Quantifying Nitrogen Removal by Oysters

Workshop Report

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Executive Summary

The NOAA Chesapeake Bay Office sponsored a workshop with the goal of evaluating the current state of knowledge related to the capacity for oysters to remove nitrogen from coastal waters. The workshop, which was held at Virginia Institute of Marine Sciences Eastern Shore Lab in Wachapreague, VA, on January 10 – 11, brought together a total of 30 resource management agency personnel, restoration practitioners and scientists with expertise in the field to (1) identify the best available values, or ranges of values, for nitrogen removal by oysters primarily focusing on denitrification and bioassimilation, (2) discuss the uncertainty associated with these estimates, (3) identify the research needed to fill data gaps and (4) discuss minimum requirements for studies to accurately measure nitrogen removal rates associated with oysters.

The workshop included five presentations by scientists detailing their field studies that have measured nitrogen dynamics associated with oyster reefs and oyster aquaculture in Chesapeake Bay and elsewhere. The range of observed values for nitrogen (1) assimilated in oyster soft tissue and shell and (2) removed from the system via coupled nitrification/denitrification processes were summarized. Workshop participants engaged in fruitful discussions about the sources of variation in the observed values, the generality of the findings, and the conditions under which it is appropriate to apply the few estimates that currently exist.

The overarching finding of the workshop was that our current state of knowledge on the effects of oysters, both on reefs and in aquaculture, on nitrogen dynamics is incomplete in many respects. Removal of particulate nitrogen from the water column via filtration, incorporation into oyster tissues and biodeposition of nitrogen are all relatively straightforward to quantify, though the rates vary with environmental conditions and oyster growth. Determining the portion of that nitrogen that is returned to the atmosphere or sequestered for a significant period of time is less straightforward. No published rates exist for burial of nitrogen associated with oyster reefs or oyster aquaculture. Four separate studies conducted in a total of 14 different tributaries and sub-estuaries from Cape Cod, MA, Great Bay, NH and Chesapeake Bay provide very similar estimates of amount of nitrogen as a percent of dry weight found in soft tissue ($8.22 \pm 0.89\%$ SD) of the Eastern Oyster, *Crassostrea virginica*, across a range of conditions, including subtidal reefs, floating aquaculture, high and low flow regimes, and varying degrees of eutrophication. Two of these studies, both conducted in Chesapeake Bay, also measured the nitrogen content of oyster shell. They provide similar estimates (0.19% and 0.21%) of the amount of nitrogen as a percent of oyster shell dry weight. The consistency of these values suggests that reasonably accurate estimates can be made of the amount of nitrogen removed via oyster harvest. This finding is tempered, however, by the results of one study in Mobile Bay, AL which found that nitrogen comprised 11.8% of oyster soft tissue dry weight, suggesting that the percentage of nitrogen in oyster tissue can vary by location. We also caution that estimating <u>potential</u> nitrogen removal via oyster harvest is subject to a much greater uncertainty related to the variance associated with oyster growth and survival rates, necessitating precise measures of oyster abundance and biomass at harvest. In addition, the ratio of oyster tissue to oyster shell dry weight varies widely with location, growing conditions and physiological status of oysters, with aquacultured oysters tending to have higher tissue to shell ratios than wild oysters.

Data collected thus far on denitrification associated with either intensive oyster aquaculture or oyster reefs show much greater variability than data on nitrogen bioassimilation. To date, no study has shown significant net annual enhancement of denitrification associated with intensive oyster aquaculture. At present, we recommend assigning a value of zero for nitrogen removal via denitrification associated with aquacultured oysters. However, we note that the only existing data come from studies of the sediments beneath oysters growing in aquaculture floats. As additional data become available for other types of intensive aquaculture and/or for nitrogen dynamics within aquaculture floats, this recommendation should be reviewed and revised as needed.

Although data on denitrification associated with oyster reefs suggest that they significantly enhance net annual denitrification rates over those at reference sites, the degree of enhancement is highly variable. Denitrification rates vary with season, tidal regime, oyster biomass density and other unidentified factors. Additional studies are needed to better understand the sources of this variation prior to assigning a value to the nitrogen removal capacity of oyster reefs attributable to denitrification. Although it is not possible at present to provide generalized relationships for estimating the enhancement in denitrification associated with oyster reefs under varying conditions, reliable methods do exist for measuring these rates, interest in clarifying these relationships is growing and new research is underway.

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Background

It has long been recognized that suspension-feeding bivalves can alter water quality, through top-down control of phytoplankton, biodeposition of suspended sediments and alteration of nutrient dynamics (Officer et al. 1982; Newell 1988, 2004; Newell et al. 2002, 2005; Newell and Koch 2004). By calculating that the summertime filtration capacity of the oyster population of Chesapeake Bay in late 19th Century would have allowed them to filter a volume of water equivalent to the entire Bay in 3 – 6 days, Newell (1988) sparked public interest in the role of oysters in controlling water quality, providing much of the impetus for numerous restoration programs by environmental groups and government agencies (Brumbaugh and Coen 2009, Kennedy et al. 2011). It also generated considerable scientific debate about the capacity of oysters, either historical or restored populations, to affect phytoplankton dynamics at a bay-wide or tributary level (e.g., Pomeroy et al. 2006, 2007, Coen et al. 2007, Newell et al. 2007, Fulford et al. 2007). In its focus on the ability of oysters to filter phytoplankton from the water column, this debate has generally overlooked the fact that far greater uncertainty about the role of oysters in improving water quality surrounds the fate of the nitrogen contained in phytoplankton filtered by oysters.

As federal, state and local governments seek ways to meet new water quality improvement goals for the Bay, there is growing interest in incorporating the effects of public oyster restoration projects and private aquaculture into Bay water quality models, load reduction strategies and nutrient trading markets. Doing so requires an understanding of the effects of oysters on the removal of nitrogen, the primary pollutant of concern.

Nitrogen entering an estuary from the watershed and airshed stimulates phytoplankton growth, excesses of which can result in eutrophication and oxygen-depleted dead zones (Kemp et al. 2005). Benthic grazers, including oysters, remove a portion of the phytoplankton from the water column and facilitate the transformation of nitrogen into other forms, some of which do not support phytoplankton growth (Fig. 1 and Appendix Fig. A1-A5). Importantly, there are three primary means by which nitrogen can be removed from the water column for a significant amount of time: 1) conversion to a gaseous form with subsequent return to the atmosphere, 2) conversion to animal tissue or shell and 3) deep burial in sediments. The primary pathway of conversion from organic nitrogen to gaseous forms on oyster reefs is thought to be microbially-

mediated coupling of nitrification-denitrification, although the role of anammox and release of nitrous oxide have yet to be elucidated. Bioassimilation of nitrogen into the tissues of oysters, other grazers and higher trophic levels represents a more or less ephemeral pool of nitrogen within the system. However, bioassimilation into oyster shell may represent a means of longer term or permanent sequestration if shells are removed from the estuary or deeply buried. Harvesting oysters removes the bioassimilated nitrogen from the estuary, but as seen in the case of oysters in the Chesapeake Bay, overharvesting leads to population decline and system degradation. Burial of oyster biodeposits and shell can remove nitrogen, but the timescale of this removal has yet to be estimated and is likely to vary widely among sites with the majority of biodeposits being buried at some sites and being remineralized at others.

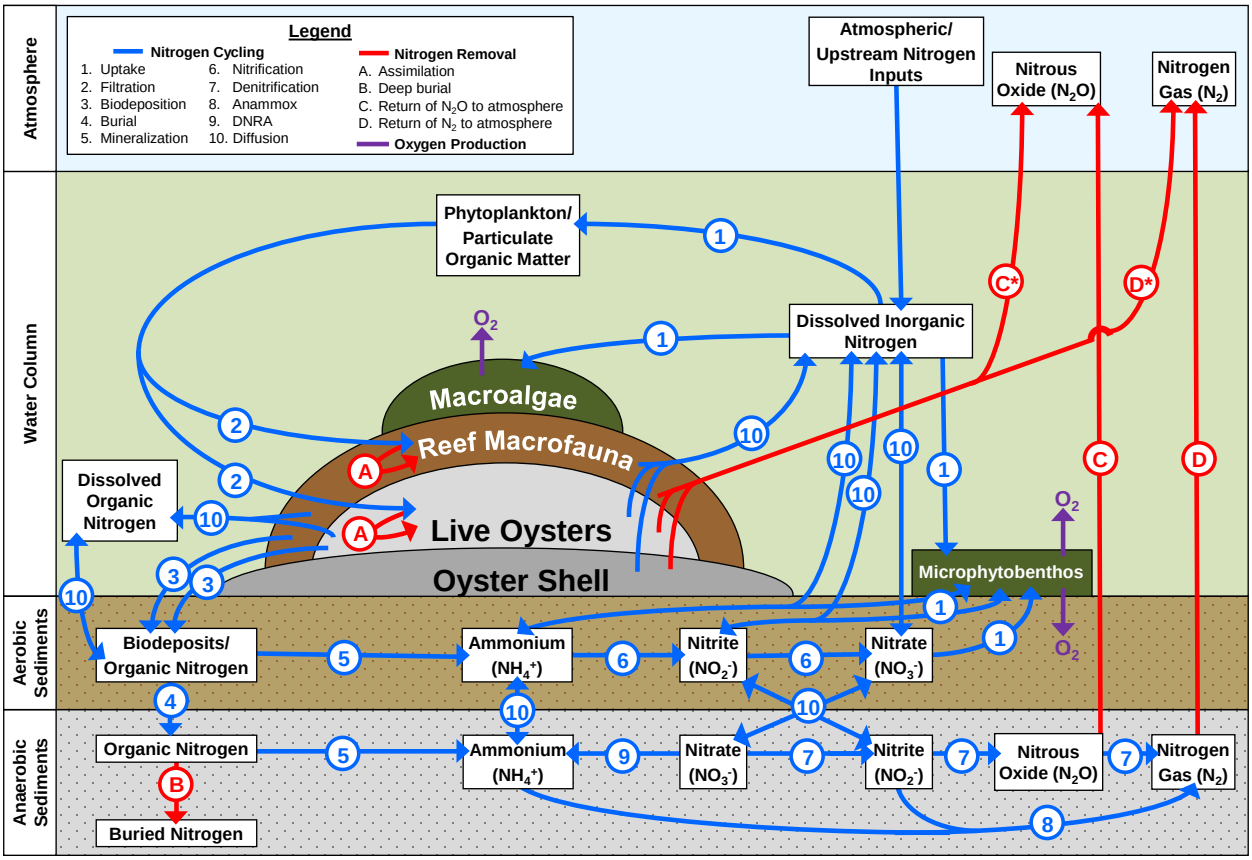

Figure 1. Primary nitrogen cycling and nitrogen removal pathways for a shallow subtidal or submerged intertidal oyster reef in the euphotic zone.

