

1 **Title:**

2 Meta-analysis of oyster impacts on coastal biogeochemistry

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17 **Abstract**

18 Overfishing, nutrient fueled hypoxia, and habitat destruction have reduced oyster populations to
19 a fraction of their former abundance. Over the last two decades there has been widespread effort
20 to restore oyster reefs and develop oyster aquaculture. Yet it remains unclear how re-introduction
21 of large oyster populations will change coastal biogeochemistry. Of particular interest is whether
22 oysters may help offset excess nitrogen loading, which is responsible for widespread coastal
23 water quality degradation, low oxygen conditions, and biodiversity declines. Here, we used a
24 meta-analysis approach to assess how oysters alter inorganic nutrient cycling, with a focus on
25 nitrogen removal. Additionally, we examined how oysters alter greenhouse gas emissions. We
26 demonstrate that oysters enhance removal of excess nitrogen by stimulating denitrification,
27 promote efficient nutrient recycling, and may have a negligible greenhouse gas footprint.
28 Further, oyster reefs and oyster aquaculture appear to have similar biogeochemical function,
29 suggesting the potential for sustainable production of animal protein alongside environmental
30 restoration.

31

32 Approximately 40% of the world's population lives within 100 km of the coast¹. These
33 2.4 billion people exert immense pressure on the ecology of coastal ecosystems, perhaps most
34 dramatically by altering the availability of nitrogen (N)²⁻⁴, phosphorus (P)^{5,6}, and silica (Si)^{7,8}.
35 Excess nutrient loading to coastal systems has led to a series of deleterious consequences (e.g.,
36 eutrophication^{5,6}, low oxygen conditions⁹, and reduced productivity of valuable fisheries^{6,10}). The
37 negative impacts of excess nutrients have been exacerbated by the decimation of coastal shellfish
38 populations through overharvest¹¹, pollution¹², and disease¹³.

39 Historically, oysters were found in large populations in many coastal systems, and
40 sustainably supported human populations for millennia¹⁴. Today over 85% of reefs have been
41 lost globally¹⁵, and less than 1% of wild populations remain in many locations¹⁶. Returning
42 oysters to human dominated coastal systems may help negate some deleterious anthropogenic
43 impacts and achieve Sustainable Development Goal 14 of the United Nations "To conserve and
44 sustainably use the oceans, seas and marine resources for sustainable development"¹⁷.
45 Specifically, rebuilding large and well managed oyster populations can promote conservation
46 and sustainable use of the seas by creating new habitat and maintaining productivity, cleaning
47 water through removal of suspended particulates and excess nutrients^{18,19}, and providing a source
48 of animal protein for those living in coastal communities with limited access to livestock protein
49 sources, at a low greenhouse gas cost²⁰. Additionally, development of the oyster aquaculture
50 industry provides economic benefits for small-scale fishers and coastal communities.
51 Introduction, or re-introduction of oysters to coastal systems may also be associated with
52 negative ecological consequences (e.g. competition for phytoplankton with other filter-feeders,
53 introduction of disease), particularly if a non-native species is used in restoration or
54 aquaculture²¹⁻²³.

55 In this paper, we examine the role oysters play in regulating coastal biogeochemical
56 cycling. In coastal ecosystems, N and P availability is controlled by external (e.g., sewage
57 discharge) and internal (e.g., recycling of nutrients from the sediments to the water column)
58 sources²⁴. Oysters enhance recycling of nutrients by stimulating sediment decomposition
59 processes following deposition of feces and pseudofeces (collectively “biodeposits”)¹⁸.
60 Biodeposition can also reduce eutrophication by stimulating N loss through denitrification (the
61 microbial conversion of biologically reactive N to non-reactive dinitrogen (N₂) gas)²⁵. Oysters
62 themselves alter nutrient cycling by excreting N and P and denitrification occurs in their
63 digestive system²⁶.

64 Since the 1990s, there have been significant efforts to restore oyster habitat²⁷ and develop
65 the oyster aquaculture industry for economic benefit and to regain lost ecosystem services. For
66 example, New York City is working to return 1 billion oysters to its waters by 2035²⁸, and oyster
67 aquaculture is practiced on every continent except Antarctica. In 2016, global harvest of oysters
68 raised in aquaculture exceeded 5438 million tonnes – an 8% increase since 2014²⁹ – and there is
69 still tremendous potential for expansion³⁰. To date, studies of the biogeochemical impact of
70 oysters have focused on denitrification or nutrient regeneration at a single oyster farm or reef,
71 making extrapolation to larger scales difficult. Here, we used a meta-analysis approach to
72 quantify the role oysters play in regulating coastal biogeochemical cycles at a global scale.
73 Specifically, we focused on oyster driven: regeneration of N, P, and Si, N removal via
74 denitrification, and greenhouse gas (GHG) emissions. We then compare the biogeochemical
75 impact of different oyster habitats and species. Finally, we suggest future studies that will
76 advance the field and clarify uncertainties identified in this meta-analysis.

77

78 **Results & Discussion**

79 Our meta-analysis considered 1012 studies, and after screening included 45
80 (Supplemental Figure 1). Included studies spanned 5 continents and 7 species of oyster, and most
81 ($n = 28$) were from the Atlantic Coast of North America (Figure 1). In total, 17 studies reported
82 fluxes from oysters^{20,26,31-45} with 626 individual flux measurements, and 31 studies^{20,37,41,46-73}
83 reported fluxes from sediments, providing 4328 individual flux measurements. Below, all effect
84 sizes for sediment fluxes are reported as mean estimated Hedge's $g \pm 95\%$ confidence interval
85 and a p-value for significance. For context, small effect: $g = 0 - 0.2$, medium effect: $g = 0.2 - 0.5$,
86 and a strong effect: $g > 0.5$ ⁷⁴. For fluxes from oysters, we report the mean \pm standard error (μmol
87 $\text{ind}^{-1} \text{hr}^{-1}$) for market size *Crassostrea virginica*. As oyster fluxes are not compared with a
88 control, no g can be calculated.

89

90 **Oyster Regulation of Nutrient Recycling**

91 Both the magnitude and ratio of oyster-mediated nutrient regeneration are necessary
92 considerations when discussing how oysters regulate coastal nutrient availability. Enhanced
93 nutrient regeneration maintains a productive ecosystem, but changes in the availability of N
94 relative to P and Si, or NH_4^+ relative to NO_x can lead to a phytoplankton community dominated
95 by dinoflagellates and cyanobacteria^{9,75,76} – groups often associated with harmful algal blooms
96 and less efficient transfer of energy across trophic levels.

97 Sediments beneath oysters return significantly more NH_4^+ to the water column than bare
98 sediments ($g = 0.750 \pm 0.377$ ($p < 0.001$), $n = 25$; Figure 2). In contrast, oysters have a highly
99 variable effect on sediment NO_x fluxes, and one that is not significant across studies ($g = 0.406 \pm$
100 1.577 ($p = 0.583$), $n = 12$; Figure 2). NO_3^- fluxes were also statistically unchanged in the

101 presence of oysters ($g = 0.012 \pm 0.478$ ($p = 0.956$), $n = 9$). No conclusions could be drawn for
102 oyster effect on NO_2^- release due to small sample size ($g = 0.784 \pm 0.927$ ($p = 0.059$), $n = 2$), but
103 these limited data suggest it may be enhanced in oyster habitats. Oysters also have a strong effect
104 on sediment PO_4^{3-} regeneration (Figure 2; $g = 0.561 \pm 0.319$ ($p = 0.003$), $n = 12$). Oysters may
105 have a net positive effect on sediment Si flux ($g = 0.375 \pm 0.974$ ($p = 0.123$), $n = 2$), but again
106 the data are too limited to draw a definitive conclusion.

107 We can consider the ratio of the calculated g values for two sediment nutrient fluxes to
108 determine how oysters may change water column nutrient availability. For example, diatoms
109 require an N:Si ratio ≤ 1 to dominate. A $g(\text{N}):g(\text{Si})$ equal to 1 would indicate that oysters
110 stimulate sediment regeneration of N and Si at the same rate, and do not change the ratio of
111 sediment N and Si regeneration. If $g(\text{N}):g(\text{Si}) > 1$ then oysters drive greater N than Si
112 regeneration relative to bare sediments, and if $g(\text{N}):g(\text{Si}) < 1$, than Si is regenerated more
113 rapidly than N. Oysters only slightly change the ratio of sediment N and P regeneration ($g(\text{NH}_4^+$
114 $+ \text{NO}_x):g(\text{PO}_4^{3-}) = 1.31$), and likely do not drive either nutrient to become limiting to production.
115 However, oysters may drive greater regeneration of N relative to Si ($g(\text{NH}_4^+ + \text{NO}_x):g(\text{Si}) =$
116 2.17), though again only two studies compared sediment Si fluxes between bare sediment and
117 sediment beneath oysters. In areas with large oyster populations, sediments will likely regenerate
118 at least twice as much NH_4^+ as NO_x ($g(\text{NH}_4^+):g(\text{NO}_x) = 2.38$). It appears oysters could
119 potentially lead to shifts in phytoplankton community structure that preferentially use NH_4^+ to
120 NO_x , such as dinoflagellates and cyanobacteria^{75,77}.

