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Impacts of New England Oyster Aquaculture on Sediment Nitrogen Cycling: Implications for Nitrogen Removal and Retention

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Impacts of New England Oyster Aquaculture on Sediment Nitrogen Cycling:
Implications for Nitrogen Removal and Retention

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M.A., Boston University, 2013

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Impacts of New England Oyster Aquaculture on Sediment Nitrogen Cycling:
Implications for Nitrogen Removal and Retention

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List of Abbreviations

%	percent
%OC	percent organic carbon
‰	per mille, parts per thousand
°C	degrees Celsius
°N	degrees North
°W	degrees West
±	plus or minus
Anammox	anaerobic ammonium oxidation
C	carbon
cm	centimeter
cm ²	square centimeter
δ	delta
DIN	dissolved inorganic nitrogen
DE	denitrification efficiency
DNRA	dissimilatory nitrate reduction to ammonium
e.g.	exempli grata [Latin: "for example"]
et al.	et alia [Latin: "and others"]
Fig.	figure
GF/F	glass fiber filter
h	hour

i.e	id est [Latin: "that is:]
L	liter
m	meter
M	molar
m ²	square meter
MIMS	membrane inlet mass spectrometer
min	minute
mL	mililiter
N	nitrogen
n	number of samples
N ₂	di-nitrogen gas
N ₂ -N	di-nitrogen gas in terms of units of nitrogen
NH ₄ ⁺	ammonium
NO ₂ ⁻	nitrite
NO ₃ ⁻	nitrate
¹⁴ N	light isotope of N, 7 neutrons
¹⁵ N	heavy isotope of N, 8 neutrons
OM	organic matter
<i>p</i>	p-value
ppt	parts per thousand
vs	versus
μg	microgram

μL	microliter
μM	micromolar, or micromoles per liter
μm	micrometer
μmol	micromole

Abstract

In this three-year, seasonal study of sediment nitrogen (N) biogeochemistry, we used stable isotope techniques to measure the N cycling reactions nitrification, denitrification (DNF), and dissimilatory nitrate reduction to ammonium (DNRA). Rates were compared to dissolved inorganic nitrogen exchanges between the sediment and overlying water to assess denitrification efficiency (DE). All measurements were performed on sediments underlying (oyster) and removed from (control) oyster aquaculture in two oyster farms in the Northeast, USA. Site 1 is a brackish, enclosed coastal pond with oysters growing in nets suspended from the surface, while Site 2 is a fully saline coastal lagoon with oysters growing directly on the sediment in bags and cages. In both sites, there was generally net nitrate (NO_3^-) uptake from and net ammonium (NH_4^+) release to the water column. Despite NO_3^- uptake, the water column ^{15}N - NO_3^- pool was isotopically diluted by 2 to 68 at%, indicating high rates of gross nitrification. Calculated nitrification rates ranged from 5.86 to 1597 $\mu\text{mol m}^{-2} \text{h}^{-1}$, and there was no significant effect of oyster aquaculture on nitrification. High nitrification rates supported largely coupled-dominated DNF at both sites, comprising 80% of total DNF on average. Total DNF rates were significantly greater in the oyster cores compared to controls in the summer at Site 1 and in the fall at Site 2. DNRA rates ranged from non-detect to 130 $\mu\text{mol m}^{-2} \text{h}^{-1}$ and were not significantly different between oysters and controls; however, ratios of DNF/DNRA were significantly larger in oyster cores by 6-25-fold, suggesting that DNF was the dominant NO_3^- transformation process over DNRA. Overall, DNF was small relative to release of DIN from sediment to the

overlying water. Based on DE, both sites were found to favor N recycling over N removal, releasing 81% to 93% more N back to the overlying water than was removed via DNF. However, DE was enhanced by 50 to 100% in the oyster cores at both sites. Collectively, these data indicate that presence of oysters enhances DNF in warmer seasons and favors DNF over DNRA, but this enhancement of N removal via DNF is modest relative to the amount of DIN released to the overlying water at these sites. Results also show that oysters affect the balance of some, but not all, N cycle processes simultaneously. As a result, DNF should be concurrently measured with other N cycle processes in order to more accurately assess changes to the sediment N-cycle resulting from oyster biodeposition.