The potential for shellfish aquaculture to participate in nutrient trading markets has been suggested in the scientific literature (e.g., Newell 2004, Lindhal et al. 2005, Shabman and Stevenson 2007) and is currently being considered by resource management agencies in both

Virginia and Maryland. Ongoing publically- and privately-funded oyster restoration efforts are, in part, based upon the expectation of water quality benefits and oyster restoration is being considered by some local governments as a Best Management Practice (BMP) to meet their Total Maximum Daily Load (TMDL) allocations (e.g., VA Beach: http://www.vbgov.com/ government/offices/eso/ Documents/tmdl-local-strategy.pdf). Recognition of the need for well-supported, consensus-based values for the nutrient removal capacity of oysters in support of these considerations served as the impetus for the workshop.

Purpose of the Workshop

The NOAA Chesapeake Bay Office sponsored the workshop with the goal of evaluating the current state of knowledge related to the capacity for oysters to remove nitrogen from coastal waters. The workshop, which was held at Virginia Institute of Marine Sciences Eastern Shore Lab in Wachapreague, VA, on January 10 – 11, 2013, brought together a total of 30 scientists, resource management agency personnel and restoration practitioners with expertise in the field to (1) identify the best available values, or ranges of values, for nitrogen removal by oysters primarily focusing on denitrification and bioassimilation, (2) discuss the uncertainty associated with these estimates, (3) identify the research needed to fill data gaps and (4) discuss minimum requirements for studies to accurately measure nitrogen removal rates associated with oysters.

Workshop Scope and Structure

Prior to the workshop a literature review was conducted and relevant publications made available to all participants. This initial review included data on nutrient content in numerous bivalve species, including oysters (*Crassostrea gigas, C. virginica, Pinctada imbricata*) and mussels (*Mytilis edulis, M. galloprovincialis*, *Perna canaliculus* and *Geukensia demissa*) (reviewed in Carmichael et al. 2012), and denitrification rates measured in large-scale aquaculture of clams (*Tapes philippinarum*) in the Saca di Goro lagoon in Italy (Nizolli et al. 2011). After reviewing these data the decision was made that the workshop would best address its charge by focusing only on those data obtained for the Eastern oyster *Crassostrea virginica*. The workshop included five presentations by scientists (Brown, Carmichael, Cornwell, Kellogg and Phieler) detailing their past and on-going field studies that have measured nitrogen dynamics associated with *C. virginica* in Chesapeake Bay and elsewhere. The range of observed values for

nitrogen (1) assimilated in oyster soft tissue and shell and (2) removed from the system via coupled nitrification/denitrification processes were summarized. Workshop participants engaged in fruitful discussions about the sources of variation in the observed values, the generality of the findings, and the conditions under which it is appropriate to apply the few estimates that currently exist to oyster-related nutrient removal in other locations or under other conditions.

Through these discussions three additional limitations were placed on the scope and the eventual conclusions and recommendations from the workshop. Two pertained to the definition of aquaculture under consideration and one to policy recommendations. With regard to oyster aquaculture, definitions and approaches commonly used in the Chesapeake Bay vary (Table 1). Though frequently considered aquaculture, *extensive aquaculture* practices, which rely on the recruitment of wild oysters onto planted shell or the transplantation of wild juvenile oysters are fundamentally extensions of wild fishery practices and are not included in our recommendations related to aquaculture. Further, though *spat-on-shell aquaculture* shares some techniques with restoration approaches used in recruitment-limited areas, it varies sufficiently in harvest practices and population age structure that data on enhanced denitrification rates derived from sanctuary reefs constructed in this manner are not applicable to this form of aquaculture. Finally, though our discussions frequently turned to policy implications of the findings, we concluded that the groups' expertise lay primarily outside that arena and limited our conclusions and recommendations to interpreting the data and determining how they can appropriately be used.

Table 1. Summary of aquaculture practices in the Chesapeake Bay.

Type	**Definition**	**Approach**	**Description**
Extensive	Cultivation of natural wild stocks	Shell planting	Oyster shell placed on the bottom to attract recruitment of wild oysters
		Seed relay	Transplant wild juvenile oysters from natural reefs to private leases
Intensive	Cultivation of hatchery-produced oysters	Spat-on-shell bottom aquaculture	Hatchery-produced larvae settled onto shell then planted on bottom leases
		Bottom cage or rack-and-bag aquaculture	Cultchless oysters raised in protective cages or bags near the bottom
		Suspended aquaculture	Cultchless oysters reared in floating cages near the surface

Summary and Meta-Analysis of Existing Data

A preliminary review of the published literature on the role of oysters and oyster reefs in nitrogen cycling quickly made it clear that few published data exist. One of the workshop goals was to gather scientists actively working in this field to learn more about ongoing projects and unpublished data. The studies described below span the range from completed works resulting in published peer-reviewed papers to ongoing projects that are still gathering data.

Nitrogen assimilation

Summary - We define assimilated nitrogen as the nitrogen contained in the soft tissue and/or shell of an oyster at the time of sampling. All nitrogen data considered as part of our review were reported as a percentage of the total dry weight of the material sampled. Each study is described briefly below and data are summarized in Tables 2 and 3.

Newell (2004) reported the nitrogen content of the soft tissue (7.0% N) and shells (0.3% N) of wild oysters from a natural reef in Chesapeake Bay. Because information on study site characteristics, the number of oysters analyzed and associated variance of nitrogen content are not provided, we have not included these data in Tables 2 or 3.

Higgins et al. (2011) measured the nitrogen content of the soft tissue and shell of individual oysters grown in floating aquaculture cages at two sites in Chesapeake Bay: Spencer's Creek, VA and St. Jerome Creek, MD. They found no significant differences in the percentage of nitrogen in oyster shell or tissue between sites (Tables 2 and 3). Using data on the nitrogen content of a range of oyster size classes they found that total nitrogen content of an aquacultured oyster in their study could be predicted based on its total length (i.e. shell height) using the equation:

$$TN = e^{(-14.1569+2.7994*\ln(TL))}$$

where: TN = total nitrogen content (g)

TL = total oyster shell height (mm)

Higgins et al. noted that this equation results in a total predicted nitrogen content for a harvest-sized (76.2 mm) aquacultured oyster that is one-fourth of that previously estimated for wild oysters in Chesapeake Bay (Newell 2004) and attributed the difference to a combination of the lower shell weight and the lower shell nutrient content of aquacultured oysters.

Carmichael et al. (2012) reported original measurements of the nitrogen content of oyster soft tissue and reviewed previously published studies that included measurements of the nitrogen in the soft tissue of several bivalve species. For their own study, Carmichael et al. (2012) deployed oysters at five locations near Cape Cod, MA that spanned a range of nitrogen loading levels and measured the nitrogen content of oyster soft tissue. They found no significant differences among sites (Table 2) and used the mean value (8.6% N) for all subsequent calculations.

Kellogg et al. (2013) measured the nitrogen content of the tissues and shells of oysters collected from a restored subtidal oyster reef in the Choptank River, MD (Tables 2 and 3). Each sample contained material from multiple oysters (n = 3-6). Oyster tissue nitrogen content as a percent of dry weight (9.27% N) was slightly higher than that reported by Higgins et al. (2011) but shell nitrogen content (0.21% N) was very similar. This same study sampled oyster shells used as part of the site preparation prior to restoration. These shells, assumed to be >7 years old, contained less nitrogen (0.15% N) than shells from live oysters at the same site (Table 3, Kellogg unpublished data).

Dalrymple and Carmichael measured the percentage of nitrogen in both the tissues and shells of juvenile and adult oysters (Dalrymple 2013; Dalrymple and Carmichael, In prep) and of diploid and triploid oysters (Dalrymple 2013; Dalrymple et al., In prep) held at two sites in Mobile Bay, Alabama. Sites were sampled nine times at two week intervals. Tissue nitrogen content did not differ with ploidy or site within Mobile Bay, but values (11.8% N) in soft tissue were higher than those observed in studies along the Atlantic Coast. Collection of data on oyster shell nitrogen content is complete and will be available in the near future.

Grizzle and Ward (2011) reported data on nitrogen content of two size classes of cultured oysters from six locations in Great Bay, NH. They observed significant differences in soft tissue nitrogen content across the six sites with mean values ranging from 5.64 to 9.27%. Although Grizzle and Ward characterized these data as preliminary, we chose to include them in our analysis because many of their observed values fall within the range observed in other studies (Table 3).