121 Both oyster reefs ($g = 0.684 \pm 0.428$ ($p = 0.002$), $n = 12$) and aquaculture ($g = 0.742 \pm$
122 0.482 ($p = 0.003$), $n = 11$) stimulate sediment NH_4^+ fluxes, with no statistical difference between
123 the two habitat types ($p = 0.860$). Similarly, NO_x fluxes were statistically the same ($p = 0.706$)

124 between reefs ($g = 0.673 \pm 3.043$ ($p = 0.665$), $n = 6$) and aquaculture ($g = 0.077 \pm 0.571$ ($p =$
125 0.791), $n = 4$). NO_3^- fluxes did not differ ($p = 0.063$) between habitat types, despite the
126 appearance of NO_3^- uptake by sediments beneath aquaculture ($g = -0.307 \pm 0.616$ ($p = 0.328$), n
127 $= 5$) and release from sediments adjacent to reefs ($g = 0.316 \pm 0.230$ ($p = 0.007$) $n = 4$). Too few
128 studies reported NO_2^- fluxes to allow for statistical comparison. PO_4^{3-} fluxes were also no
129 different ($p = 0.863$) from sediments beneath reefs ($g = 0.616 \pm 0.677$ ($p = 0.075$), $n = 4$) and
130 aquaculture ($g = 0.550 \pm 0.314$ ($p < 0.001$), $n = 8$).

131 Oysters themselves recycle significant quantities of NH_4^+ ($5.73 \pm 0.91 \mu\text{mol NH}_4^+ \text{ indiv}^{-1}$
132 hr^{-1}) and PO_4^{3-} ($0.82 \pm 0.1 \mu\text{mol PO}_4^{3-} \text{ indiv}^{-1} \text{ hr}^{-1}$) to the water column. NO_x fluxes were much
133 more variable ($2.64 \pm 5.74 \text{ SE } \mu\text{mol NO}_x \text{ indiv}^{-1} \text{ hr}^{-1}$), with one study reporting high NO_x
134 emission⁴³, and another reporting nearly equivalent NO_x consumption³⁹. NO_3^- fluxes were only
135 reported in two studies that met our criteria, but were slightly lower ($0.50 \pm 0.92 \text{ SE } \mu\text{mol NO}_3^-$
136 $\text{ indiv}^{-1} \text{ hr}^{-1}$) than NO_x . Three studies reported NO_2^- fluxes, and they were lower ($0.11 \pm 0.02 \text{ SE}$
137 $\mu\text{mol NO}_2^- \text{ indiv}^{-1} \text{ hr}^{-1}$) than the sum of NO_x and NO_3^- fluxes. We could not locate any studies
138 that report oyster DSi excretion. Other grazing organisms excrete DSi ⁷⁸, so it is possible oysters
139 may too. Unlike sediment nutrient regeneration, N and P excretion from oysters likely drives N
140 to become limiting in the context of the Redfield ratio (16N:1P), with an N:P molar ratio of 6.99
141 $\text{NH}_4^+ : \text{PO}_4^{3-}$.

142 It is clear oyster habitats drive substantial nutrient recycling, though they may increase
143 $\text{NH}_4^+ : \text{NO}_x$, with potentially negative consequences. There is a significant body of literature
144 focused on top-down regulation of phytoplankton biomass and community by oysters, but less
145 attention has been paid to how oysters may change phytoplankton community structure from the
146 bottom-up, despite evidence for stimulation of productivity using regenerated nutrients⁷⁹. Future

147 research should address this so we can better understand how larger oyster populations may
148 change the ecology of coastal ecosystems.

149

150 **Removal of Excess Nitrogen by Oysters via Denitrification**

151 Stimulation of sediment denitrification and denitrification in oysters can permanently
152 remove excess N from coastal systems, reducing the impact of eutrophication. There are two
153 commonly used methods for measuring rates of denitrification (N_2/Ar technique⁸⁰ and the
154 isotope pairing technique (IPT)⁸¹). The N_2/Ar technique measures net exchange of N_2 between
155 sediment and water column, and the resulting fluxes are either net positive (i.e., denitrification)
156 or net negative (i.e., nitrogen fixation). IPT requires the addition of a tracer and a series of
157 methodological assumptions to calculate sediment denitrification, and IPT rates are generally
158 lower than those measured with the N_2/Ar technique⁸².

159 When we examined denitrification rates measured using the N_2/Ar technique we found
160 that oysters have a strong positive effect on sediment denitrification ($g = 0.682 \pm 0.276$ ($p <$
161 0.001), $n = 19$; Figure 3). The effect of oysters on sediment denitrification is greater when
162 measured using the N_2/Ar method ($g = 0.682 \pm 0.284$) relative to the isotope pairing technique
163 (IPT; $g = 0.239 \pm 0.745$ ($p = 0.463$), $n = 7$), likely due to under estimation of denitrification
164 using IPT⁸³. While the magnitude of the effect of oysters on net N_2 fluxes was higher in reef
165 habitats ($g = 0.792 \pm 0.199$ ($p < 0.001$), $n = 11$) than aquaculture ($g = 0.514 \pm 0.220$ ($p < 0.001$),
166 $n = 6$), there was no statistical difference between the two habitats ($p = 0.223$). A possible
167 explanation for this discrepancy is that some studies that measured denitrification in reefs
168 occasionally included the oysters in the incubation chamber. As oysters themselves produce N_2
169 ^{26,37,39,40,42,43}, this would likely increase the measured net N_2 flux.

170 Relative to sediment denitrification measurements made using the N₂/Ar technique (403
171 individual measurements of net denitrification in sediment beneath oysters), there are only a few
172 measurements of net N₂ from oysters themselves (n = 37). Regardless, it is clear that
173 denitrification proceeds within oysters, with an average denitrification rate from oysters of 4.78
174 ± 2.46 μmol N₂ indiv⁻¹ hr⁻¹. In dense populations, denitrification in oysters could be a significant
175 pathway for N removal from coastal ecosystems²⁶, perhaps more than doubling total removal of
176 excess N.

177 Despite a large number of studies that quantified denitrification from sediments in oyster
178 habitats, every study that used the N₂/Ar method was conducted on either the Atlantic or Gulf
179 Coast of the United States, and used the oyster native to this region (*Crassostrea virginica*). The
180 only study conducted elsewhere used the IPT method⁴¹. While it is unlikely that other oyster
181 species will have different effects than *C. virginica*, it is necessary to collect similar
182 measurements in other locations and with other oyster species.

183 It is important to consider the simultaneous removal and recycling of N in oyster habitats
184 in an ecosystem context. Greater N release from oyster habitats may at first seem to suggest that
185 oysters worsen water quality, but this may not be the case, as any N oysters excrete or that is
186 regenerated from sediments beneath oysters was previously held in phytoplankton and detritus
187 processed by the oysters. Simply, oysters generally do not add new nutrients to the system, and
188 recycled nutrients cannot support more phytoplankton than would be supported by external
189 nutrient loading to the system¹⁸. Instead, oysters promote a second round of primary production
190 and efficient re-use of nutrients already in the system, while removing some excess N (Figure 6).
191 Specifically, any increase in water column primary productivity likely increases deposition of
192 organic matter to sediments. In turn, because sediment denitrification is positively correlated

193 with organic matter, nitrogen removal is enhanced (Figure 5)^{84–86}. Simultaneous promotion of N
194 recycling and removal by oysters may not reduce the trophic status of a coastal system – and
195 may in fact increase it through enhanced primary production and carbon fixation – but the
196 presence of oysters in the system may help to reduce the effects associated with cultural
197 eutrophication, such as high phytoplankton biomass, reductions in harvestable fish and shellfish,
198 and decreased water clarity.

199

200 **Greenhouse Gas Release from Oyster Habitats**

201 Oyster biodeposition may promote release of carbon dioxide (CO₂), methane (CH₄), and
202 nitrous oxide (N₂O) from sediments. Oysters can also release these gases during respiration,
203 digestion, and from the metabolism of the biofilm living on their shell. If oysters promote the
204 production of these greenhouse gases, it may offset benefits associated with nutrient recycling
205 and denitrification.

206 Very few studies report the impact of oysters on greenhouse gas fluxes. This limited data
207 demonstrates high variability between studies, and no conclusions could be made for the effect
208 of oysters on sediment CO₂ ($g = 0.620 \pm 1.194$ ($p = 0.155$), $n = 3$; Figure 6), CH₄ ($g = 0.432 \pm$
209 1.592 ($p = 0.364$), $n = 3$), or N₂O fluxes ($g = -0.771 \pm 2.969$ ($p = 0.469$), $n = 4$). There was not
210 enough data to compare whether oyster reefs or aquaculture have a greater impact on sediment
211 GHG fluxes. Quantifying how oysters may change sediment greenhouse gas production is an
212 important step as aquaculture continues to expand and restoration projects are planned and
213 completed.

214 Only three studies reported CO₂ and CH₄ fluxes from sediments beneath oysters and a
215 control site, and those studies were conducted in two locations. Two were conducted at the same

216 location in Ireland ^{48,71} and the other on the Atlantic Coast of North America²⁰. The two studies
217 in Ireland were conducted by building oyster reefs, then destructively sampling them a few
218 months later, effectively creating a perturbation experiment. Ray et al.²⁰ measured sediment
219 GHG fluxes at an oyster farm along a 7-yr chronosequence, and demonstrated an initial
220 stimulation in sediment CO₂ and CH₄ release after the installation of oyster aquaculture,
221 followed by a return to baseline conditions. Together, these results suggest that oysters may
222 stimulate sediment GHG release, but only for a brief period.