Introduction

Increasing coastal urbanization has led to increased nitrogen (N) delivery to many coastal ecosystems. This increased N load has resulted in dramatic shifts in estuarine community structure and function, which have widespread ecological, economic, and human health impacts on both local and global scales (Howarth 2008). These far-reaching consequences of N pollution are results of changes in the balance between N loads and N removal in these ecosystems. As a result, much effort has been spent on reducing point and non-point source N loads (e.g. Driscoll et al. 2003, Kaushal et al. 2011). Additional work has focused on biological N removal, whereby as much as 75% of reactive N is removed before it reaches the open ocean (Nixon et al. 1996, Seitzinger 2008, Fennel et al. 2009).

There are two primary biological N removal pathways in sediments: denitrification (DNF) and anaerobic oxidation of ammonium (anammox). However, DNF is generally the dominant removal process in coastal systems (e.g. Dalsgaard et al. 2005, Trimmer & Engström 2011, Plummer et al. 2015). DNF converts biologically available nitrate (NO_3^-) to dinitrogen (N_2) gas. This is a heterotrophic process utilizing organic matter (OM) as a reductant and NO_3^- as a terminal electron acceptor. The NO_3^- for DNF can be supplied via diffusion from the overlying water into sediments; this process is referred to as ‘direct DNF.’ Alternately, nitrification (ammonium oxidation + nitrite oxidation) in the sediment can be the source of NO_3^- for DNF, resulting in a tight coupling of the processes of nitrification and denitrification, also known as, ‘coupled DNF’. In the case of coupled DNF, sediment OM provides both the source of NH_4^+ that is nitrified, and the electrons needed to reduce NO_3^- during denitrification (Burgin and Hamilton 2007). Regardless of the NO_3^- source, DNF removes reactive and biologically

available N from the ecosystem. As a result, DNF is considered an ecosystem service, and is the focus of many N and eutrophication mitigation strategies (Piehler and Smyth, 2011; Seitzinger 2008).

One proposed strategy to enhance whole system DNF is to increase oyster populations in heavily eutrophied coastal systems. It is widely acknowledged that oysters benefit coastal ecosystems by improving water clarity through filtration, and serving as an N-sink when harvested (e.g. Kimberly et al. 2004, Higgins et al. 2013, Carmichael et al. 2012, Pollack et al. 2013). However, linkages between oysters and N cycling and removal rates are less clear (e.g. Costa-Pierce 2002, Forest et al. 2009).

The idea that oysters might alter N biogeochemistry is not a new one. N cycling and oysters have been studied together for nearly six decades. Early studies demonstrated that oysters enhanced benthic pelagic coupling by efficiently filtering particles from the water column, resulting in high sediment remineralization rates and increased fluxes of NH_4^+ back to the overlying water. This NH_4^+ was then available again to fuel pelagic phytoplankton production (Hammen et al. 1966, Dame et al. 1984). Additionally, it was proposed that these high rates of remineralization also lead to ample NH_4^+ available for nitrification, and therefore, coupled nitrification-denitrification. As a result, it was estimated that as much as one third of the total N in oyster biodeposits could ultimately be denitrified (Dame et al. 1989). Later studies elicited the hypothesis that oysters could increase DNF rates by providing a more labile carbon source to the sediments in the form of biodeposits, which would also stimulate removal of N from the system via coupled DNF (Newell et al. 2002, Newell 2004, Cerco and Noel 2007).

This work set the stage for the existing idea that oysters increase denitrification rates in sediments. While several studies have now observed increased DNF with oysters (Piehler and

Smyth 2011, Kellogg et al. 2013, Smyth et al. 2013, Humphries et al. 2016), others have seen the opposite (Higgins et al. 2013, Hoellein and Zarnoch 2014). Perhaps the reason for such variable results in such a wide range of studies is that the majority examine how oysters impact only denitrification rates without measuring other major, concomitant N cycle processes (Piehler and Smyth 2011, Higgins et al. 2013, Kellogg et al. 2013, Humphries et al. 2016). For example, dissimilatory nitrate reduction to ammonium (DNRA) is an alternate NO_3^- reduction pathway which can compete with DNF for an OM-derived electron donor. DNRA has been found to be favored over denitrification in sediments with high labile carbon availability (Kelso et al. 1997, Silver et al. 2001, Hardison et al. 2015) such as those receiving oyster biodeposits. This evidence suggests that, in at least some cases, high oyster density could promote DNRA, a N retention process, over DNF, a N removal process. This shift to DNRA has been observed in various other aquaculture settings, which might serve as an analog for sediments modified by oyster aquaculture (Chutivisut et al. 2014, Murphy et al. 2016).