Table 2. Nitrogen content of oyster tissue as a percentage of dry weight. N = number of oysters sampled, SH = Shell height, * All values calculated using raw data provided in report appendix.

Source	Growing Conditions	Study Site and Environmental Conditions	% Nitrogen Mean	% Nitrogen Range	N
Higgins et al. (2011)	Floating aquaculture cages Oysters per cage = 200 Cage area = 0.5 m^{-2} Mean SH = 44 – 118 mm	Spencer's Creek, VA Salinity = 5 – 15 Low flow, high sedimentation	8.10 ± 0.13 SE	5.80 – 9.97	47
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation	7.37 ± 0.19 SE	5.43 – 10.36	37
Carmichael et al. (2012)	Cages 6 cm off bottom Oysters per cage = 67 Cage area = 0.15 m^{-2} SH = 8.2 ± 0.2 mm at start of study Maximum SH ~68 mm at end of study	Sage Lot Pond, Cape Cod, MA Salinity = 28 N load =14 x 10^{-4} kg N m^{-2} y^{-1}	8.47 ± 0.09 SE	N/A	160
		Wild Harbor, Cape Cod, MA Salinity = 26 N load = 65 x 10^{-4} kg N m^{-2} y^{-1}	8.95 ± 0.16 SE	N/A	160
		Green Pond, Cape Cod, MA Salinity = 28 N load = 178 x 10^{-4}kg N $m^{-2}y^{-1}$	8.04 ± 0.24 SE	N/A	160
		Snug Harbor, Cape Cod, MA Salinity = 25 N load = 236 x 10^{-4}kg N $m^{-2}y^{-1}$	9.19 ± 0.15 SE	N/A	160
		Childs River, Cape Cod, MA Salinity = 26-27 N load = 601 x 10^{-4}kg N $m^{-2}y^{-1}$	8.37 ± 0.27 SE	N/A	160
Kellogg et al. (2013)	Restored oyster reef Oyster density = 131 m^{-2} Mean SH = 114 mm	Choptank River, MD Salinity = 7.0-11.6 Subtidal reef	9.27 ± 0.60 SD	8.58 – 9.71	15[b]
Dalrymple and Carmichael (In prep)	Cages ~10-20 cm off bottom Cage area = 0.65 m^{-2} Mean juvenile SH = 42mm Mean adult SH = 98mm	Mobile Bay, AL 2 study sites	11.8 ± 0.1 SE	9.10 – 13.54	108
Grizzle and Ward (2011) [a]	Cages ~10-20 cm off bottom Oyster density per cage: "Seed" = 1,000 indiv. 1-yr olds= 200 indiv.	Adams Point, Great Bay, NH	7.20 ± 1.61 SD	5.20 - 9.56	10
		Bellamy River, Great Bay, NH	6.63 ± 2.13 SD	3.00 - 9.87	10
		Oyster River, Great Bay, NH	7.55 ± 2.14 SD	3.23 - 9.55	9
		Fox Point, Great Bay, NH	5.64 ± 1.70 SD	3.85 - 9.07	10
		Nannie Island, Great Bay, NH	7.39 ± 2.07 SD	3.70 - 10.66	10
		Squamscott R., Great Bay, NH	9.27 ± 2.38 SD	5.13 - 14.01	10

[a] All values calculated using raw data provided in report appendix

[b] Three samples composed of five individuals per sample

Table 3. Nitrogen content of shell as a percentage of dry weight. SH = Shell height, N = number of oysters sampled.

Source	Growing Conditions	Study Site and Environmental Conditions	% Nitrogen Average	% Nitrogen Range	N
Higgins et al. (2011)	Floating cages 200 oysters per bag Bag size = 100 cm L x 50 cm W x 8 cm D	Spencer's Creek, VA Salinity = 5 – 15 Low flow, high sedimentation	0.20 ± 0.01 SE	0.11 – 0.39	47
		St. Jerome Creek, MD Salinity = 12 – 15 High flow, low sedimentation	0.20 ± 0.02 SE	0.11 – 0.48	37
Kellogg et al. (2013)	Restored oyster reef Oyster density = 131 m^{-2} Mean SH = 114 mm	Choptank River, MD Salinity = 7.0-11.6	0.21 ± 0.08 SD	0.16-0.30	16[a]
Kellogg et al. (Unpublished data)	Restored oyster reef Aged shell (presumed to be >7 years old)	Choptank River, MD Salinity = 7.0-11.6	0.15 ± 0.02 SD	0.13-0.17	15[b]

[a] Three samples composed of four to six individuals per sample
[b] Three samples composed of five individuals per sample

Meta-analysis - The studies included in our meta-analysis measured nitrogen content of oyster tissue at a total of 16 sites. Six of these sites were in close proximity to one another in New Hampshire, another five in Cape Cod, three sites were in Chesapeake Bay and two sites were in Mobile Bay. Mean values for soft tissue at individual sites range from 7.20 to 11.8% N with the highest values measured at Mobile Bay. Averaging across studies conducted on the Atlantic Coast yielded a mean oyster tissue nitrogen content of 8.22 ± 0.89% N. Although differences in the nitrogen content of oyster tissue across sites on the Atlantic Coast appears to be relatively small, it should be noted that no studies have been conducted on the Atlantic coast of the United States south of Chesapeake Bay and none of these studies have explicitly considered the impact of oyster reproductive condition on nitrogen content.

The two recent studies measured the nitrogen content of the shells of living oysters from three sites in Chesapeake Bay produced similar estimates of 0.20 and 0.21% N (Higgins et al. 2011 and Kellogg et al. 2013, respectively; Table 3). Lower nitrogen content in aged shell suggests that the nitrogen content of shell declines through time (Kellogg, unpublished data; Carmichael, unpublished data).

Although variation in the percentage of nitrogen in the tissues and shell of aquacultured oysters versus those collected from natural or restored oyster reefs is relatively small, the ratio of oyster tissue dry weight to oyster shell dry weight varies widely. The significantly higher ratio of tissue dry weight to shell dry weight for aquacultured oysters likely reflects differences in growing conditions.

Denitrification

Summary - Few studies have been conducted that directly measure the impacts of oysters on denitrification rates. Below are brief summaries of studies published to date and descriptions of ongoing work. To facilitate comparisons across studies, only data resulting from studies using membrane inlet mass spectrometery (MIMS) to assess net fluxes of N_2 gas in the water column are included below, although Higgins et al. (2013) also made measurements using ^{15}N. All values have been converted to the same units (μmol N_2-N m^{-2} h^{-1}) and reported in terms of enhancement of denitrification over that measured at an appropriate reference site. For the purposes of this review, we define enhancement of denitrification as a net increase in flux of nitrogen gas from the sediments into the water column as compared to a reference site. Data from sites without an appropriate reference site nearby have been excluded.

Laboratory Experiments

Newell et al. (2002) simulated the effects of oyster biodeposition on estuarine nitrogen dynamics in the laboratory. Under aerobic conditions in the absence of light, they found that ~20% of the nitrogen in simulated bivalve biodeposits (pelletized *Thallasosira pseudonana*) was converted to nitrogen gas and returned to the atmosphere via denitrification. Under anoxic conditions, organic nitrogen was returned to the water column as ammonium. Under oxic conditions with sufficient light, a benthic algal and cyanobacterial community developed that both absorbed inorganic nitrogen and fixed nitrogen. Many subsequent estimates of the influence of oysters on nitrogen cycling and related modeling efforts have been based upon the results of this laboratory simulation of the effects of oyster biodeposition.

Aquaculture

Only two studies addressing the effects of intensive aquaculture of *Crassostrea virginica* on nitrogen dynamics currently exist. In both cases nitrogen fluxes from sediments into the water column were measured beneath oysters in suspended aquaculture and at adjacent sites without oyster cultivation that served as reference sites. No data currently exist on nitrogen dynamics associated with other forms of intensive oyster aquaculture (see Table 1).

Holyoke (2008) studied the impact of oysters growing in aquaculture floats on nitrogen dynamics in underlying sediments during four sampling periods at each of three sites in La

Trappe Creek, MD. Impacts were highly variable across sampling periods and under both light and dark conditions. Of the 24 estimates of denitrification rates produced by this study (12 under light conditions and 12 under dark conditions), half indicate reduced denitrification at the aquaculture site compared to the reference site. Of the 12 estimates that indicate enhanced denitrification, only three rates were >50 µmol N_2-N m^{-2} h^{-1} (>0.7 mg N_2-N m^{-2} h^{-1}).

Higgins et al. (2013) studied the impacts of oysters growing in aquaculture floats on nitrogen dynamics in underlying sediments. Studies were conducted at a low flow, high sedimentation site in Spencer's Creek, VA and a high flow, low sedimentation site in St. Jerome Creek, MD. In addition to an oyster treatment, which included oysters in floats at common aquaculture densities, this study included a "biodeposit fence" treatment that prevented dispersal of biodeposits, concentrating them immediately beneath the floats. To facilitate comparisons with other studies, our meta-analysis below only considers the data from their oyster treatment and reference sites. Similar to Holyoke (2008), this study found both positive and negative impacts on denitrification. Denitrification rates at St. Jerome Creek were reduced compared to the reference site in May but enhanced in August. The effect of oysters on denitrification ranged from a reduction of -59.2 µmol N_2-N m^{-2} h^{-1} (0.8 mg N_2-N m^{-2} h^{-1}) to an enhancement of 95.0 µmol N_2-N m^{-2} h^{-1} (1.3 mg N_2-N m^{-2} h^{-1}) relative to reference sites (Table 4).