223 Oysters themselves release N₂O ($0.00027 \pm 0.00054 \mu\text{mol indiv}^{-1} \text{ hr}^{-1}$) and CH₄ ($0.0057 \pm$
224 $0.00085 \mu\text{mol indiv}^{-1} \text{ hr}^{-1}$). Oyster N₂O release is dictated by the availability of dissolved N in
225 the water column, with higher rates of production when DIN concentrations are high, and N₂O
226 consumption in the absence of DIN⁴⁴. There are at least two, not mutually exclusive, possibilities
227 as to how N₂O production associated with large oyster populations will interact with enhanced
228 nutrient recycling and denitrification. Enhanced regeneration of dissolved NH₄⁺ from sediments
229 followed by transformation to NO_x via water column nitrification may lead to N₂O release.
230 Alternatively, as DIN loading to coastal systems is reduced or removed via denitrification, there
231 will be less N₂O production by oysters. Two of four studies reported enhanced uptake of N₂O in
232 sediments beneath oyster aquaculture which generally occurs in estuarine sediments when nitrate
233 is limiting to denitrification. If site specific conditions influence how oysters alter sediment N₂O
234 fluxes, smart installation of oyster restoration and aquaculture could be an important strategy to
235 help reduce estuarine N₂O emissions, particularly in systems anthropogenically enriched with N.
236 It is unclear what controls oyster CH₄ production and consumption. Oyster respiration of CO₂
237 varies by size, water temperature, and food availability, though when estimating the greenhouse
238 gas footprint of animals in food production systems CO₂ release is typically ignored as it is a

239 return of photosynthetically fixed carbon to the atmosphere^{87,88}. Oysters also release some CO₂
240 to the atmosphere during precipitation of calcium carbonate in shell formation^{89,90}.

241 Jackson et al.⁴³ showed a 1:1 relationship between DIC fluxes in oyster only incubations
242 relative to oyster and sediment incubations, indicating that the majority of CO₂ release in oyster
243 reefs comes from the oysters themselves. Oysters may also influence the C budget over space
244 and time. Primary production and CO₂ sequestration may be higher downstream of oyster
245 habitats than upstream, driven by enhanced light and nutrient availability⁹¹, that could offset
246 oyster associated CO₂ release. As oyster reefs accrete over time, they switch from a net source of
247 C to the atmosphere driven by respiration and shell formation, to a net sink as organic material
248 accumulates in the reef habitat and is buried⁸⁹. It is necessary to consider these spatial and
249 temporal factors alongside direct measurements of CO₂ flux from oyster habitats.

250

251 **Comparison Between Oyster Species**

252 In some regions native oyster species are used in restoration and aquaculture (e.g.
253 *Crassostrea virginica* on the Atlantic Coast of the United States), and the biogeochemical
254 changes associated with expanding this population may be considered beneficial. In other areas
255 non-native oysters have either invaded, or been introduced. For example, *Crassostrea gigas* has
256 largely replaced *Ostrea edulis* populations in Europe, both in natural habitats and in oyster
257 farms⁹². *O. edulis* also threatens *Saccostrea glomerate* populations in Australia⁹³. Non-native
258 introductions have been associated with negative consequences, such as reduced ecosystem
259 carrying capacity and alteration of food web structure^{92,94}. It is less clear how non-native oysters
260 may alter biogeochemical function relative to the native species because of a paucity of data.
261 Here we were only able to compare the biogeochemical impacts of *C. gigas* with *C. virginica* on

262 sediment NH_4^+ and PO_4^{3-} fluxes as there were not enough studies that met our criteria for other
263 fluxes or species. Nevertheless, this preliminary analysis affords some insight. We found that
264 sediment NH_4^+ fluxes were higher ($p = 0.749$) under both *C. gigas* ($g = 0.836 \pm 0.295$ ($p <$
265 0.001), $n = 5$) and *C. virginica* habitats ($g = 0.742 \pm 0.497$ ($p = 0.003$), $n = 19$). *C. gigas* appears
266 to enhance sediment PO_4^{3-} fluxes ($g = 0.847 \pm 0.124$ ($p < 0.001$), $n = 3$) more than *C. virginica* (g
267 $= 0.451 \pm 0.473$ ($p = 0.062$), $n = 7$), though the two species were not significantly different in
268 regard to their effect on sediment PO_4^{3-} release ($p = 0.113$).

269 There is also limited information for direct fluxes from oysters apart from *C. virginica*
270 (Table 1). For this reason, we could not conduct statistical comparisons, though qualitatively it
271 appears that most fluxes were of a similar range across species. There is a clear need for more
272 studies of the impact of oysters on sediment biogeochemical fluxes as well as direct fluxes from
273 oysters species besides *C. virginica*. With this limited evidence available, we cautiously suggest
274 similar effects of oysters on coastal biogeochemistry across species, consistent with a recent
275 study that reported no significant difference in GHG production by native (*C. virginica*) and non-
276 native (*O. edulis*) in the north-eastern United States⁴⁵.

277

278 **Similar Biogeochemistry in Oyster Reefs and Aquaculture**

279 Oyster reefs and aquaculture had similar effects on sediment NH_4^+ and PO_4^{3-} recycling,
280 as well as denitrification. Here we show that – while not identical – oyster aquaculture may have
281 similar value in recovering ecosystem services associated with biogeochemical cycling as oyster
282 reefs. Additionally, oyster reefs and aquaculture provide similar quality habitat⁹⁵⁻⁹⁷ and both may
283 help to protect shorelines from storm surge^{98,99}. Considering these benefits, we cautiously
284 suggest that financial investment in native oyster aquaculture may provide similar benefits as

285 oyster reefs, while also expanding production of a sustainable food resource and enhancing local
286 economies^{29,100}. A recent study suggests oceans can be restored by 2050²⁷, and includes oyster
287 reef restoration as a major step in that effort. Several roadblocks to oyster reef restoration were
288 noted, including poor management of fisheries on remaining reefs and the economic cost of
289 restoration. We propose that promoting oyster aquaculture may allow for these two roadblocks to
290 be side stepped while still achieving similar restoration goals and benefits.

291 Human demand for animal protein is increasing^{101,102}. Oyster aquaculture provides a
292 viable alternative source of animal protein with the added benefit of promoting coastal
293 productivity, and does not require the addition or exploitation of limited resources. Additionally,
294 like other bivalves, oysters have adapted to live in estuarine systems where they experience
295 diurnal, seasonal, and annual patterns of temperature, salinity, pH, and oxygen fluctuations,
296 potentially making adult oysters more resilient to the impacts of a changing climate. Further,
297 oysters are a low greenhouse gas emitting protein source and even a small change in their
298 consumption could lead to a significant reduction in GHG emissions²⁰.

299 An important next step in understanding the effect of oysters on coastal biogeochemistry
300 is the development of predictive models that use local environmental characteristics, such as
301 temperature, salinity, water column dissolved nutrient concentrations, and/or sediment physical
302 and chemical properties. To do this, future studies must thoroughly report environmental data
303 associated with flux measurements, and publish full datasets alongside manuscripts and theses.

304

305 **Methods**

306

307 **Literature Search**

308 We aggregated studies measuring oyster fluxes and fluxes from sediments beneath
309 oysters and a control site from peer-reviewed studies and non-reviewed literature. Peer-reviewed
310 study identification was finalized on September 23, 2019 using *Web of Science* (Clarivate
311 Analytics) and the following searches and terms: search 1. (oyster) AND (sediment) AND
312 (nitrogen OR denitrification OR phosphorus OR silica OR methane OR nitrous oxide OR carbon
313 dioxide); search 2. (oyster) AND (nitrogen OR denitrification OR phosphorus OR silica OR
314 methane OR nitrous oxide OR carbon dioxide). Together, these searches yielded 998 results
315 (Supplementary Figure 1). In addition to the initial literature search, we added published journal
316 articles, dissertations and theses, and journal articles in review that we were aware of (n = 14)
317 that did not appear in the *Web of Science* search, for a total of 1012 studies.

318

319 **Study Selection Criteria**

320 We applied two screening steps to select studies for inclusion in our analysis. In the first
321 step, we read the abstract of each study. If the abstract did not include a flux measurement from
322 oysters or sediment beneath oysters, or indicate that such a measurement might be found in the
323 manuscript, the study was rejected for further analysis. Next, we read all studies that passed the
324 first screening step. If the study did not measure a net-flux from adult oysters, report fluxes from
325 both control sediment and sediment beneath or directly adjacent to oysters, or report sample
326 sizes, standard deviation, or standard error, we excluded it. Studies that manually added organic
327 material or biodeposits to sediments were excluded. If the same data set was reported in multiple
328 studies, only one version of that data set was included (the earliest paper that reported the data).
329 We also excluded published abstracts for conference talks, and studies not published in English.

330 In some cases, studies reported sample size, but mean values and standard deviation or
331 error had to be estimated from figures in the paper. These studies were included in the analysis,
332 and the missing values were estimated by extracting them from plots and images embedded
333 within the manuscript using WebPlotDigitizer Version 3.9 software. When the error bar did not
334 pass the edge of the bar (or did not exist), error was calculated as 0, but an SD value of 1 was
335 used in order to calculate g values in the meta-analysis. When sample size was reported as a
336 range, we used the median possible sample size (i.e. if the study reported $n = 3-4$, we assigned n
337 a value of 3.5). Following the second extraction step, 45 studies met our criteria for inclusion in
338 the analysis.

339

340 **Data Analysis – Sediment Fluxes**

341 When studies reported standard error, we converted it to standard deviation (Equation 1).
342 We converted all flux rates to either $\mu\text{mol g DTW}^{-2} \text{ hr}^{-1}$ (oysters) or $\mu\text{mol m}^{-2} \text{ hr}^{-1}$ (sediments). If
343 rates were reported seasonally or monthly, but no study mean was reported, we manually
344 calculated a pooled study mean (Equation 2) and variance (Equation 3). In studies that compared
345 two habitat types to one control site, we used the same control site for both habit types. Two
346 studies reported the sample size as “ $n = 3$ or 4 ” for each sampling month. In this case, we
347 assigned an n value of 3.5, and used that while combining monthly mean and error. In all cases,
348 we considered each sediment flux as an independent sample.