This increase in benthic remineralization, nitrification rates, and sediment carbon lability suggests that oysters have the potential to foster an environment that supports higher rates of N removal via DNF. However, this potential can only be realized under certain conditions. First, the NH_4^+ resulting from increased remineralization of oyster biodeposits must fuel relatively greater nitrification rates compared to NH_4^+ efflux to the water column. If a greater proportion of NH_4^+ is released to the water column than is nitrified, pelagic primary production, and not nitrification will be enhanced. Second, if nitrification rates do increase, enough of the resulting NO_3^- needs to be taken up by DNF so that DNF comprises the majority of the NO_3^- sink, relative to DNRA and other exchanges of NO_3^- . This, then, will stimulate N removal relative to N retention. Therefore, the effect that oysters have on N removal in coastal systems is ultimately

dependent on the balance of several processes including, OM remineralization, nitrification, and DNRA, as well as DNF.

In the northeast USA, natural oyster populations are currently ~1% of maximum historic levels thus, most commercial oyster harvest is via aquaculture (Jackson et al. 2001). In 2013, 73% of the \$143 million-dollar US oyster harvest came from aquaculture of the eastern oyster (*Crassostrea virginica*, NMSF 2015; FAO 2015). Further, many heavily eutrophied coastal systems including the Chesapeake Bay, and New York Harbor are currently implementing plans to increase oyster density for water quality benefits. Therefore, there is a clear need for establishing the efficacy of oyster aquaculture or oyster gardens on enhancing N removal via denitrification.

Here I present N cycling rates from two different New England oyster farms, with two different oyster grow-out strategies over the course of three years. Nitrogen isotope tracer techniques were used in sediment core incubations to simultaneously quantify rates of coupled and direct DNF, DNRA, and nitrification seasonally in two aquaculture systems. Oysters were not included in the incubations; therefore, we do not consider any direct effects of oyster filtration on water column N cycling. This work focuses on the balance between nitrogen recycling and removal processes to gain a deeper understanding of how oysters effect overall N biogeochemistry in sediments affected by oyster aquaculture.