Table 4. Effects of intensive oyster aquaculture on denitrification (DNF) rates calculated as the flux of nitrogen gas from the sediments to the atmosphere at the aquaculture site minus the flux at a reference site. Positive values indicate enhanced DNF rates and negative values reduced DNF rates relative to the reference site. LTC = La Trappe Creek.

Source	Growing Conditions	Study Site and Environmental Conditions	Month(s) Sampled	Incubation Type	Average Effect on DNF (µmol N_2-N m^{-2} h^{-1})
Holyoke (2008)	Floating cages Oyster density = 184-216 m^{-2}	Lowry Cove, LTC, MD Salinity = 13.25 ± 0.96 PAR: ~70-80 µmol m^{-2} s^{-1}	Jul. Aug. Sep.	Light	-36.1 – 25.2
				Dark	-86.1 – 343.5
		Mainstem, LTC, MD Salinity = 6.75 ± 1.50 PAR: ~70-80 µmol m^{-2} s^{-1}	Jul. Aug. Sep.	Light	-7.1 – 117.6
				Dark	-103.1 – 48.0
		Pier, LTC, MD Salinity = 5.50 ± 1.29 PAR: 5-25 µmol m^{-2} s^{-1}	May Jun. Jul.	Light	-118.6 – 6.0
				Dark	-146.3 – -32.6
Higgins et al. (2013)	Floating cages 200 oysters per bag Cage area = 0.5 m^{-2} Max. oyster density = 286 m^{-2}	Spencer's Creek, VA Salinity = 5-15 Low flow, high sedimentation	Aug.	Dark	70.8
		St. Jerome Creek, MD Salinity = 12-15 High flow, low sedimentation	May Aug.	Dark	-59.2 – 95.0

Oyster Reefs

Estimates of denitrification rates associated with oyster reefs have been made in five recently completed studies and one on-going study (Table 5). The study sites spanned a wide range of conditions from intertidal to subtidal below the euphotic zone and had salinities ranging from 7 to 36. The sites included natural intertidal reefs, reefs formed from shell plantings and wild recruitment on leased bottom (i.e. extensive aquaculture), a restored reef produced by multiple year classes of spat-on-shell addition to a sanctuary area, and replicated, experimental reefs constructed at varying densities. Some of these studies were conducted in a single season, whereas others span a greater portion of the year. Reference sites included nearby soft sediment areas and unrestored reefs.

Piehler and Smyth (2011) collected sediment cores from within and adjacent to natural intertidal oyster reefs in Bogue Sound, NC during four sampling periods distributed throughout the year. Denitrification rates were higher for the oyster reef than for the control site with an annual enhancement rate of 2.7 g N m^{-2} y^{-1}(22.0 µmol N_2-N m^{-2} h^{-1}). In both spring and summer, all samples indicated enhanced denitrification rates at the oyster reef site. In winter and fall, results were mixed with some samples demonstrating enhanced rates and others showing reduced rates.

Sisson et al. (2011) measured denitrification rates on intertidal oyster reefs of varying density in the Lynnhaven River, VA during a single sampling period. These reefs were the result of wild oyster settlement onto shell plantings on a privately-held lease and thus fall into the fishing practice sometimes referred to as extensive aquaculture (see discussion on pg. 4). Samples encompassed a range of oyster biomass densities (35 – 218 g tissue DW m^{-2}). With the exception of the lowest oyster density under dark conditions, all samples indicated enhanced denitrification rates and there was a tendency for denitrification rates to increase with increasing oyster biomass density.

Smyth et al. (2013) used the same methods and sites as those used by Piehler and Smyth (2011). These studies indicated net enhancement (3.2 g N m^{-2} y^{-1} = 26.1 µmol N_2-N m^{-2} h^{-1}) of annual denitrification rates associated with oyster reef. Similar to the previous study, they found enhancement for all samples collected in spring and summer. In contrast to previous work, all samples collected in winter indicated enhanced denitrification compared to the reference site whereas all samples collected in fall indicated reduced denitrification rates.

Kellogg et al. (2013) compared nitrogen dynamics at a mature, subtidal restored oyster reef to a nearby reference site that was suitable for oyster reef restoration in the Choptank River, MD. Both sites lay beneath the euphotic zone. Rates of denitrification at the restored site were enhanced over those at the reference site during all seasons. The degree of enhancement varied between seasons with greatest enhancement in August (1486 µmol N_2-N m^{-2} h^{-1} = 20.8 mg N_2-N m^{-2} h^{-1}). Net annual enhancement was estimated at 55.6 g m^{-2} y^{-1} (453.1 µmol N_2-N m^{-2} h^{-1}) for this site.

Kellogg et al. (In prep) used experimental reefs to examine the relationship between oyster biomass density and denitrification rates at a shallow subtidal site in Onancock Creek, VA. Although studies had been planned to encompass four seasons, a die-off of >99% of the oysters limited sampling to April. Results from the single sampling period indicate a significant positive relationship between oyster biomass density and denitrification rates. However, the relationship appears to be non-linear with limited increases in denitrification per unit oyster biomass above ~100 g tissue dry weight m^{-2}.

Kellogg et al. (Ongoing) are using experimental reefs to examine the relationship between oyster biomass density and denitrification rates at an intertidal site at the Hillcrest Oyster Sanctuary in the Virginia Coast Reserve near Oyster, VA. Thus far, studies indicate that there may be a positive relationship between oyster biomass density and denitrification rate. However, preliminary data suggest that this relationship may be more variable for intertidal reefs than for subtidal reefs, change with season and require relatively high oyster biomass density to enhance denitrification rates.

Table 5. Effects of oyster reefs on denitrification rates. Calculations as in Table 4.

Source	Incubation Chamber Contents and Reef Characteristics	Study Site and Environmental Conditions	Month(s) Sampled	Incubation Type	Average Effect on DNF ($\mu mol\ N_2\text{-}N\ m^{-2}\ h^{-1}$)
Piehler and Smyth (2011)	Sediments collected within and adjacent to natural oyster reefs Intertidal Oyster biomass density = ~39 g DW m^{-2}	Bogue Sound, NC Salinity = 27 – 36 Temp. = 11 – 24 ºC	February May July October	Dark	-58.4 – 128
Sisson et al. (2011)	Intact section (0.1 m^2) of reef from extensive oyster aquaculture site Intertidal Oyster biomass density = 35.4 – 217.8 g DW m^{-2}	Humes Marsh, Lynnhaven River, VA Salinity = 29.4 Intertidal	October	Light	48 – 217.8
				Dark	-21.0 – 209.5
Smyth et al. (2013)	Sediments collected within and adjacent to natural oyster reefs Intertidal reef Oyster biomass density = ~39 g DW m^{-2}	Bogue Sound, NC Salinity = 29 – 32 Temp. = 3 – 30 ºC	January March July November	Dark	-58.4 – 422.5
Kellogg et al. (2013)	Intact section (0.1 m^2) of experimentally restored oyster reef Subtidal Oyster biomass density = 262 – 382 g DW m^{-2}	Choptank River, MD Salinity = 7.0-11.6 Temp. = 13.5 – 27.4 ºC	April June August November	Dark	199.2 – 1486.4
Kellogg et al. (In prep)	Intact section (0.1 m^2) of experimental oyster reef treatments Subtidal Oyster biomass density = 42.8 – 533.0 g DW m^{-2}	Onancock Creek, VA Salinity = 16 Temp. = 14 ºC	April	Light	235.3 – 533.5*
				Dark	273.9 – 767.8*
Kellogg et al. (Ongoing study)	Intact section (0.1 m^2) of experimental oyster reef treatments Intertidal Oyster biomass density = 0.0 – 345.2 g DW m^{-2}	Hillcrest Oyster Sanctuary, Oyster, VA Salinity = 32 - 34 Temp. = 18.0 – 26.5 ºC	August October	Light	-178.3 – 329.7*
				Dark	0 – 709.5*

* Study included a "shell only" treatment created by placing a layer of oyster shell at the site without adding live oysters. Because oyster biomass density was zero for the entire experimental plot, resulting data have not been included in the table.

Meta-analysis – A total of eight studies have measured oyster-associated denitrification rates in the field. The geographic range of these measurements is narrow, including five sites in Maryland, four sites in Virginia and one site in North Carolina. To date, measurements have been made at five aquaculture sites and five oyster reef sites.

Aquaculture

The two existing studies from suspended oyster aquaculture in Chesapeake Bay provide little evidence for significant net annual increase in denitrification. Although there is a slight trend towards increasing rates of denitrification later in the year (Fig. 2), this pattern explains very little of the overall variation. Denitrification rates have not been assessed for either the material within aquaculture floats or for any other type of intensive aquaculture.