349 Next, we calculated Hedge’s g effect size (Equation 4) and variance (Equation 2 and 3;
350 same method as for pooling means and variance within a study, but in this case the mean of
351 sediments beneath oysters (X_E) and control sediments (X_C) for whole studies were pooled as

352 were all variances) using a fixed effects model approach in the *metafor* package¹⁰³ in R
353 Statistical Software version 3.6.0, followed similar methods as Harrer et al¹⁰⁴ and Anton et al¹⁰⁵.

354 The J value in Equation 4 controls bias associated with different sample sizes between
355 studies (Equation 5). When interpreting g , the value reflects the effect size of the experimental
356 treatment (in this case presence or absence of oysters on sediment net N_2 flux) in terms of
357 number and direction of standard deviations the experimental treatment is from the control. A g
358 value between 0 – 0.2 is typically considered a small effect, 0.2-0.5 a medium effect, 0.5-0.8 a
359 large effect, and >0.8 a very large effect⁷⁴.

360

361 Equation 1:

$$362 \quad s.d. = s.e. \times \sqrt{n}$$

363

364 Equation 2:

$$365 \quad X_{pooled} = ((X_a \times n_a) + (X_b \times n_b)) / (n_a + n_b)$$

366

367 Equation 3:

$$368 \quad s.d._{pooled} = \sqrt{((n_a - 1) \times (s.d._a)^2 + (n_b - 1) \times (s.d._b)^2) / (n_a + n_b - 2)}$$

369

370 Equation 4:

$$371 \quad g = ((X_E - X_C) \times J) / s.d._{pooled}$$

372

373 Equation 5:

$$374 \quad J = 1 - (3 / (4 \times (N_E + N_C - 2) - 1))$$

375

376 To compare the influence of oysters on sediment fluxes between oyster reefs and oyster
377 aquaculture we used a random effects meta-analysis model via the *dmetar* package¹⁰⁶. In this
378 model we used the Sidik-Jonkman method for determining τ^2 and the Hartung-Knapp
379 adjustment¹⁰⁴. We repeated this approach to compare sediment fluxes in oyster habitats with *C.*
380 *gigas* and *C.virginica*.

381 In regard to denitrification, we excluded all studies that used acetylene techniques as this
382 acetylene is known to alter sediment microbial community function and provide inaccurate
383 measurements^{107,108}. While only net N₂ fluxes from sediments and oyster matter in an ecosystem
384 context, we also accepted studies that used the isotope pairing technique (IPT)⁸¹ in order to
385 compare this direct measurement with a measurement of the net N₂ flux (N₂/Ar technique)⁸⁰
386 Studies using IPT were not included in the total estimate of oyster effects on sediment
387 denitrification or comparison between habitat types.

388

389 **Publication Bias – Sediment Fluxes**

390 We followed the same methods used by Anton et al.¹⁰⁵ to test for publication bias among
391 sediment flux studies that may distort the results of the meta-analysis. We evaluated bias using
392 Egger's Test, which estimates a regression using standard error of study specific effect sizes, and
393 is considered significant when the model intercept is statistically different from zero¹⁰⁴.
394 Additionally, we identified outlier studies that may exert substantial influence on the direction of
395 the meta-analysis as those where the upper bound of the study 95% CI was lower than the lower
396 bound of the pooled 95% CI (i.e. an extremely small effect), or the lower bound of the study
397 95% CI was higher than the upper bound of the pooled 95% CI (i.e. an extremely large effect)¹⁰⁴.

398 Both of these tests are available in the *dmetar* package¹⁰⁶. When Eggers test indicated significant
399 publication bias ($p \leq 0.05$) for models that included possible outlier studies in the meta-analysis,
400 we removed them and report results from the newer, outlier free test. We also excluded
401 significant outliers from our habitat comparison. We only had to remove one outlier from the
402 N₂/Ar meta-analysis (Supplementary table 12), indicating a generally robust dataset free of
403 publication bias.

404

405 **Data Analysis – Oysters**

406 Oyster fluxes were reported in various units. We elected to convert all fluxes to $\mu\text{mol ind}^{-1}$
407 hr^{-1} , as some studies reported this rate without information about oyster tissue mass. For studies
408 that reported flux as $\mu\text{mol g}^{-1} \text{hr}^{-1}$, we assigned a value of 2.93 g ind^{-1} to convert the rates, as this
409 is the dry tissue mass of commercial size oysters where most of the measurements included in
410 this meta-analysis were made (Atlantic Coast of the United States). We opted to report rates in
411 units of $\mu\text{mol ind}^{-1} \text{h}^{-1}$ so that we could maximize the amount of data included in this study and
412 because reporting on a spatial scale (e.g., $\mu\text{mol m}^{-2} \text{h}^{-1}$) is less descriptive than reporting on an
413 individual basis for several reasons. For example, there is and can be high variance in oyster
414 stocking density in reefs and aquaculture dependent on habitat, oyster size, food availability,
415 farm management practices, etc. Additionally, the vertical structure of the reef or oyster
416 aquaculture gear (for example cages in deep water that are several m high can hold several bags
417 of oysters) will have a different oyster density (and flux m^{-2}) than gear in shallow water with
418 only one bag of oysters. However, this choice also likely introduces some bias as oyster weight
419 and life stage vary across reef and aquaculture communities. To help reduce this bias we
420 specifically excluded juvenile and larval oysters from this meta-analysis. After converting flux

421 rates to $\mu\text{mol ind}^{-1} \text{ h}^{-1}$, we calculated pooled means and variance across studies using Equation 2
422 and 3. We report all oyster flux values as mean \pm standard error. We did not perform publication
423 bias analyses on fluxes from oysters alone, as these studies only report rates and do not compare
424 oyster fluxes to anything.

425

426 **Data Availability**

427 All data used in this study is available in the Figshare repository under the access number
428 <https://doi.org/10.6084/m9.figshare.12488753>.

429

430 **Code Availability**

431 The R script used in this meta-analysis is available in the Github community repository
432 (<https://github.com/nray17/Meta-analysis-oyster-impacts-on-biogeochemistry>).

433 Correspondence and requests for materials should be addressed to NER.

434

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441

442 *Author Contributions*

443 NER and RWF conceived and designed the study. NER constructed the dataset and
444 performed statistical analyses. Both authors interpreted the results. NER wrote the manuscript
445 with significant contribution from RWF. NER and RWF edited the manuscript. Both authors
446 take full responsibility for the contents of the manuscript.

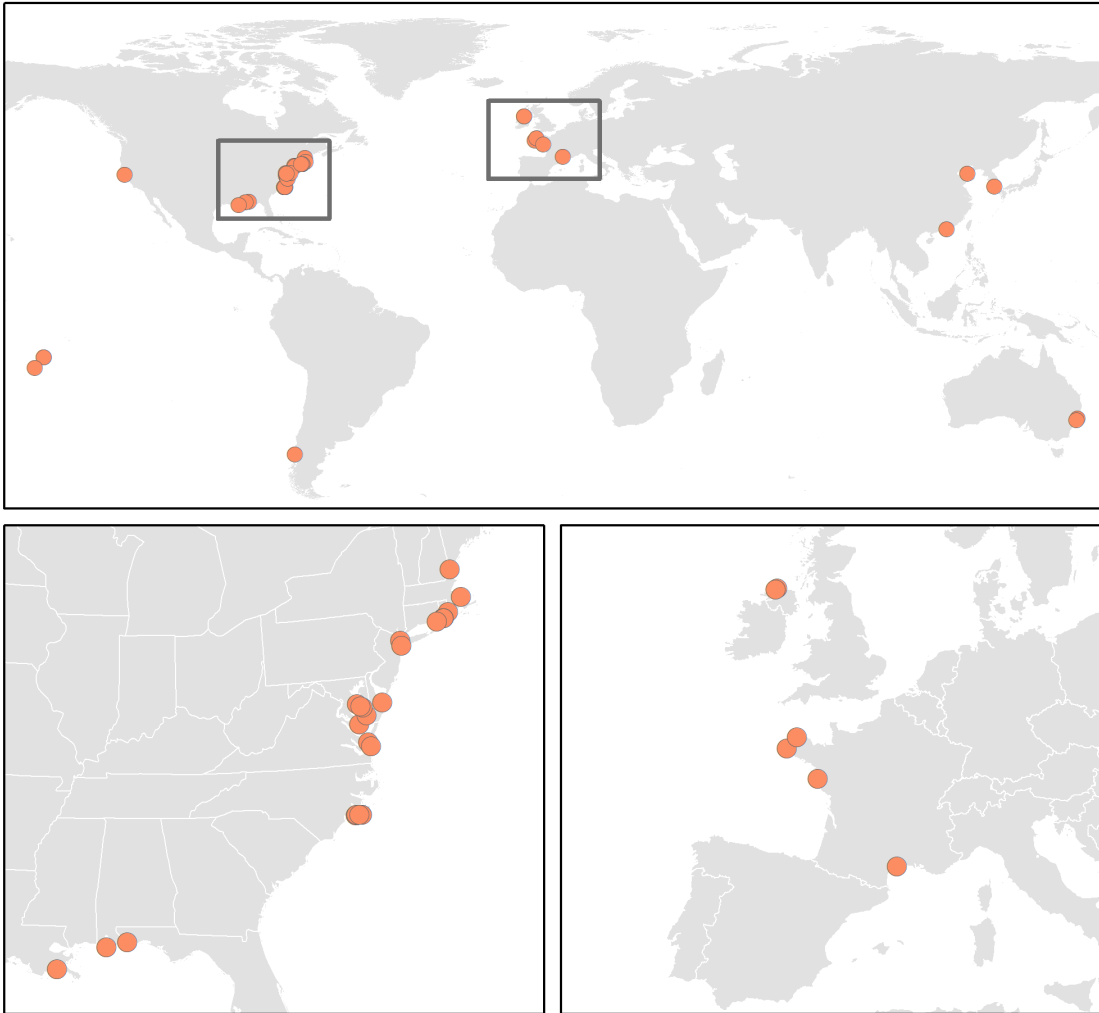
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448 *Competing Interests*