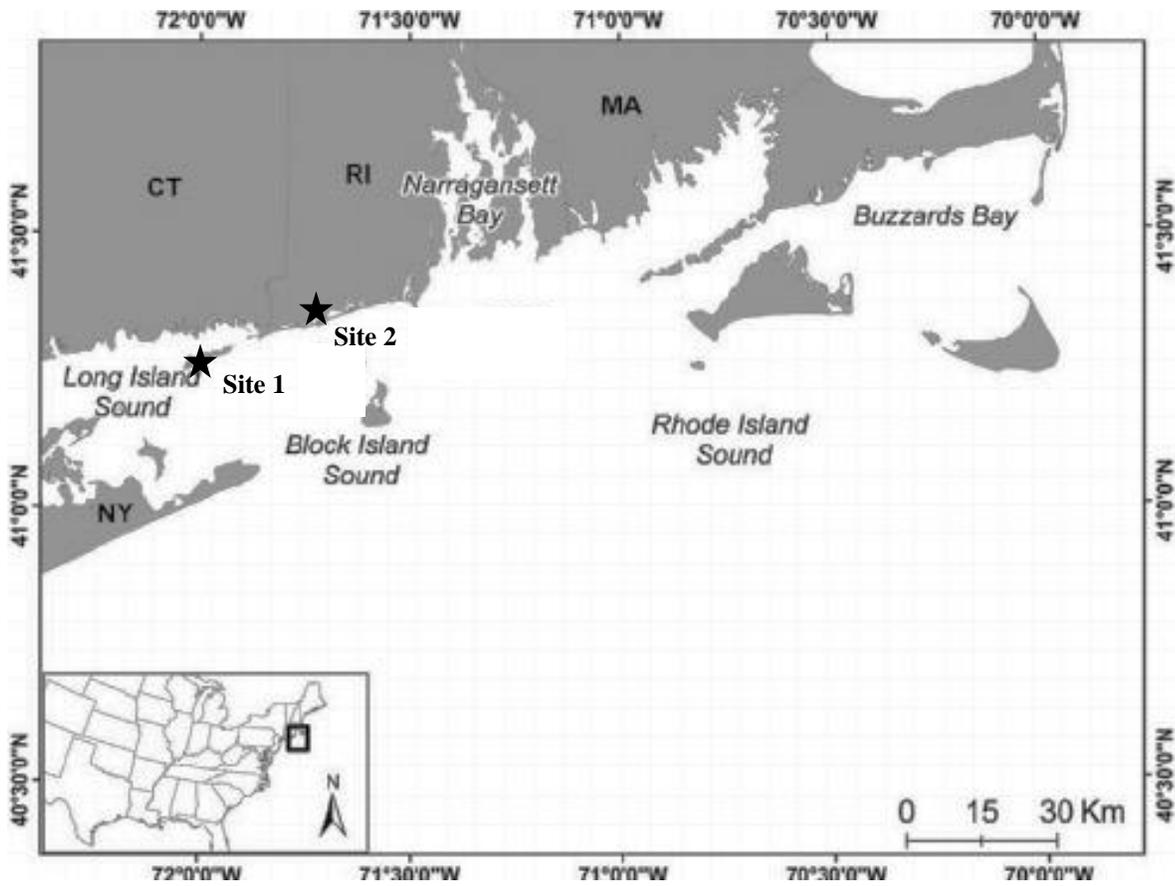


Figure 1. Map of southern New England, USA, including study sites for this work. Site 1 is Fishers Island Oyster Farm, Fishers Island NY (41.2693, -71.9850) and Site 2 is Walrus and Carpenter Oysters, Charlestown, RI (41.3551, -71.6750)

Methods

Study Sites

In this study samples sediment from two oyster farms in southern New England were collected (Fig. 1). Site 1 is in Fishers Island, NY. The oysters in this farm grew in lantern nets suspended from surface floats in an enclosed, brackish coastal pond. Oysters did not contact the sediment directly, which had an organic carbon content (%OC) of approximately 6%. Site 2 was in Charlestown, RI and had oysters growing in bottom bags and cages that rested directly on the bottom of a fully saline coastal lagoon. This site was well flushed, and had sandy sediment with less than 1% OC. Oyster aquaculture has occurred at sites 1 and 2 for 30 and 10 years, respectively.

Sampling and Analysis

Field Collection - Each oyster farm was sampled in the spring, summer, and fall from October 2013 to July 2016. During each sampling event, five, 10cm diameter, intact sediment cores were collected from areas where oysters were grown (either directly under suspended lantern nets at Site 1 or directly adjacent to bottom cages at Site 2), and from oyster-free control areas.

¹⁵NO₃⁻ Tracer Incubation - Cores were transported directly to the seawater laboratory at the University of Connecticut in Groton, CT, placed in an ambient temperature water bath, and allowed to settle with gentle, surface bubbling over night. The following morning the overlying water was siphoned carefully from cores so as to keep the sediment surface intact, and replaced with site water which had been spiked with ¹⁵N-NO₃⁻ at 99 at% ¹⁵N to a target concentration of 85μM NO₃⁻. After a 30-minute equilibration period, initial samples were taken for water column

concentration and ^{15}N enrichment of dissolved inorganic nitrogen (DIN; NH_4^+ , NO_3^- , NO_2^-) and N_2 . Each core was then capped with a gas tight top equipped with a magnetic stir bar. Incubations lasted 12-48hr, depending on temperature, and were carried out in the dark so as to minimize the parallel impact of any benthic micro or macroalgae. Additionally, dissolved oxygen (DO) concentration was monitored throughout to ensure it remained at or above $2\text{mg O}_2 \text{ L}^{-1}$ as to avoid hypoxia. Time series sampling was done whereby cores were first sampled for overlying water O_2 and DIN concentrations, then individually sacrificed by slurry while the core was sealed. The slurried core was allowed to settle for 90s, then additional samples were collected. Aqueous samples for DIN concentration and $^{15}\text{N}\text{-NO}_3^-$ enrichment were passed through a $0.7\mu\text{m}$ GF/F filter into 20ml polypropylene bottles and immediately frozen until analysis. Aqueous samples for dissolved $^{15}\text{N}\text{-N}_2$ isotopologues ($^{29}\text{N}_2$ and $^{30}\text{N}_2$) were siphoned into 12mL glass exetainer vials with zero headspace, preserved with approximately $75\mu\text{L}$ of concentrated zinc chloride, and refrigerated, underwater until analysis. Sediment samples for natural abundance $\delta^{13}\text{C}$, and C/N analyses were collected and frozen. Approximately 10cm^3 of sediment was then collected from each core in triplicate for total extractable NH_4^+ concentrations and determination of $^{15}\text{N}\text{-NH}_4^+$ enrichment. These samples were added to 20ml of 2M potassium chloride (KCl) and shaken vigorously for 2hr, centrifuged, passed through $0.2\mu\text{m}$ GF/F filters, and frozen until analysis. Additional sediment samples were collected in the field and frozen for analysis of porosity and %OC.