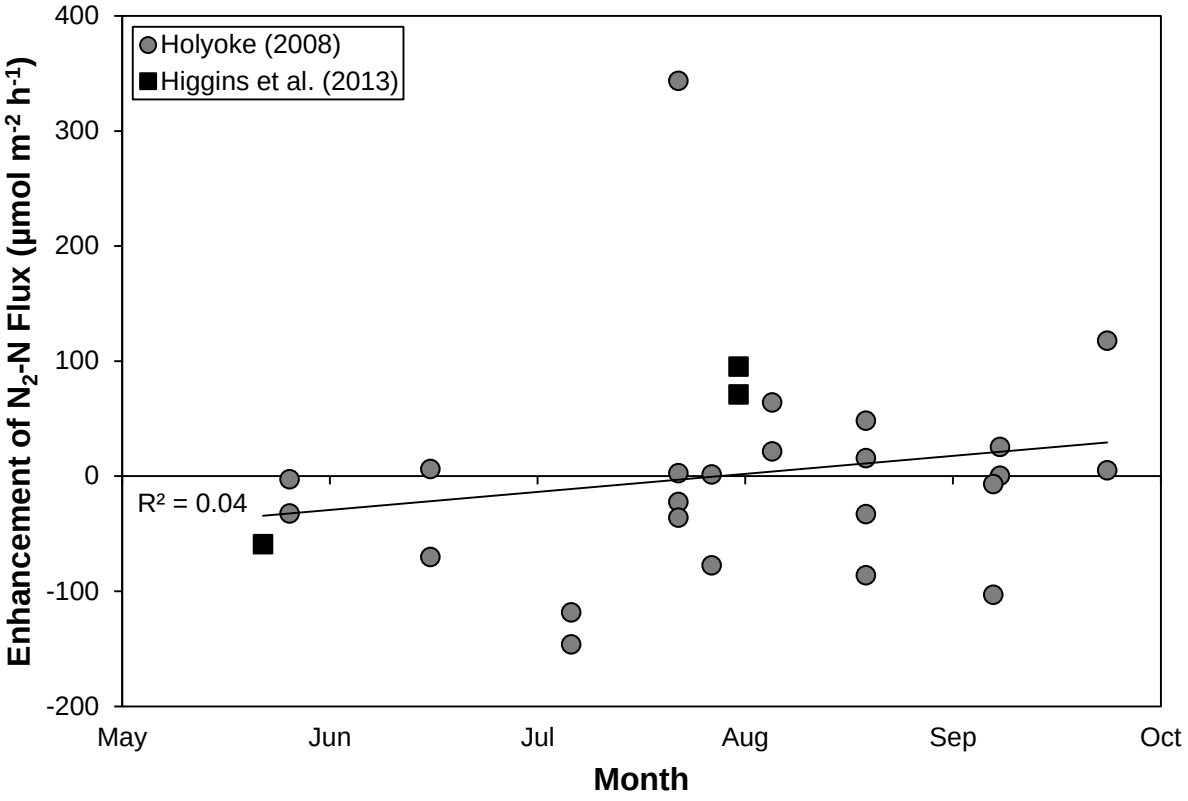

Figure 2. Seasonal denitrification rates for intensive aquaculture. Data generated by MIMS from sediment cores collected beneath oysters in growing in aquaculture floats. Holyoke (2008) data include both light and dark incubations. Higgins et al. (2012) data include only dark incubations.

Oyster Reefs

At present, five completed studies and one ongoing study have measured denitrification rates associated with oyster reefs with varied results. Across all studies, seasons and oyster biomass levels, the degree to which denitrification is enhanced over nearby reference sites varies by four orders of magnitude and includes both positive (an increase relative to the reference site) and negative values (a decrease relative to the reference site, Fig. 3). Within individual studies, enhancement of denitrification often varies by three orders of magnitude and sometimes includes both positive and negative values. Although we suggest possible sources of variation in denitrification rates below, we emphasize that these apparent patterns are based on very few data points.

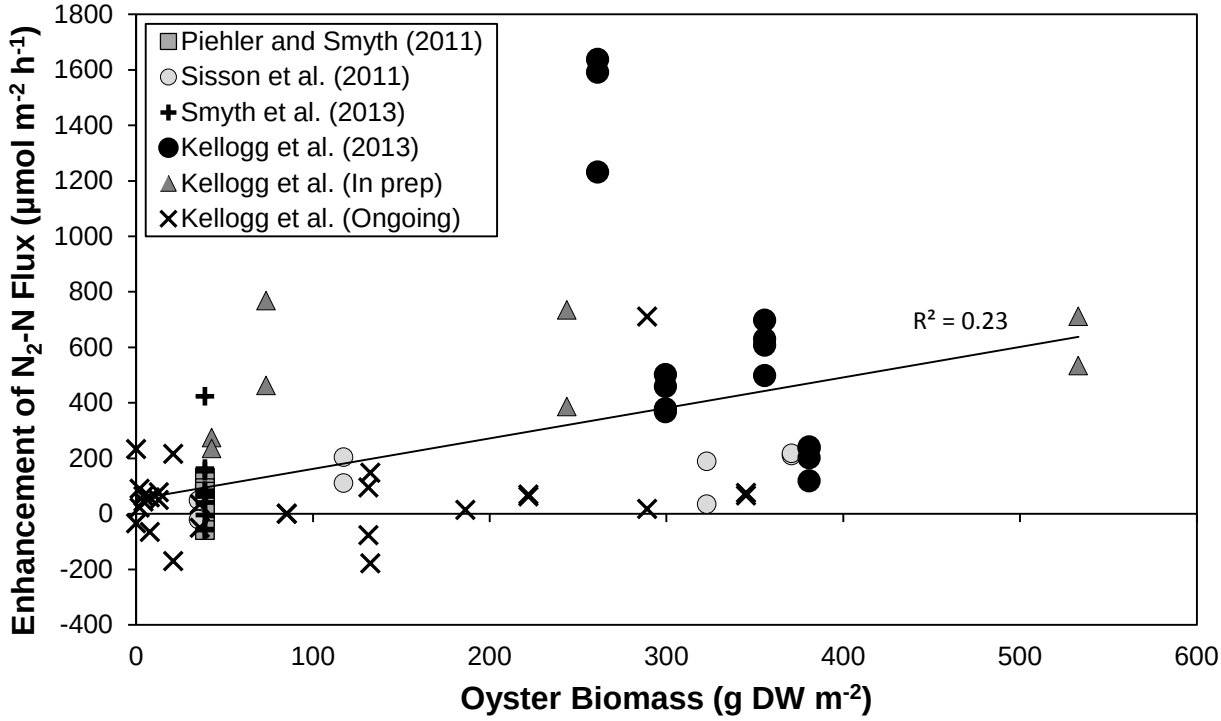

Figure 3. Enhancement of denitrification rates in relation to oyster biomass density. Biomass density for Piehler and Smyth (2011) and Smyth et al. (2013) are approximate and based on estimated adult oyster density at the study site combined with size and biomass distribution data from Kellogg et al. (Ongoing).

Three completed studies have estimated annual enhancement of denitrification rates by oyster reefs compared to reference sites (Table 6). Each of these studies measured denitrification rates a four time points within a single year and extrapolated these values to produce annual rates. For the purposes of this review, we define annual enhancement of denitrification as the net annual increase in N_2-N flux from the sediments to the atmosphere. For the two studies of

intertidal reefs in North Carolina, annual enhancement ranged from 2.7 to 3.2 g N_2-N m^{-2} y^{-1} (22.0 to 26.1 µmol N_2-N m^{-2} h^{-1}). The one study of a subtidal reef in Maryland estimated annual enhancement at 55.6 g m^{-2} y^{-1}(453.1 µmol N_2-N m^{-2} h^{-1}). An ongoing study of experimental intertidal oyster reefs in the Virginia coastal bays will produce estimates of annual denitrification enhancement for that site.

Table 6. Estimated annual enhancement of denitrification rates by oyster reefs. See Table 5 for details of each study. DNF = denitrification.

Source	Number of Sampling Periods	Annual DNF Enhancement (g N_2-N m^{-2} y^{-1})	Method Used to Calculate Annual Rate
Piehler and Smyth (2011)	4	2.7	Each seasonal rate applied to three months of the year, adjusted for hours submerged per day in the dark
Smyth et al. (2013)	4	3.2	Each seasonal rate applied to three months of the year; adjusted for hours submerged per day in the dark.
Kellogg et al. (2013)	4	55.6	Values from each sampling period applied to two months of the year. Assumed no denitrification in other four months of year.

The three completed studies that measured denitrification in multiple seasons show a significant effect of season within site (Fig. 4). Highest rates are observed in summer, lower rates in spring and lowest rates in fall or winter. This pattern is likely driven by a combination of water temperature and the supply of organic material.

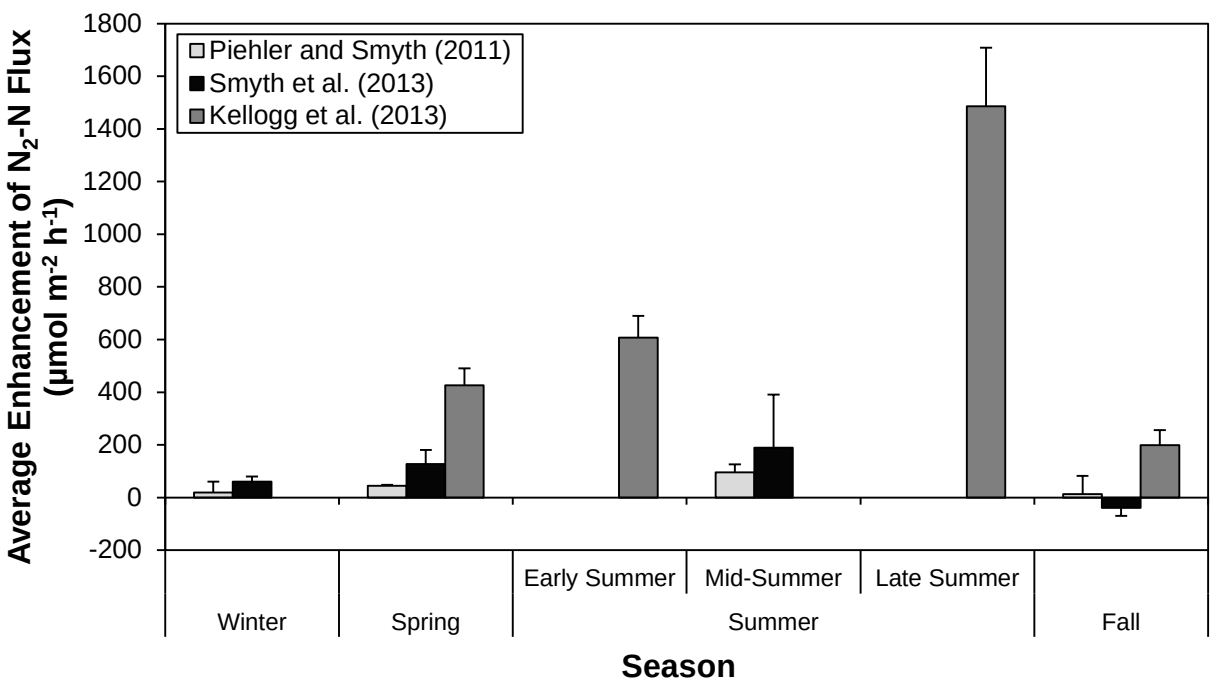

Figure 4. Seasonal patterns of denitrification enhancement relative to reference sites. Error bars represent standard deviaiton.