449 The authors declare no competing interests.

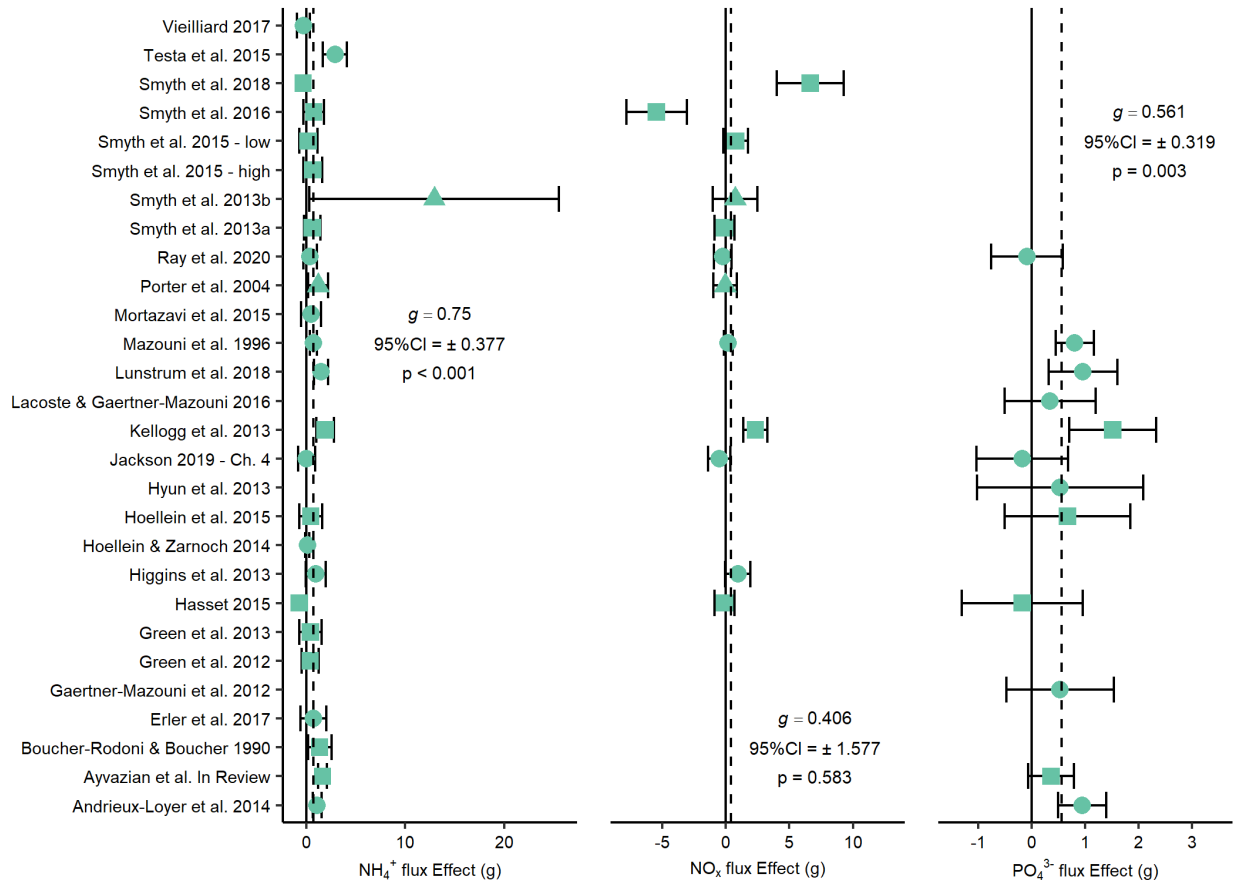
450

451 Figure 1: Location of studies used in this meta-analysis. Map created by Emily Moothart using
452 ArcGIS software with data collected during the literature review.



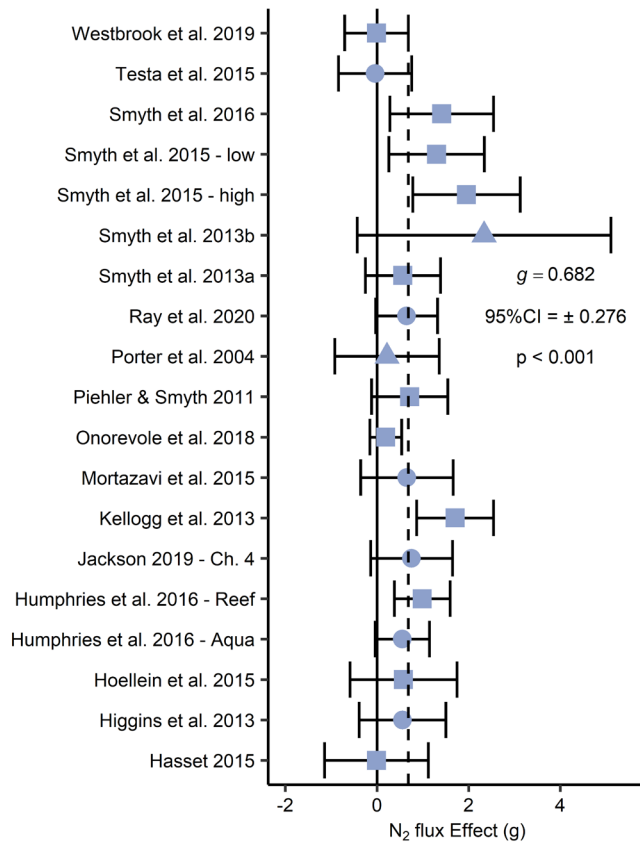
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457 Figure 2: Effect size (Hedge's g , 95% confidence interval, and random effect meta-analysis
 458 model p-value) of oysters on rates of sediment regeneration of the nutrients ammonium (NH_4^+),
 459 combined nitrate + nitrite (NO_x), and phosphate (PO_4^{3-}), for oysters from aquaculture studies
 460 (circles), reef studies (squares), and mesocosm studies (triangles). Effect size describes the
 461 magnitude of increase ($g > 0$) or decrease ($g < 0$) of a flux beneath oysters relative to bare
 462 sediment. All points to the right of the solid line indicate a net positive effect, and points to the
 463 left of the solid line indicate a net negative effect. The dashed line indicates the mean effect size
 464 (g) of all studies together. For context, small effect: $g = 0 - 0.2$, medium effect: $g = 0.2 - 0.5$, and
 465 a strong effect: $g > 0.574$.



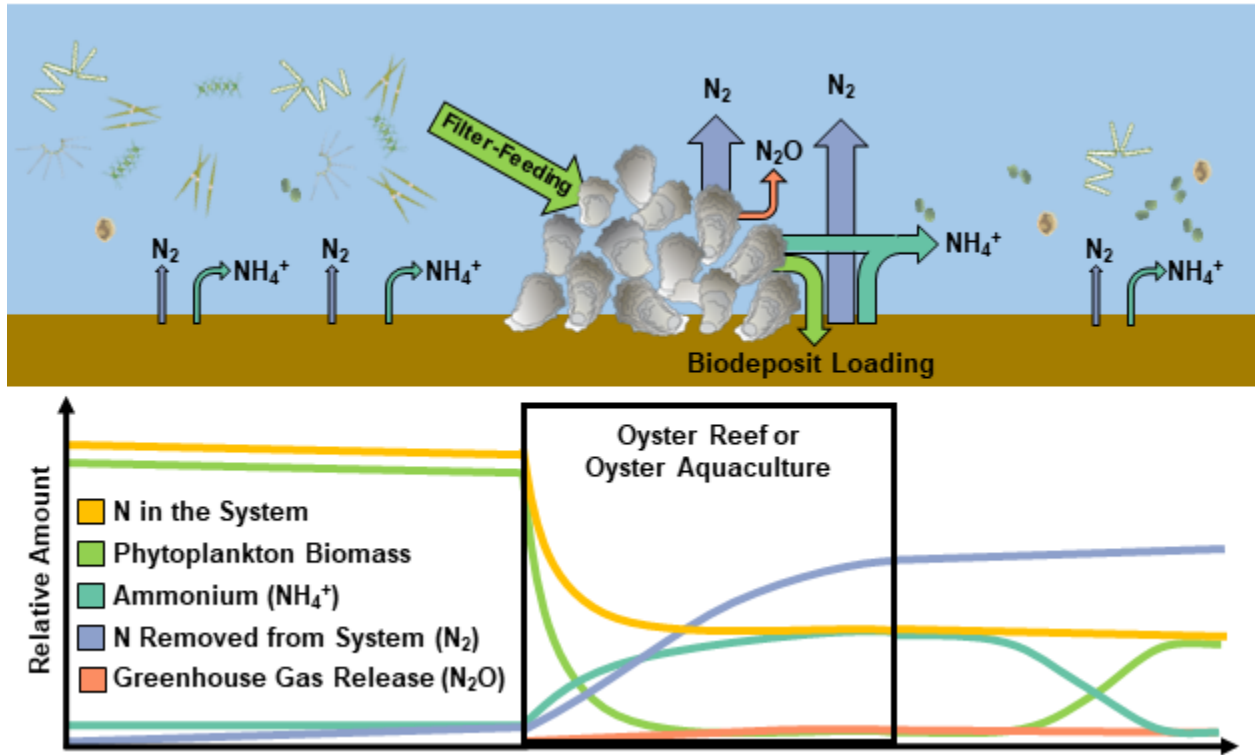
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470 Figure 3: Effect size (Hedge's g , 95% confidence interval, and random effect meta-analysis
 471 model p-value) of oysters on rates of sediment denitrification (N_2 flux), for oysters from
 472 aquaculture studies (circles), reef studies (squares), and mesocosm studies (triangles). Details as
 473 in Figure 2.