Sample Analysis - Sediment samples were thawed and dried for the calculation of porosity as well as %OC via loss on ignition (LOI). DIN samples, including KCl extracts were analyzed on a Smartchem auto analyzer (WestCo.), for dissolved nitrate, nitrite, and ammonium. Dissolved $^{15}\text{N}\text{-NO}_3^-$ enrichments were measured using the denitrifier method (Christensen et al.

1988, Sigman and Cassiotti 2001), and then analyzed as nitrous oxide (N_2O) on an isotope ratio mass spectrometer (IRMS) at the US Geological Survey Reston Stable Isotope Lab (USGS). The dissolved concentration of each of the N_2 isotopologues ($^{29,30}\text{N}_2$) was measured on a membrane inlet mass spectrometer (MIMS) fitted with a 600 °C reduction furnace to prevent oxygen effects on mass 30 (Eyre et al. 2003). The KCl extracted NH_4^+ was isolated for ^{15}N - NH_4^+ analysis using the alkaline acid-trap diffusion method (Sørensen and Jensen 1991, Hannon and Böhlke 2008). The NH_4^+ , trapped on GF/D filters was analyzed on an elemental analyzer (EA) coupled to an IRMS for ^{15}N - NH_4^+ enrichment. Sediment samples left over after KCl extraction, as well as those sampled directly from slurried cores, were freeze dried, acidified to remove any carbonate, and analyzed on the EA/IRMS for sediment $\delta^{13}\text{C}$ and C:N. Isotope values were two-point normalized to the known glutamic-acid reference materials USGS 40 and 41.

Rate Calculation - DIN flux rates were calculated using linear regressions fit to time series changes in NH_4^+ , NO_2^- , and NO_3^- concentrations over the course of the core incubation. These are net rates, that result from the balance of DIN into (negative values) and released out of the sediment (positive values).

Four variants of DNF were calculated using the isotope pairing technique (IPT; Nielsen 1992): coupled, direct, total (coupled + direct), and potential DNF. The coupled, direct, and total DNF rates refer to denitrification of ambient N in the incubation. The potential DNF rate includes denitrification of the added ^{15}N - NO_3^- tracer. This rate is used for direct comparison to DNRA, which also draws on the added ^{15}N - NO_3^- tracer and is considered a potential rate. The IPT calculation uses the production rates of $^{29}\text{N}_2$ and $^{30}\text{N}_2$, as well as measured ^{15}N - NO_3^- enrichments at the beginning of the incubation. The $^{29}\text{N}_2$ and $^{30}\text{N}_2$ production rates were derived

from linear regressions of the time series $^{29}\text{N}_2$ and $^{30}\text{N}_2$ measurements. Error for the DNF rates was determined from the root mean square error (RMSE) of the linear regression fits.

DNRA rates were calculated using a linear regression model of the change in $^{15}\text{N-NH}_4^+$ mass over time, determined from the ^{15}N mole fraction and the total inventory of extractable NH_4^+ in the sediment bound ammonium pool. The $^{15}\text{N-NH}_4^+$ mass production was normalized to mean $^{15}\text{N-NO}_3^-$ enrichment to yield the total DNRA rate. Nitrification rates were calculated via an isotope dilution model, modified from Wessel and Tietema 1992, which includes the natural log of the isotopic dilution of the $^{15}\text{N-NO}_3^-$ pool normalized to the natural log of the fractional NO_3^- concentration change over the course of each incubation (Tobias et al. 2003). This method yields an estimate of gross nitrification for each incubation.

