_4 release, followed by an increase in methanotroph activity, leading to a return to near baseline conditions.

In our study, we measured sediment GHG fluxes in an aquaculture system where oysters are suspended in bags and physically removed from the sediment. Some oyster farmers plant their oysters directly on the sediment, which could potentially yield changes in sediment GHG release. There are few studies in which both oysters and sediments have been incubated together,[63,64] and we could only locate one that measured the flux of a GHG between the sediment and water column.[64] In that study, Jackson et al.[64] found that dissolved inorganic carbon (DIC; which includes CO_2) flux in chambers with living oysters and sediment was driven by the oysters themselves. Future studies that measure GHG release in the field with oysters and sediment in the same chamber will help to better refine estimates of GHG release from oyster culture.

Comparison with Terrestrial Livestock Protein Production. We compared the data generated in this study with literature values of N_2O, CH_4, and CO_2 releases from livestock production (SI Text 1) as protein from terrestrial livestock is the biggest contributor to protein in diets in the United States.[49,50] We estimated that oysters release a total 0.13 kg CO_2-eq kg $protein^{-1}$ (Figure 2). This value is orders of magnitude less than the GHG-cost of protein from beef (465.5 kg CO_2-eq kg $protein^{-1}$), small ruminants (203.1.0 kg CO_2-eq kg $protein^{-1}$), pork (51.8 kg CO_2-eq kg $protein^{-1}$), or poultry (39.5 kg CO_2-eq kg $protein^{-1}$) (Figure 2). N_2O release from oysters made up

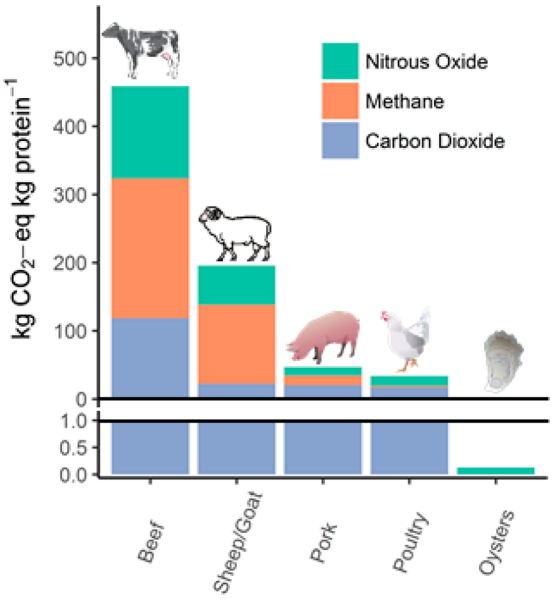

Figure 2. Estimated greenhouse gas release for oysters and terrestrial livestock products from fodder production, enteric fermentation, and manure management, which together make up 94% of the greenhouse gas release from terrestrial livestock systems. Oyster values were quantified in this study; beef and small ruminant values are from Opio et al. (2013), and values for pork and poultry are from MacLeod et al. (2013). Animal symbols on the figure are courtesy of the Integration and Application Network, University of Maryland Center for Environmental Science (ian.umces.edu/symbols/).

100% of the oyster GHG-cost, as sediment GHG-fluxes were unchanged, and there is no GHG-cost associated with production of feed for oysters raised in culture. Despite CO_2 respiration at significant rates, these emissions were excluded from our comparison as CO_2 from animal respiration is not included in estimates of livestock GHG-release.[21,22] Additionally, we elected to ignore CO_2 release and sequestration during shell formation as there is still debate as to whether these should be included, and knowledge of the future use of the shell is needed for accurate assessment.[18,65,66] Environmental conditions at the oyster farm can also regulate the ratio of C sequestration and release, with greater release in warmer, less saline waters with lower pH.[66] In this study, if CO_2 from shell formation was included, total emissions increase by 15.59 kg CO_2-eq kg $protein^{-1}$ to a total of 15.72 kg CO_2-eq kg $protein^{-1}$ (SI Text 2). We estimated CO_2 sequestration during shell formation to be equivalent to 22.99 kg CO_2-eq kg $protein^{-1}$ (SI Text 2). The sum of sequestration and release yields net negative GHG-emissions (−7.27 kg CO_2-eq kg $protein^{-1}$). Since we ended our study at the farm gate, we have elected to exclude the impact of CO_2 release and sequestration by the shell, though this merits future study.

Our estimates indicate that oysters have about 0.04%, 0.09%, 0.25%, and 0.33% of the GHG-cost per kg protein of beef, small ruminants, pork, and poultry, respectively. To put the difference in GHG-cost in perspective, we conducted a thought experiment to estimate the impact of a 10% change in diet from beef to oysters on GHG-release. We elected to compare oysters to beef for two reasons: first, terrestrial animal protein makes up a significant portion of protein in the American diet, and second, of terrestrial livestock, beef has the highest GHG-cost per kg protein, so reductions in beef consumption will have significant impacts on global food-based GHG release.[67] In 2016, per capita