All three studies in Virginia were explicitly designed to examine the relationship between oyster biomass density and enhancement of denitrification. These studies suggest that there is a positive but non-linear relationship between oyster soft tissue biomass and denitrification rates within a site. However, the exact nature of this relationship appears to vary widely among sites and among seasons within site (Fig. 5).

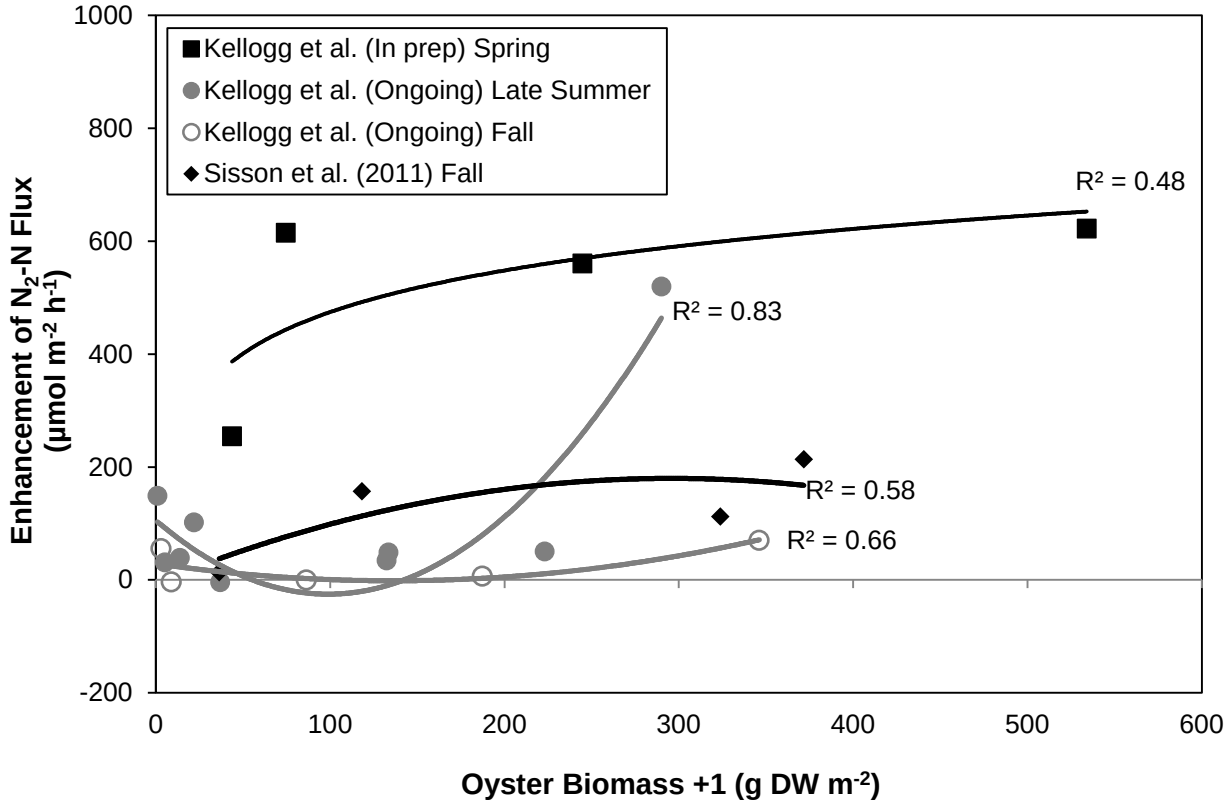

Figure 5. Enhancement of denitrification in relation to oyster biomass density. Biomass data have been +1 transformed to allow fitting of a logrithmic function. Enhancement rates are the average of the enhancement under light and dark conditions.

The two studies of intertidal reefs in North Carolina measured denitrification rates for sediments collected within or adjacent to oyster reefs. The other four studies in the Chesapeake Bay region (two subtidal and two intertidal) measured denitrification rates from intact sections of oyster reef that included sediments, oysters and the associated macrofaunal community. Research by Kellogg et al. (unpublished data) using samples from a subtidal restored reef in Maryland found significant levels of nitrification and denitrification associated with oyster clumps in the absence of underlying sediments, suggesting that fluxes measured for oyster reef sediments alone could underestimate actual denitrification rates.

Gaps in Existing Data

Our literature review along with the workshop presentations and discussions make it clear that few data on the impacts of oysters on nitrogen cycling exist. Below we list some of the primary gaps in existing data. This is not a comprehensive list, but rather highlights gaps in our current knowledge that significantly limit our ability to assign values for nitrogen removal to oysters growing in aquaculture or reef settings.

Nitrogen assimilation

1. Impact of reproductive state on the nitrogen content of oyster soft tissue: No published data exist for seasonal patterns in reproductive state on nitrogen content.
2. Percentage of nitrogen in the soft tissues and shells of intertidal oysters: All published data come from oysters growing either in aquaculture cages or on subtidal reefs.
3. Geographic variation in percentage of nitrogen in oyster soft tissue and shell: At present, data for oyster shell have been gathered at three sites in Chesapeake Bay and will be available soon for Mobile Bay, AL. Data on oyster tissue have been gathered in Chesapeake Bay, Cape Cod, MA, Mobile Bay, AL and Great Bay, NH. More data are needed to understand and define the importance of regional differences in relation to environmental conditions.

Denitrification

4. Denitrification rates for common forms of aquaculture: Data exist for the sediments underlying aquaculture floats but no data exist for the material inside aquaculture cages or for other types of intensive aquaculture (e.g. bottom cage and spat-on-shell culture techniques).
5. Factors controlling the relationship between oyster biomass density and denitrification rates: Recently completed and ongoing studies find positive relationships between oyster biomass density and denitrification but these relationships are non-linear and vary between sites and seasons. Research is needed to clarify the roles of biotic and abiotic factors which affect the relationship between oyster biomass density and denitrification rates.

Other nitrogen removal processes

6. Nitrogen removal via burial of biodeposits or shells: To date, no data have been published for nitrogen burial rates associated with oysters. Quantifying burial rates of both shell and

biodeposits under a variety of environmental conditions will establish the conditions, if any, under which these pathways lead to significant removal of nitrogen from the system.

7. Nitrogen removal via anammox or nitrous oxide release: At present, no published data exist to determine whether nitrous oxide release represents a significant mechanism for the return of nitrogen to the atmosphere. Although data on net fluxes of nitrogen gas (collected using MIMS) exist for both oyster reefs and oyster aquaculture, the relative roles of denitrification and anammox in producing nitrogen gas cannot be distinguished using these data alone. A better understanding relative importance of each pathway leading to the return of nitrogen to the atmosphere is needed.

Methodology Recommendations for Future Studies

Nitrogen assimilation

1. Nitrogen content should be reported as a percentage of dry weight for oyster tissues and shell.

Denitrification

2. For the purposes of estimating water quality benefits, measurement of denitrification rates as the net flux of di-nitrogen gas in the water column using membrane inlet mass spectrometry is the most appropriate method available at present. However, we note that this method does not allow identification of the process by which nitrogen gas is generated (e.g. microbially mediated denitrification versus anammox) and that other techniques (e.g. stable isotope analyses) or a combination of techniques are more suited to identifying the role of various potential nitrogen cycling pathways.
3. All studies seeking to assess the impacts of oyster aquaculture, oyster reef restoration or similar management actions should include a nearby reference site with similar physical and environmental conditions to allow calculation of resulting denitrification enhancement. Because oysters only survive in areas not prone to extensive periods of anoxia, consideration of the impacts of concentrating biodeposits in these areas versus alternate fates including deposition in deep channels prone to anoxia where denitrification is unlikely is also warranted.
4. Whenever feasible, reef materials should be incorporated into samples intended to estimate denitrification rates for oyster reefs.

Recommendations for Application of Existing Data

Nitrogen assimilation

1. Because oyster growth and survival can vary widely spatially and temporally, as well as with grow-out method, accurate estimates of the removal of assimilated nitrogen via harvest of cultured oysters will require collecting site-specific data on the tissue and shell biomass of oysters harvested.
2. Because length to dry weight relationships can vary significantly with environmental conditions, food quality, oyster reproductive state and oyster health, the most accurate estimates of nitrogen assimilation will be derived using length to biomass relationships from the oyster population of interest at the time of interest. Data from other locations or sampling periods should be extrapolated with caution, especially for older animals.
3. Variation in the amount of nitrogen (as a percentage of dry weight) in oyster tissue and shell is relatively low for a range of sites and environmental conditions along the mid-Atlantic and northeast coasts of the United States. We recommend using the mean of existing values (8.22% N in oyster tissue and 0.20% N in oyster shell) when making estimates for oysters from these regions only. However, we caution that these estimates should be revised as additional data become available.