Results

Site Characteristics

Site 1 - Site 1 was a brackish, coastal pond with mid water lantern net oyster grow-out. Water temperature ranged from 8°C in early spring to 25°C in late summer with salinity ranging from 15-23 ppt (Table 1). Sediments were generally high in organic carbon, with mean %OC values ranging from 1.2 to 8.3% (mean= 5.6%). Seasonally averaged C/N ratios ranged from 8.7 to 54.7, and $\delta^{13}\text{C}$ at this site ranged from -26.0 to -21.1‰, suggesting a mix of terrestrial and marine carbon sources. Both oyster (sediment collected beneath oyster grow-out) and control cores had similar temperature and salinity. While not significantly different, $\delta^{13}\text{C}$ was isotopically lighter in the oyster cores, compared to the controls, in the fall season. Oyster cores also had significantly lower C/N, on average, than the control cores ($p < 0.05$).

Site 2 - Site 2 was a sandy, coastal lagoon with bottom cage oyster grow-out.

Throughout the study water temperature at this site ranged from 5°C in the spring to 22°C in the summer with salinity ranging from 25 to 31ppt (Table 1). Site 2 sediments were lower in organic carbon than Site 1, with %OC ranging from 0.2 to 2.0% (mean= 0.9%). C/N ratios were also significantly lower than Site 1, ranging from 2.1 to 8.7 ($p < 0.05$, Table 1). The $\delta^{13}\text{C}$ values at Site 2 were 2-8‰ heavier than those of Site 1, ranging from -19.9 to -18.0‰ (Table 1). As in Site 1, both oyster and control sites had consistent temperature, salinity. $\delta^{13}\text{C}$ and %OC at Site 2 were also not significantly different between oyster and control cores.

Dissolved Inorganic Nitrogen

Site 1 - This site generally had net NO_3^- uptake (negative values) by the sediment with flux values ranging from -129.9 to -26.1 $\mu\text{mol m}^{-2} \text{h}^{-1}$; however, in the spring of 2014, the control cores exhibited net NO_3^- release. NH_4^+ was released from the sediment (positive values) with rates ranging from 131.6 to 395.1 $\mu\text{mol m}^{-2} \text{h}^{-1}$. There was also net release of NO_2^- from sediments to water column (excepting two incubations), with flux values ranging from -1.9 to 29.8 $\mu\text{mol m}^{-2} \text{h}^{-1}$ (Table 2). The isotope dilution of ^{15}N - NO_3^- over the course of the incubation, indicative of nitrification, ranged from 2 to 68 at% (Table 2). Both the ammonium fluxes and isotope dilution of ^{15}N - NO_3^- from nitrification were not significantly different between the oyster and control cores, but the oyster cores had significantly greater net NO_3^- uptake and NO_2^- release than controls ($p < 0.05$).

Table 1. Site characteristics including temperature, salinity, $\delta^{13}\text{C}$ (‰), percent organic carbon, and carbon to nitrogen ratios averaged seasonally for both sites, data are mean \pm standard error (SE).

Site	Season	Temperature (°C)	Salinity	$\delta^{13}\text{C}$	%OC	C/N
Site 1 Oyster	Spring	10.1 \pm 1.6	15.8 \pm 0.6	-25.4 \pm 0.1	5.5 \pm 0.1	20.7 \pm 0.3
	Summer	24.5 \pm 0.5	20.0 \pm 1.0	-24.9 \pm 0.0	6.3 \pm 0.6	10.1 \pm 0.6
	Fall	18.0 \pm 0.6	20.3 \pm 1.5	-24.9 \pm 0.1	4.9 \pm 0.0	8.7 \pm 0.4
Site 1 Control	Spring	10.1 \pm 1.6	15.8 \pm 0.6	-26.0 \pm 0.2	8.3 \pm 0.0	12.5 \pm 0.7
	Summer	24.5 \pm 0.5	20.0 \pm 1.0	-25.0 \pm 0.1	5.3 \pm 1.3	54.7 \pm 1.3
	Fall	18.0 \pm 0.6	20.3 \pm 1.5	-21.1 \pm 0.2	1.2 \pm 0.1	16.9 \pm 1.8
Site 2 Oyster	Spring	5.7 \pm 0.7	25.3 \pm 0.3	-19.9 \pm 0.3	1.1 \pm 0.2	8.7 \pm 0.9
	Summer	21.6 \pm 0.5	29.7 \pm 0.4	-18.3 \pm 0.2	0.6 \pm 0.1	2.1 \pm 0.3
	Fall	17.7 \pm 0.7	30.7 \pm 0.8	-18.3 \pm 0.0	0.9 \pm 0.1	4.5 \pm 0.2
Site 2 Control	Spring	5.7 \pm 0.7	25.3 \pm 0.3	-18.0 \pm 0.0	0.7 \pm 0.1	4.4 \pm 0.7
	Summer	21.6 \pm 0.5	29.7 \pm 0.4	-18.7 \pm 0.2	0.2 \pm 0.1	2.2 \pm 0.1
	Fall	17.7 \pm 0.7	30.7 \pm 0.8	-18.8 \pm 0.0	2.0 \pm 1.7	8.7 \pm 0.0