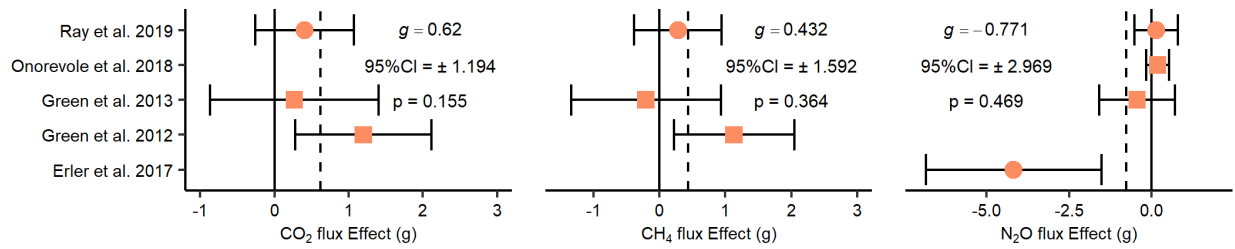
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478 Figure 4: Transport of nitrogen (N) through coastal ecosystems with oyster habitats. Oysters
 479 consume phytoplankton and move biodeposits containing N to the sediments. Oysters excrete
 480 ammonium (NH_4^+) and drive NH_4^+ regeneration from sediments, supporting water column
 481 primary production. Oysters also enhance N removal from the system through denitrification
 482 (N_2 production) in their digestive system and by stimulating sediment denitrification. A very
 483 small amount of nitrous oxide (N_2O) is released from the oyster digestive system, likely due to
 484 inefficient denitrification. Larger arrows indicate greater rates. Oyster and phytoplankton
 485 symbols courtesy of the Integration and Application Network, University of Maryland Center for
 486 Environmental Science (ian.umces.edu/symbols).
 487



488
 489

490 Figure 5: Effect size (Hedge's g , 95% confidence interval, and random effect meta-analysis
 491 model p-value) of oysters on rates of sediment production of the greenhouse gases carbon
 492 dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), for oysters from aquaculture studies
 493 (circles) and reef studies (squares). Details as in Figure 2.



494
 495
 496

497 **Table 1:** Mean (\pm standard error) flux of dissolved nutrients and gases from various oyster species. All
 498 values reported in $\mu\text{mol ind}^{-1} \text{hr}^{-1}$, except N_2O and CH_4 fluxes which are in $\text{nmol ind}^{-1} \text{hr}^{-1}$. N_2 fluxes only
 499 report values measured using the N_2/Ar methods. All rates were collected from previous studies, and are
 500 available in the published dataset accompanying this manuscript. *n.d.* indicates no published rates met our
 501 study selection criteria.

Species	N_2 Flux	NH_4^+ Flux	NO_x Flux	NO_3^- Flux	NO_2^- Flux	PO_4^{3-} Flux	N_2O Flux	CH_4 Flux
<i>C. gigas</i>	<i>n.d.</i>	2.81 ± 0.42	0.20 ± 0.05	<i>n.d.</i>	0.08 ± 0.01	0.87 ± 0.09	<i>n.d.</i>	<i>n.d.</i>
<i>C. virginica</i>	4.78 ± 2.46	9.44 ± 2.56	2.89 ± 6.33	0.80 ± 1.46	0.11 ± 0.03	1.33 ± 0.67	0.11 ± 0.76	2.93 ± 1.23
<i>O. chilensis</i>	<i>n.d.</i>	0.89 ± 0.20	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>
<i>O. edulis</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	<i>n.d.</i>	0.47 ± 0.04	0.21 ± 0.97
<i>S. glomerata</i>	<i>n.d.</i>	5.65 ± 0.10	<i>n.d.</i>	-0.03 ± 0.01	<i>n.d.</i>	0.38 ± 0.06	67.66 ± 16.46	<i>n.d.</i>
All species	4.78 ± 2.46	5.73 ± 0.91	2.64 ± 5.74	0.50 ± 0.92	0.11 ± 0.02	0.82 ± 0.10	0.27 ± 0.54	1.84 ± 0.85

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504 References:

- 505 1. United Nations. Factsheet: People and Oceans. (2017).
- 506 2. Vitousek, P. M. *et al.* Human alteration of the global nitrogen cycle: sources and
507 consequences. *Ecol. Appl.* **7**, 737–750 (1997).
- 508 3. Galloway, J. N. *et al.* Transformation of the nitrogen cycle: Recent trends, questions, and
509 potential solution. *Science*. **320**, 889–892 (2008).
- 510 4. Canfield, D. E., Glazer, A. N. & Falkowski, P. G. The evolution and future of earth's
511 nitrogen cycle. *Science*. **330**, 192–196 (2010).
- 512 5. Ryther, J. & Dunstan, W. Nitrogen, phosphorus, and eutrophication in the coastal marine
513 environment. *Science*. **171**, 1008–1013 (1971).
- 514 6. Conley, D. J. *et al.* Controlling eutrophication: Nitrogen and phosphorus. *Science*. **323**,
515 1014–1015 (2009).
- 516 7. Downing, J. A., Cherrier, C. T. & Fulweiler, R. W. Low ratios of silica to dissolved
517 nitrogen supplied to rivers arise from agriculture not reservoirs. *Ecol. Lett.* **19**, 1414–1418
518 (2016).
- 519 8. Carey, J. C. & Fulweiler, R. W. Human activities directly alter watershed dissolved silica
520 fluxes. *Biogeochemistry* **111**, 125–138 (2012).
- 521 9. Turner, R. E. *et al.* Fluctuating silicate:nitrate ratios and coastal plankton food webs. *Proc.*
522 *Natl. Acad. Sci. U. S. A.* **95**, 13048–13051 (1998).
- 523 10. Nixon, S. W. Coastal marine eutrophication: A definition, social causes, and future
524 concerns. *Ophelia* **41**, 199–219 (1995).
- 525 11. Kirby, M. X. Fishing down the coast: Historical expansion and collapse of oyster fisheries
526 along continental margins. *Proc. Natl. Acad. Sci. U. S. A.* **101**, 13096–13099 (2004).
- 527 12. Mackenzie, C. L. Causes underlying the historical decline in Eastern oyster (*Crassostrea*
528 *virginica* Gmelin, 1791) landings. *J. Shellfish Res.* **26**, 927–938 (2007).
- 529 13. Powell, E., Ashton-Alcox, K., Kraeuter, J., Ford, S. & Bushek, D. Long-term trends in
530 oyster population dynamics in Delaware Bay: regime shifts and response to disease. *J.*
531 *Shellfish Res.* **27**, 729–755 (2008).
- 532 14. Rick, T. *et al.* Millennial-scale sustainability of the Chesapeake Bay Native American
533 oyster fishery. *Proc. Natl. Acad. Sci. U. S. A.* **113**, 6568–6573 (2016).
- 534 15. Beck, M. W. *et al.* Oyster reefs at risk and recommendations for conservation, restoration,
535 and management. *Bioscience* **61**, 107–116 (2011).
- 536 16. Zu Ermgassen, P. S. E. *et al.* Historical ecology with real numbers: Past and present extent
537 and biomass of an imperilled estuarine habitat. *Proc. R. Soc. B Biol. Sci.* **279**, 3393–3400
538 (2012).
- 539 17. United Nations. *Transforming Our World: The 2030 Agenda for Sustainable*
540 *Development*. (2015) doi:10.1201/b20466-7.
- 541 18. Newell, R., Fisher, T., Holyoke, R. & Cornwell, J. Influence of Eastern Oysters on
542 Nitrogen and Phosphorus Regeneration in Chesapeake Bay, USA. in *The Comparative*
543 *Roles of Suspension-Feeders in Ecosystems* vol. 47 93–120 (Springer, 2005).
- 544 19. Kellogg, M. L. *et al.* Use of oysters to mitigate eutrophication in coastal waters. *Estuar.*
545 *Coast. Shelf Sci.* **151**, 156–168 (2014).
- 546 20. Ray, N. E., Maguire, T. J., Al-Haj, A., Henning, M. & Fulweiler, R. W. Low greenhouse
547 gas emissions from oyster aquaculture. *Environ. Sci. Technol.* **53**, 9118–9127 (2019).
- 548 21. Carman, M. R., Morris, J. A., Karney, R. C. & Grunden, D. W. An initial assessment of
549 native and invasive tunicates in shellfish aquaculture of the North American east coast. *J.*