Denitrification

4. To date, no studies show significant net annual enhancement of denitrification associated with intensive oyster aquaculture. At present, we recommend assigning a value of zero for nitrogen removal via denitrification associated with aquacultured oysters. However, we note that the only data that exist at present come from studies of the sediments beneath oysters growing in aquaculture floats. As data become available for other types of intensive aquaculture and/or for nitrogen dynamics within aquaculture floats, this recommendation should be reviewed and revised as needed.
5. Studies estimating annual enhancement of denitrification rates associated with oyster reefs suggest that they significantly enhance denitrification rates relative to appropriate reference sites. However, rates of enhancement vary with season, tidal regime, oyster biomass density and other unidentified factors. We recommend conducting additional

studies to better understand the sources of this variation prior to assigning values to the nitrogen removal capacity of oyster reefs attributable to denitrification.

6. Although it is not possible at present to provide generalized relationships for estimating the enhancement in denitrification associated with oyster reefs under varying conditions, reliable methods do exist for measuring these rates and applying them to the regions within which the measurements are made.

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Appendix

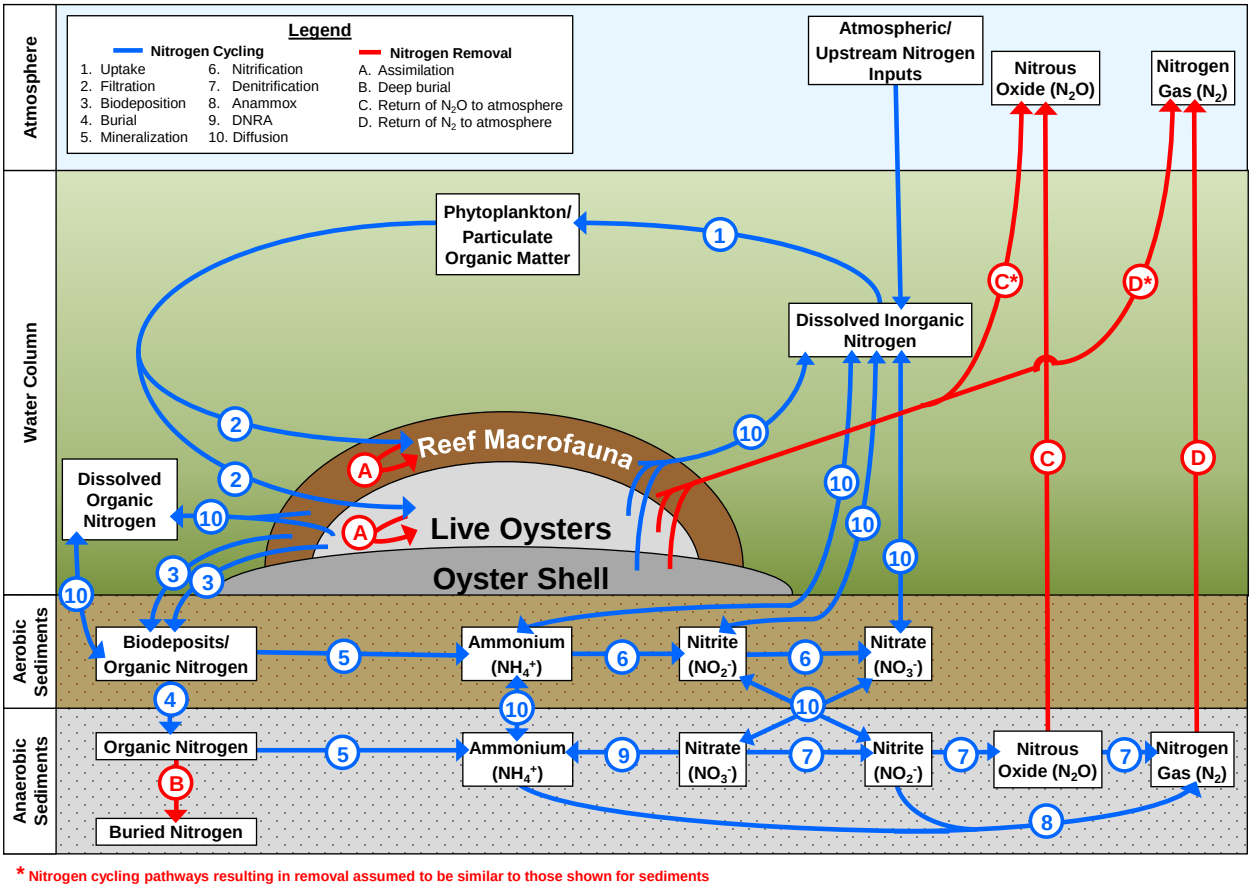

Figure A1. Primary nitrogen cycling and nitrogen removal pathways for a subtidal reef below the euphotic zone.

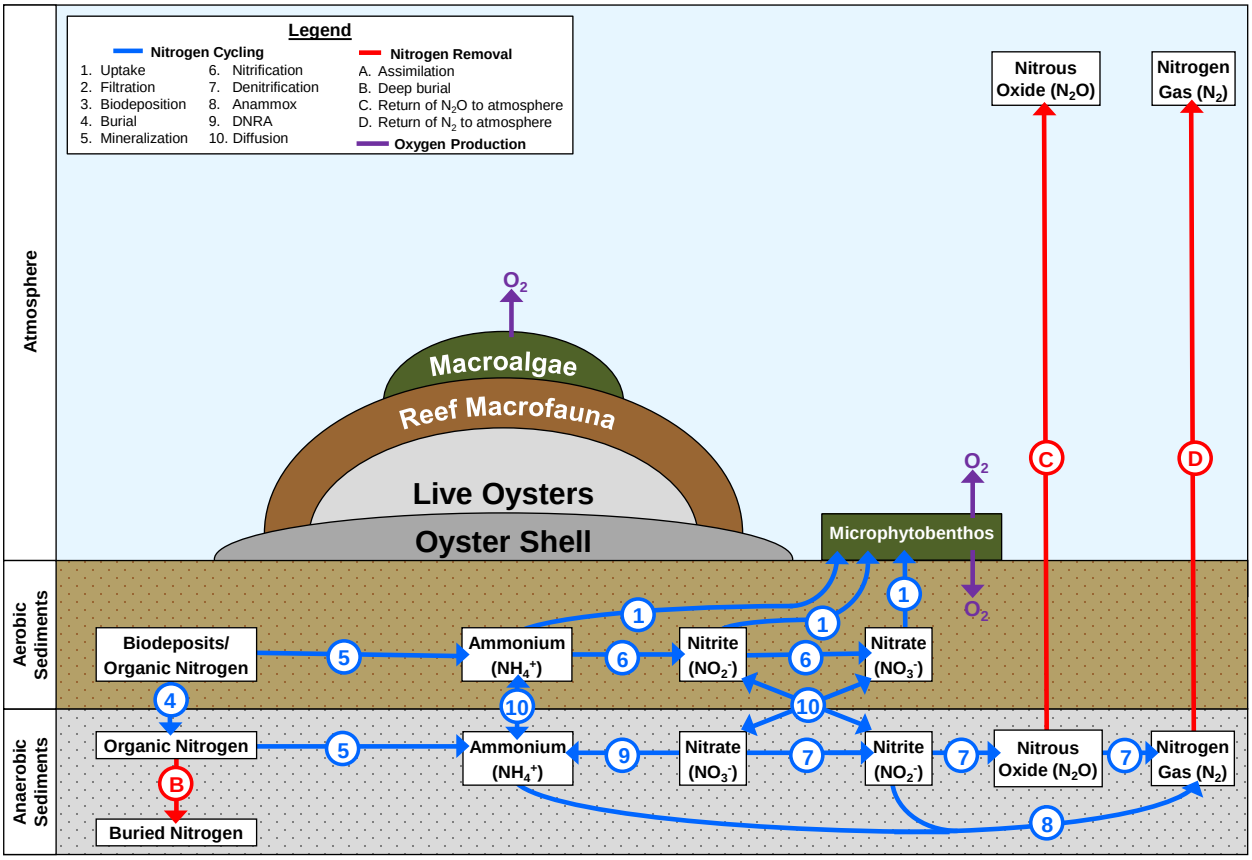

Figure A2. Primary nitrogen cycling and nitrogen removal pathways for an aerially exposed intertidal oyster reef.