Table 2. DIN fluxes across the sediment water interface in micromoles of N per meter squared per hour, as well as isotope dilution displayed as the change in $^{15}\text{N-NO}_3^-$ over the course of the incubation ($\Delta^{15}\text{N-NO}_3^-$). All values are averaged seasonally for both sites and data are mean \pm SE.

Site	Season	NO_3^- Flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	NO_2^- Flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	NH_4^+ Flux ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	$\Delta^{15}\text{N-NO}_3^-$ (at%)
Site 1 Oyster	Spring	-40.2 \pm 5.3	7.40 \pm 5.9	286.7 \pm 130	6.19 \pm 2.3
	Summer	-129.9 \pm 18.9	29.8 \pm 4.3	395.1 \pm 50.1	60.5 \pm 0.5
	Fall	-81.4 \pm 5.2	4.80 \pm 0.3	290.1 \pm 70.1	45.7 \pm 6.3
Site 1 Control	Spring	22.8 \pm 35.5	-1.37 \pm 0.1	131.6 \pm 41.4	19.8 \pm 8.1
	Summer	-94.5 \pm 5.2	-1.98 \pm 0.1	367.2 \pm 25.9	67.5 \pm 0.5
	Fall	-26.1 \pm 2.1	4.86 \pm 0.4	256.6 \pm 60.1	57.9 \pm 6.1
Site 2 Oyster	Spring	-234.8 \pm 207	32.8 \pm 30.1	267.6 \pm 150	9.43 \pm 7.6
	Summer	377.4 \pm 680	27.4 \pm 18.3	-228.1 \pm 668	14.5 \pm 4.1
	Fall	-223.4 \pm 140	23.6 \pm 8.6	172.6 \pm 53.4	24.0 \pm 2.5
Site 2 Control	Spring	-242.3 \pm 217	5.84 \pm 5.2	297.6 \pm 168	20.1 \pm 3.0
	Summer	-96.9 \pm 14.5	30.7 \pm 11.5	149.7 \pm 101	10.0 \pm 7.4
	Fall	-59.5 \pm 3.4	5.77 \pm 0.5	632.7 \pm 142	26.0 \pm 1.0

Site 2 - This site also largely exhibited net NO_3^- uptake, however there was an instance of NO_3^- release in the summer of 2014. Overall, DIN fluxes had a larger range here than at Site 1 with NO_3^- ranging from -242.3 to 377.4 $\mu\text{mol m}^{-2} \text{h}^{-1}$, NO_2^- from 5.77 to 32.8 $\mu\text{mol m}^{-2} \text{h}^{-1}$, and NH_4^+ from -228 to 632.7.2 $\mu\text{mol m}^{-2} \text{h}^{-1}$ (Table 2). As in Site 1, there was generally net release of ammonium and nitrite from the sediment as well as net uptake of nitrate; however, there were no significant differences between oyster and control net DIN fluxes at Site 2.

Nitrification

Site 1 - Despite net NO_3^- uptake from the water column into sediments, there was substantial dilution of the water column $^{15}\text{N-NO}_3^-$ pool by as much as 68 at% at Site 1 and 28 at% at Site 2 (Table 2). This isotopic dilution is a result of $^{14}\text{N-NO}_3^-$ produced by nitrification being released from the sediment. Nitrification rates ranged from 5.86 to 1597 $\mu\text{mol m}^{-2} \text{h}^{-1}$ at Site 1 with annual averages of $383.8 \pm 152 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster cores and $561.0 \pm 176 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the control cores (Fig. 2a). Nitrification rates were not significantly different between oyster and control cores.

Site 2 - Nitrification rates at Site 2 were less variable, ranging from 52.0 to 809.6 $\mu\text{mol m}^{-2} \text{h}^{-1}$, with annual average rates of $168.8 \pm 48.6 \mu\text{mol m}^{-2} \text{h}^{-1}$ and $265.3 \pm 91.5 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster and control cores, respectively (Fig. 2b). As in Site 1, nitrification rates were not significantly different between oyster and control cores at this site.