- 550 *Appl. Ichthyol.* **26**, 8–11 (2010).
- 551 22. Guy-Haim, T. *et al.* Diverse effects of invasive ecosystem engineers on marine
552 biodiversity and ecosystem functions: A global review and meta-analysis. *Glob. Chang.*
553 *Biol.* **24**, 906–924 (2018).
- 554 23. Murray, A. G., Munro, L. A. & Matejusova, I. The network of farmed Pacific oyster
555 movements in Scotland and routes for introduction and spread of invasive species and
556 pathogens. *Aquaculture* **520**, 734747 (2020).
- 557 24. Rowe, G., Clifford, C. & Smith Jr, K. Benthic nutrient regeneration and its coupling to
558 primary productivity in coastal waters. *Nature* **255**, 215–217 (1975).
- 559 25. Seitzinger, S. P. Denitrification in freshwater and coastal marine systems: Ecological and
560 geochemical significance. *Limnol. Oceanogr.* **334**, 702–724 (1988).
- 561 26. Ray, N. E., Henning, M. C. & Fulweiler, R. W. Nitrogen and phosphorus cycling in the
562 digestive system and shell biofilm of the Eastern oyster (*Crassostrea virginica*). *Mar.*
563 *Ecol. Prog. Ser.* **621**, 95–105 (2019).
- 564 27. Duarte, C. M. *et al.* Rebuilding marine life. *Nature* **580**, 39–51 (2020).
- 565 28. Billion Oyster Project. <https://billionoysterproject.org/>.
- 566 29. FAO. *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable*
567 *development goals.* (2018).
- 568 30. Gentry, R. R. *et al.* Mapping the global potential for marine aquaculture. *Nat. Ecol. Evol.*
569 **1**, (2017).
- 570 31. Lacoste, E., Gueguen, Y., Moullac, G. Le, Koua, M. S. & Gaertner-Mazouni, N. Influence
571 of farmed pearl oysters and associated biofouling communities on nutrient regeneration in
572 lagoons of French Polynesia. *Aquac. Environ. Interact.* **5**, 209–219 (2014).
- 573 32. Buzin, F., Dupuy, B., Lefebvre, S., Barillé, L. & Haure, J. Storage of Pacific oysters
574 *Crassostrea gigas* in recirculating tank: Ammonia excretion and potential nitrification
575 rates. *Aquac. Eng.* **64**, 8–14 (2015).
- 576 33. Han, T. *et al.* Interactive effects of oyster and seaweed on seawater dissolved inorganic
577 carbon systems: Implications for integrated multi-trophic aquaculture. *Aquac. Environ.*
578 *Interact.* **9**, 469–478 (2017).
- 579 34. Kesarcodi-Watson, A., Klumpp, D. W. & Lucas, J. S. Comparative feeding and
580 physiological energetics of diploid and triploid Sydney rock oysters, *Saccostrea*
581 *commercialis* I. Effects of oyster size. *Aquaculture* **203**, 195–216 (2001).
- 582 35. Winter, J., Acevedo, M. & Navarro, J. Quempillen estuary, an experimental oyster
583 cultivation station in southern Chile. Energy balance in *Ostrea chilensis*. *Mar. Ecol. Prog.*
584 *Ser.* **20**, 151–164 (1984).
- 585 36. Sma, R. F. & Baggaley, A. Rate of excretion of ammonia by the hard clam *Mercenaria*
586 *mercenaria* and the American oyster *Crassostrea virginica*. *Mar. Biol.* **36**, 251–258
587 (1976).
- 588 37. Jackson, M. Characterization of Oyster-Associated Biogeochemical Processes in Oyster
589 Restoration and Aquaculture. (2019).
- 590 38. Mao, Y., Zhou, Y., Yang, H. & Wang, R. Seasonal variation in metabolism of cultured
591 Pacific oyster, *Crassostrea gigas*, in Sanggou Bay, China. *Aquaculture* **253**, 322–333
592 (2006).
- 593 39. Smyth, A. R., Gerald, N. R. & Piehler, M. F. Oyster-mediated benthic-pelagic coupling
594 modifies nitrogen pools and processes. *Mar. Ecol. Prog. Ser.* **493**, 23–30 (2013).
- 595 40. Caffrey, J. M., Hollibaugh, J. T. & Mortazavi, B. Living oysters and their shells as sites of

- 596 nitrification and denitrification. *Mar. Pollut. Bull.* **112**, 86–90 (2016).
- 597 41. Erler, D. V. *et al.* The impact of suspended oyster farming on nitrogen cycling and nitrous
598 oxide production in a sub-tropical Australian estuary. *Estuar. Coast. Shelf Sci.* **192**, 117–
599 127 (2017).
- 600 42. Arfken, A., Song, B., Bowman, J. S. & Piehler, M. Denitrification potential of the eastern
601 oyster microbiome using a 16S rRNA gene based metabolic inference approach. *PLoS*
602 *One* **12**, e0185071 (2017).
- 603 43. Jackson, M., Owens, M. S., Cornwell, J. C. & Kellogg, M. L. Comparison of methods for
604 determining biogeochemical fluxes from a restored oyster reef. *PLoS One* **13**, 1–14
605 (2018).
- 606 44. Gárate, M., Moseman-Valtierra, S. & Moen, A. Potential nitrous oxide production by
607 marine shellfish in response to warming and nutrient enrichment. *Mar. Pollut. Bull.* **146**,
608 236–246 (2019).
- 609 45. McCarthy, G., Ray, N. E. & Fulweiler, R. W. Greenhouse gas emissions from native and
610 non-native oysters. *Front. Environ. Sci.* **7**, (2019).
- 611 46. Kellogg, M. L., Cornwell, J. C., Owens, M. S. & Paynter, K. T. Denitrification and
612 nutrient assimilation on a restored oyster reef. *Mar. Ecol. Prog. Ser.* **480**, 1–19 (2013).
- 613 47. Higgins, C. B. *et al.* Effect of aquacultured oyster biodeposition on sediment N₂
614 production in Chesapeake Bay. *Mar. Ecol. Prog. Ser.* **473**, 7–27 (2013).
- 615 48. Green, D. S., Rocha, C. & Crowe, T. P. Effects of non-indigenous oysters on ecosystem
616 processes vary with abundance and context. *Ecosystems* **16**, 881–893 (2013).
- 617 49. Hyun, J. *et al.* Impacts of long-line aquaculture of Pacific Oysters (*Crassostrea gigas*) on
618 sulfate reduction and diffusive nutrient flux in the coastal sediments of Jinhae-Tongyeong,
619 Korea. *Mar. Pollut. Bull.* **74**, 187–198 (2013).
- 620 50. Hoellein, T. J. & Zarnoch, C. B. Effect of eastern oysters (*Crassostrea virginica*) on
621 sediment carbon and nitrogen dynamics in an urban estuary. *Ecol. Appl.* **24**, 271–286
622 (2014).
- 623 51. Andrieux-Loyer, F. *et al.* Impact of oyster farming on diagenetic processes and the
624 phosphorus cycle in two estuaries (Brittany, France). *Aquat. Geochemistry* **20**, 573–611
625 (2014).
- 626 52. Hoellein, T. J., Zarnoch, C. B. & Grizzle, R. E. Eastern oyster (*Crassostrea virginica*)
627 filtration, biodeposition, and sediment nitrogen cycling at two oyster reefs with
628 contrasting water quality in Great Bay Estuary (New Hampshire, USA). *Biogeochemistry*
629 **122**, 113–129 (2015).
- 630 53. Smyth, A. R., Piehler, M. F. & Grabowski, J. H. Habitat context influences nitrogen
631 removal by restored oyster reefs. *J. Appl. Ecol.* **52**, 716–725 (2015).
- 632 54. Mortazavi, B. *et al.* Evaluating the impact of oyster (*Crassostrea virginica*) gardening on
633 sediment nitrogen cycling in a subtropical estuary. *Bull. Mar. Sci.* **91**, 323–341 (2015).
- 634 55. Testa, J. M. *et al.* Modeling the impact of floating oyster (*Crassostrea virginica*)
635 aquaculture on sediment-water nutrient and oxygen fluxes. *Aquac. Environ. Interact.* **7**,
636 205–222 (2015).
- 637 56. Smyth, A. R., Gerald, N. R., Thompson, S. P. & Piehler, M. F. Biological activity
638 exceeds biogenic structure in influencing sediment nitrogen cycling in experimental oyster
639 reefs. *Mar. Ecol. Prog. Ser.* **560**, 173–183 (2016).
- 640 57. Humphries, A. T. *et al.* Directly measured denitrification reveals oyster aquaculture and
641 restored oyster reefs remove nitrogen at comparable high rates. *Front. Mar. Sci.* **3**, 74