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availability adjusted for loss (PCAAL; this value is assumed equivalent to consumption) of beef in the United States was 18.4 kg,[68] containing 3.6 kg protein and releasing 1663.4 kg CO_2-eq. The PCAAL of all shellfish was just 1.3 kg,[68] containing less than 0.1 kg protein with an associated GHG-cost of 0.01 kg CO_2-eq. If just 10% (0.36 kg) of beef protein was replaced by protein from oysters raised in culture, the total GHG-cost would drop from 1663.4 to 1508.1 kg CO_2-eq, a savings of 9.3%. Scaling up, if the entire population of the United States (~321 million in 2015) were to replace 10% of their beef protein consumption with protein from oysters, this would lead to a CO_2 reduction of 49.9×10^{12} g CO_2-eq annually, approximately the same emissions savings as keeping 10.8 million passenger cars off of the road each year (Text 3).[69] While this shift in diet may seem extreme, between 1970 and 2016, the PCAAL of beef decreased from 27.7 to 18.4 kg, a 34% decrease in consumption, which was made up for by an increase in chicken consumption from 10.2 kg yr^{-1} in 1970 to 23.4 kg yr^{-1} in 2016.[68] Oysters once made up an important part of the diet in coastal cities of the United States. Native Americans and colonists consumed large numbers of oysters,[70,71] and in New York City, the demand for oysters was so great that the Hudson River fishery collapsed in the early 1800s.[72] Oysters were then imported from the nearest estuary until it too collapsed, and then the next nearest estuary was harvested, effectively "fishing down the coast".[72] Despite these collapses, demand remained high through the 1800s, with an annual per capita oyster consumption in the United States of 3 lbs $indiv^{-1}$ yr^{-1} in 1880 (approximately 0.07 kg protein $indiv^{-1}$ yr^{-1} or 1.9% of the current amount of protein from beef in the American diet).[73] Demand declined by 80% between 1880 and 1925 due to a combination of factors including a change in public perception of the safety of eating oysters and competition with other foods.[73] Yet, demand stayed relatively high in coastal areas. As an example, per capita consumption in New York City in 1907 was estimated to be 0.5 lbs $indiv^{-1}$ $week^{-1}$ (approximately 0.6 kg protein $indiv^{-1}$ yr^{-1}, or 16.7% of the current amount of protein from beef in the American diet).[73] More recently, there is evidence of an increase in consumer demand for oysters: between 1995 and 2005 global oyster production rose 5% annually,[74] and in the United States and Canada, the projected growth of the industry is at least 2% per year through 2030.[75] While we have demonstrated that a change in diet from beef to oysters is associated with a large reduction in GHG release, it is important to note that diet changes of this scale require either substantial government intervention or changes in consumer perception and preference. They are often slow and can be unpredictable.[76]

We included the three drivers of GHG-release that account for 94% of emissions associated with livestock production in our estimation—fodder production, animal metabolic release, and manure management—but omitted direct and indirect energy use and post farm gate GHG-release for all protein sources in our comparison (Figure 2). While LCA can provide GHG information about supplies used on the farm and post farm gate emissions, it is likely that previous LCA of aquaculture species have missed emissions from enteric fermentation and manure management, simply because these values have not been well quantified for many species. As an example, Hu et al.[14] demonstrate that N_2O release from N waste in aquaculture is globally significant for species such as carp, salmon, and trout, yet LCA studies of these same fishes report either no emissions or insignificant emissions from the fish production process.[77−79] We expect that oysters would have a similar or smaller GHG-cost than other animal products after leaving the farm, as they require minimal preconsumer processing and simply need refrigerated transport to a retail location. The waste from oyster consumption (i.e., the shells) is also a valuable product with uses in construction, as a dietary supplement in poultry production, and as a liming material in soil that can offset other GHG-costs associated with oyster production. We also predict the direct and indirect energy use in the oyster aquaculture industry would be lower than in land-based livestock production. Direct and indirect energy costs include the energy required to cool, ventilate, and heat the farm; transport of the animals to slaughter and the processing plant; and embedded energy from the production of equipment and supplies. Oyster aquaculture does not require heating or ventilation for the animals, as oysters can live in a broad range of temperatures and cope well with large changes in temperature and prolonged periods of freezing temperatures.[33] Oysters also do not require slaughter or processing, as they can be eaten raw. However, to prolong the shelf life of oysters, they can be shucked for the meat to be stored in cans or frozen. It is most difficult to predict the GHG-cost of the supplies used on an oyster farm, and this cost likely differs by culture method and farm location. However, it is unlikely that these differences could push oyster aquaculture to having a higher GHG-footprint than terrestrial livestock. As an example, tools in carp aquaculture contributed only 0.09 kg CO_2-eq kg $product^{-1}$ or less than 2.1% of total emissions in both conventional and organic production techniques.[79] To clarify these uncertainties, a full life cycle assessment of an oyster farm would be a useful next step but one that is beyond the scope of this study.

We have demonstrated that oyster aquaculture can provide a low-GHG animal protein source relative to terrestrial livestock production. Further, relative to other aquaculture products that negatively impact local ecosystems or require terrestrial food inputs,[11,13] oyster aquaculture presents an opportunity for an ecologically beneficial industry, providing habitat for juveniles of other commercially valuable fish species, protection from storm surges and wave energy, and regulation of nutrients.[80] Despite these benefits and the low GHG-cost relative to other animal protein production methods, challenges and questions of the potential of oysters raised in culture to replace terrestrial protein remain. For example, can oyster aquaculture grow to an extent that it can feasibly replace other animal proteins at a significant scale? Will there be a demand for large quantities of oyster meat? What are the ecological consequences of large scale expansion of the oyster aquaculture industry? Will ocean acidification hinder large scale bivalve production? The answers to these questions are the keys in predicting the future of oyster aquaculture and its potential to replace or augment terrestrial livestock production.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b02965.

Calculation details, results of AIC comparisons (PDF)

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Notes

The authors declare no competing financial interest.

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