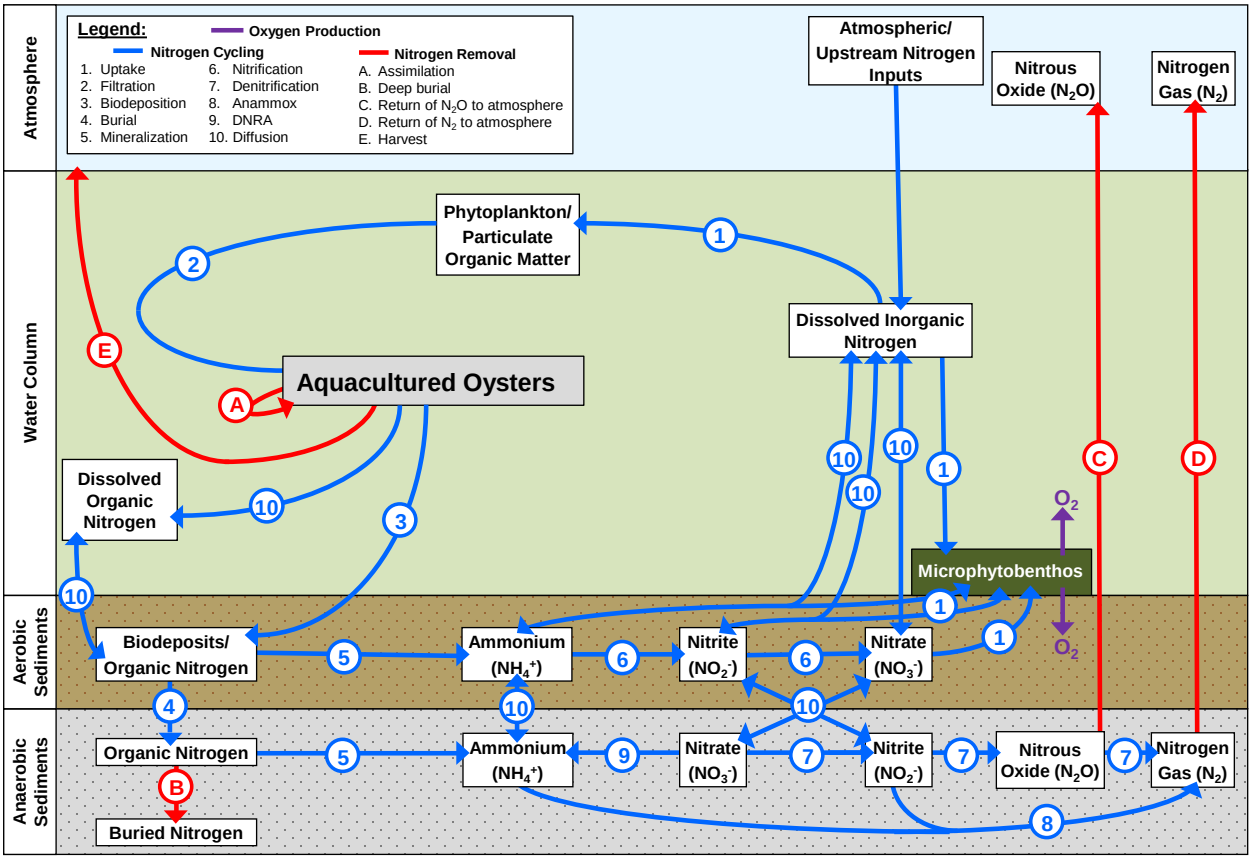

Figure A3. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over aerobic sediments within the euphotic zone.

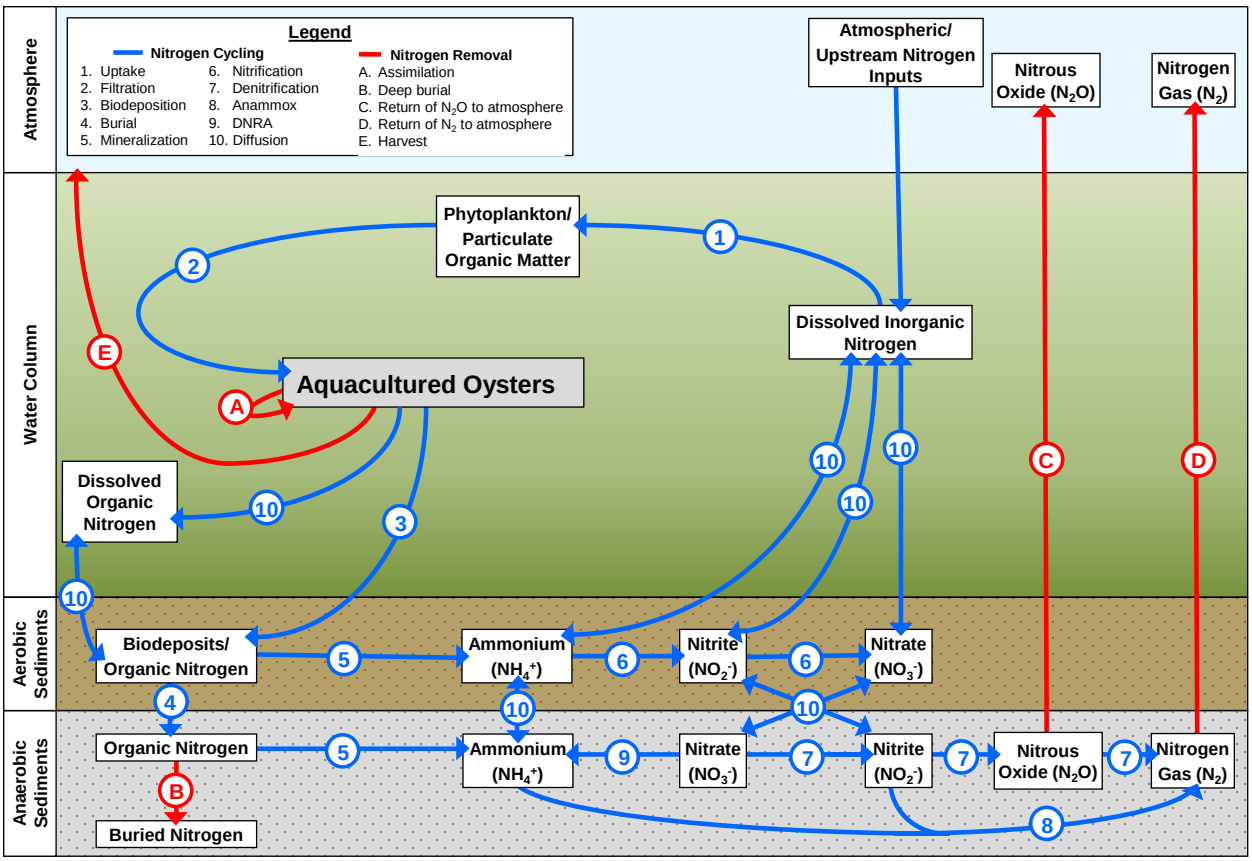

Figure A4. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over aerobic sediments beneath the euphotic zone.

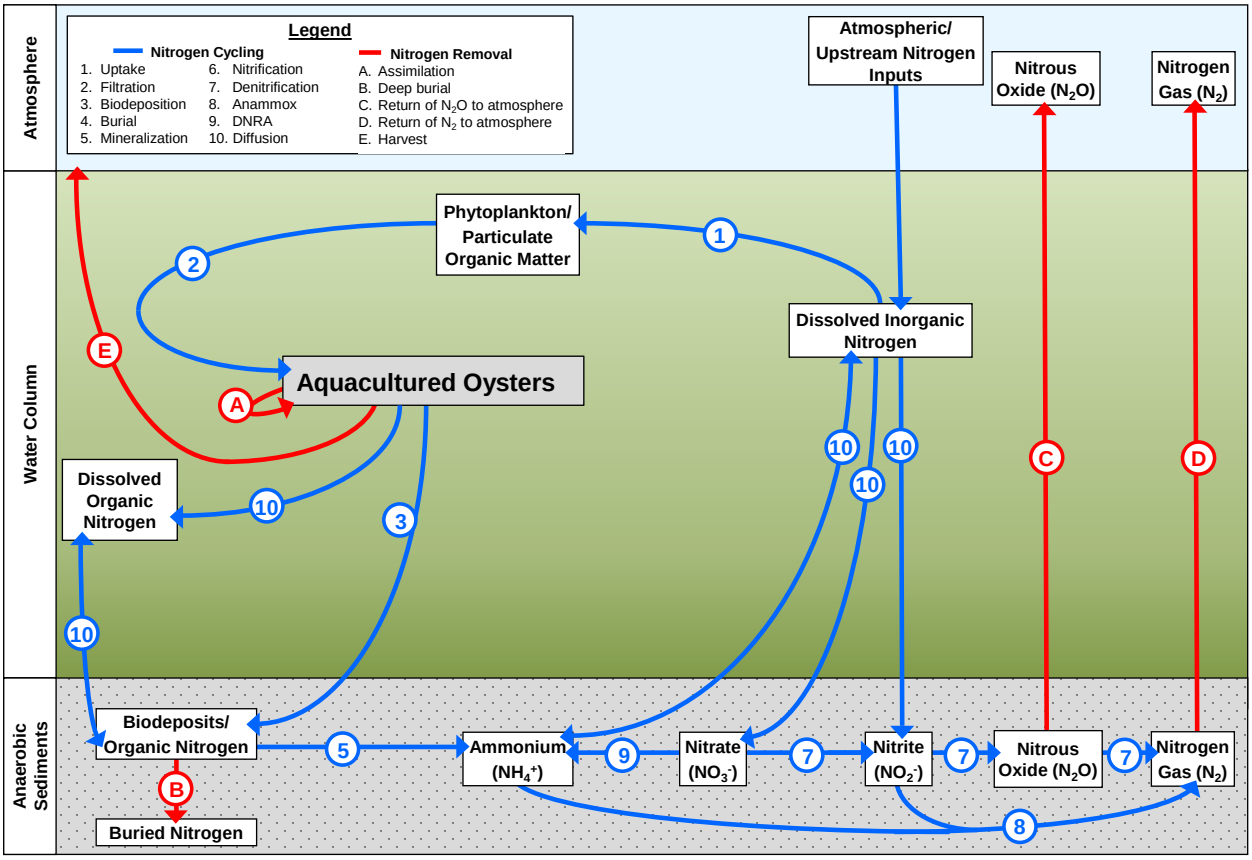

Figure A5. Primary nitrogen cycling and nitrogen removal pathways for intensive oyster aquaculture occurring over anaerobic sediments beneath the euphotic zone.