- 642 (2016).
- 643 58. Lacoste, E. & Gaertner-Mazouni, N. Nutrient regeneration in the water column and at the
644 sediment-water interface in pearl oyster culture (*Pinctada margaritifera*) in a deep atoll
645 lagoon (Ahe, French Polynesia). *Estuar. Coast. Shelf Sci.* **182**, 304–309 (2016).
- 646 59. Smyth, A. R., Murphy, A. E., Anderson, I. C. & Song, B. Differential effects of bivalves
647 on sediment nitrogen cycling in a shallow coastal bay. *Estuaries and Coasts* **41**, 1147–
648 1163 (2018).
- 649 60. Onorevole, K. M., Thompson, S. P. & Piehler, M. F. Living shorelines enhance nitrogen
650 removal capacity over time. *Ecol. Eng.* **120**, 238–248 (2018).
- 651 61. Lunstrum, A., McGlathery, K. & Smyth, A. Oyster (*Crassostrea virginica*) aquaculture
652 shifts sediment nitrogen processes toward mineralization over denitrification. *Estuaries
653 and Coasts* **41**, 1130–1146 (2018).
- 654 62. Westbrook, P., Heffner, L. & La Peyre, M. K. Measuring carbon and nitrogen
655 bioassimilation, burial, and denitrification contributions of oyster reefs in Gulf coast
656 estuaries. *Mar. Biol.* **166**, 1–14 (2019).
- 657 63. Ray, N. E., Al-Haj, A. & Fulweiler, R. W. Sediment biogeochemistry along an oyster
658 aquaculture chronosequence. *Mar. Ecol. Prog. Ser.* **646**, 13–27 (2020).
- 659 64. Hassett, M. The Influence of Eastern Oyster (*Crassostrea virginica*) Reef Restoration on
660 Nitrogen Cycling in a Eutrophic Estuary. (2015).
- 661 65. Vieillard, A. Impacts of New England Oyster Aquaculture on Sediment Nitrogen Cycling:
662 Implications for Nitrogen Removal and Retention. (2017).
- 663 66. Boucher-Rodoni, R. & Boucher, G. *In situ* study of the effect of oyster biomass on benthic
664 metabolic exchange rates. *Hydrobiologia* **206**, 115–123 (1990).
- 665 67. Ayvazian, S. G. *et al.* Evaluating connections between nitrogen cycling and the
666 macrofauna in native oyster beds in a New England estuary. (In Review).
- 667 68. Mazouni, N., Gaertner, J., Deslous-Paoli, J., Landrein, S. & D’Oedenberg, M. Nutrient
668 and oxygen exchanges at the water-sediment interface in a shellfish farming lagoon (Thau,
669 France). *J. Exp. Mar. Bio. Ecol.* **205**, 91–113 (1996).
- 670 69. Porter, E. T., Cornwell, J. C., Sanford, L. P. & Newell, R. I. E. Effect of oysters
671 *Crassostrea virginica* and bottom shear velocity on benthic-pelagic coupling and estuarine
672 water quality. *Mar. Ecol. Prog. Ser.* **271**, 61–75 (2004).
- 673 70. Piehler, M. F. & Smyth, A. R. Habitat-specific distinctions in estuarine denitrification
674 affect both ecosystem function and services. *Ecosphere* **2**, 1–17 (2011).
- 675 71. Green, D. S., Boots, B. & Crowe, T. P. Effects of non-indigenous oysters on microbial
676 diversity and ecosystem functioning. *PLoS One* **7**, 1–10 (2012).
- 677 72. Gaertner-Mazouni, N. *et al.* Nutrient fluxes between water column and sediments:
678 Potential influence of the pearl oyster culture. *Marine Pollut. Bull.* **65**, 500–505 (2012).
- 679 73. Smyth, A. R. *et al.* Assessing nitrogen dynamics throughout the estuarine landscape.
680 *Estuaries and Coasts* **36**, 44–55 (2013).
- 681 74. Borenstein, M., Hedges, L., Higgins, J. & Rothstein, H. *Introduction to Meta-Analysis*.
682 (John Wiley and Sons, 2009).
- 683 75. Egge, J. & Aksnes, D. Silicate as a regulating nutrient in phytoplankton competition. *Mar.
684 Ecol. Prog. Ser.* **83**, 281–289 (1992).
- 685 76. Glibert, P. M. *et al.* Pluses and minuses of ammonium and nitrate uptake and assimilation
686 by phytoplankton and implications for productivity and community composition, with
687 emphasis on nitrogen-enriched conditions. *Limnol. Oceanogr.* **61**, 165–197 (2016).

- 688 77. Doering, P. H. *et al.* Structure and function in a model coastal ecosystem: silicon, the
689 benthos and eutrophication. *Mar. Ecol. Prog. Ser.* **52**, 287–299 (1989).
- 690 78. Vandevenne, F. I. *et al.* Grazers: biocatalysts of terrestrial silica cycling. *Proc. R. Soc. B*
691 *Biol. Sci.* **280**, (2013).
- 692 79. Newell, R. I. E. Ecosystem influences of natural and cultivated populations of suspension-
693 feeding bivalve molluscs: A review. *J. Shellfish Res.* **23**, 51–61 (2004).
- 694 80. Kana, T. M. *et al.* Membrane inlet mass spectrometer for rapid high-precision
695 determination of N₂, O₂, and Ar in environmental water samples. *Anal. Chem.* **66**, 4166–
696 4170 (1994).
- 697 81. Nielsen, L. Denitrification in sediment determined from nitrogen isotope pairing
698 technique. *FEMS Microbiol. Lett.* **86**, 357–362 (1992).
- 699 82. Eyre, B. D., Rysgaard, S. S., Dalsgaard, T. & Christensen, P. B. Comparison of isotope
700 pairing and N₂:Ar methods for measuring sediment denitrification—Assumptions,
701 modifications, and implications. *Estuaries* **25**, 1077–1087 (2002).
- 702 83. Ferguson, A. J. P. & Eyre, B. D. Seasonal discrepancies in denitrification measured by
703 isotope pairing and N₂:Ar techniques. *Mar. Ecol. Prog. Ser.* **350**, 19–27 (2007).
- 704 84. Cornwell, J. C., Kemp, W. M. & Kana, T. M. Denitrification in coastal ecosystems:
705 methods, environmental controls, and ecosystem level controls, a review. *Aquat. Ecol.* **33**,
706 41–54 (1999).
- 707 85. Eyre, B. D. & Ferguson, A. J. P. Comparison of carbon production and decomposition,
708 benthic nutrient fluxes and denitrification in seagrass, phytoplankton, benthic
709 microalgae- and macroalgae- dominated warm-temperate Australian lagoons. *Mar. Ecol.*
710 *Prog. Ser.* **229**, 43–59 (2002).
- 711 86. Fulweiler, R. W., Nixon, S. W., Buckley, B. A. & Granger, S. L. Net sediment N₂ fluxes
712 in a coastal marine system-experimental manipulations and a conceptual model.
713 *Ecosystems* **11**, 1168–1180 (2008).
- 714 87. *PAS 2050:2011 Specification for the assessment of the life cycle greenhouse gas emissions*
715 *of goods and services.* (2011).
- 716 88. *PAS 2050-2:2012 Assessment of life cycle greenhouse gas emissions - Supplementary*
717 *requirements for the application of PAS2050:2011 to seafood and other aquatic products.*
718 (2012).
- 719 89. Fodrie, F. J. *et al.* Oyster reefs as carbon sources and sinks. *Proc. R. Soc. B Biol. Sci.* **284**,
720 20170891 (2017).
- 721 90. Ray, N. E., O’Meara, T., Williamson, T., Izursa, J.-L. L. & Kangas, P. C. Consideration of
722 carbon dioxide release during shell production in LCA of bivalves. *Int. J. Life Cycle*
723 *Assess.* **23**, 1042–1048 (2018).
- 724 91. Filgueira, R. *et al.* An integrated ecosystem approach for assessing the potential role of
725 cultivated bivalve shells as part of the carbon trading system. *Mar. Ecol. Prog. Ser.* **518**,
726 281–287 (2015).
- 727 92. Troost, K. Causes and effects of a highly successful marine invasion: Case-study of the
728 introduced Pacific oyster *Crassostrea gigas* in continental NW European estuaries. *J. Sea*
729 *Res.* **64**, 145–165 (2010).
- 730 93. Scanes, E. *et al.* Quantifying abundance and distribution of native and invasive oysters in
731 an urbanised estuary. *Aquat. Invasions* **11**, 425–436 (2016).
- 732 94. Laugen, A. T., Hollander, J., Obst, M. & Strand, Å. 10. The Pacific oyster (*Crassostrea*
733 *gigas*) invasion in Scandinavian coastal waters: Impact on local ecosystem services. in

- 734 *Biological Invasions in Changing Ecosystems: Vectors, Ecological Impacts, Management*
735 *and Predictions* (ed. Canning-Clode, J.) 230–246 (De Gruyter Open, 2015).
- 736 95. Erbland, P. J. & Ozbay, G. A comparison of the macrofaunal communities inhabiting a
737 *Crassostrea virginica* oyster reef and oyster aquaculture gear in Indian River Bay,
738 Delaware. *Journal of Shellfish Research* vol. 27 757–768 (2008).
- 739 96. Marengi, F., Ozbay, G., Erbland, P. J. & Rossi-Snook, K. A comparison of the habitat
740 value of sub-tidal and floating oyster (*Crassostrea virginica*) aquaculture gear with a
741 created reef in Delaware’s Inland Bays, USA. *Aquac. Int.* **18**, 69–81 (2010).
- 742 97. Tallman, J. & Forrester, G. Oyster grow-out cages function as artificial reefs for temperate
743 fishes. *Trans. Am. Fish. Soc.* **136**, 790–799 (2007).
- 744 98. Hossain, M. *et al.* Oyster aquaculture for coastal defense with food production in
745 Bangladesh. *Aquac. Asia* **18**, 15–24 (2013).
- 746 99. Piazza, B. P., Banks, P. D. & La Peyre, M. K. The potential for created oyster shell reefs
747 as a sustainable shoreline protection strategy in Louisiana. *Restor. Ecol.* **13**, 499–506
748 (2005).
- 749 100. *Fisheries of the United States, 2017.* (2018).
- 750 101. Delgado, C. L. Rising consumption of meat and milk in developing countries has created a
751 new food revolution. *J. Nutr* **133**, 3907–3910 (2003).
- 752 102. Sans, P. & Combris, P. World meat consumption patterns: An overview of the last fifty
753 years (1961-2011). *Meat Sci.* **109**, 106–111 (2015).
- 754 103. Viechtbauer, W. Conducting meta-analyses in R with the metafor package. *J. Stat. Softw.*
755 **36**, 1–48 (2010).
- 756 104. Harrer, M., Cuijpers, P., Furukawa, T. & Ebert, D. *Doing Meta-Analysis in R: A Hands-*
757 *On Guide.* (2019).
- 758 105. Anton, A. *et al.* Global ecological impacts of marine exotic species. *Nat. Ecol. Evol.* **3**,
759 787–800 (2019).
- 760 106. Harrer, M., Cuijpers, P., Furukawa, T. & Ebert, D. *dmetar*: Companion R package for the
761 guide ‘Doing Meta-Analysis in R’. *R Packag. version 0.0.9* (2019).
- 762 107. Rudolph, J., Frenzel, P. & Pfennig, N. Acetylene inhibition technique underestimates in
763 situ denitrification rates in intact cores of freshwater sediment. *FEMS Microbiol. Lett.* **85**,
764 101–106 (1991).
- 765 108. Fulweiler, R. W. *et al.* Examining the impact of acetylene on N-fixation and the active
766 sediment microbial community. *Front. Microbiol.* **6**, 1–9 (2015).
- 767