Denitrification: Coupled, Direct, and Total

Site 1 - Coupled DNF dominated in all oyster cores and in control cores during the spring

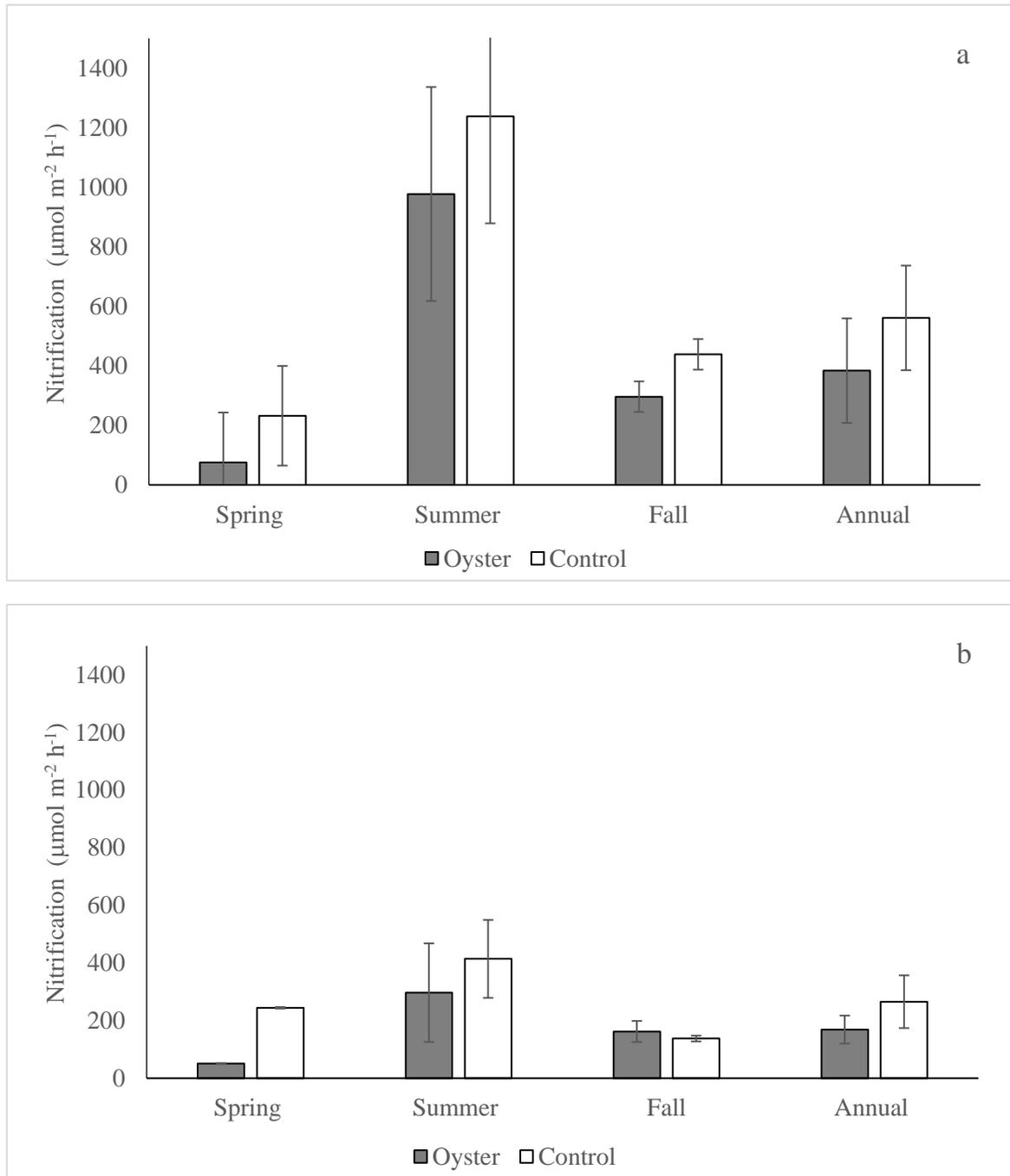


Figure 2. Seasonal and annual averages (\pm SE) of calculated nitrification rates from Sites 1 and 2 (a and b, respectively) throughout the three years of this study. Shaded bars represent oyster cores while open bars signify control cores.

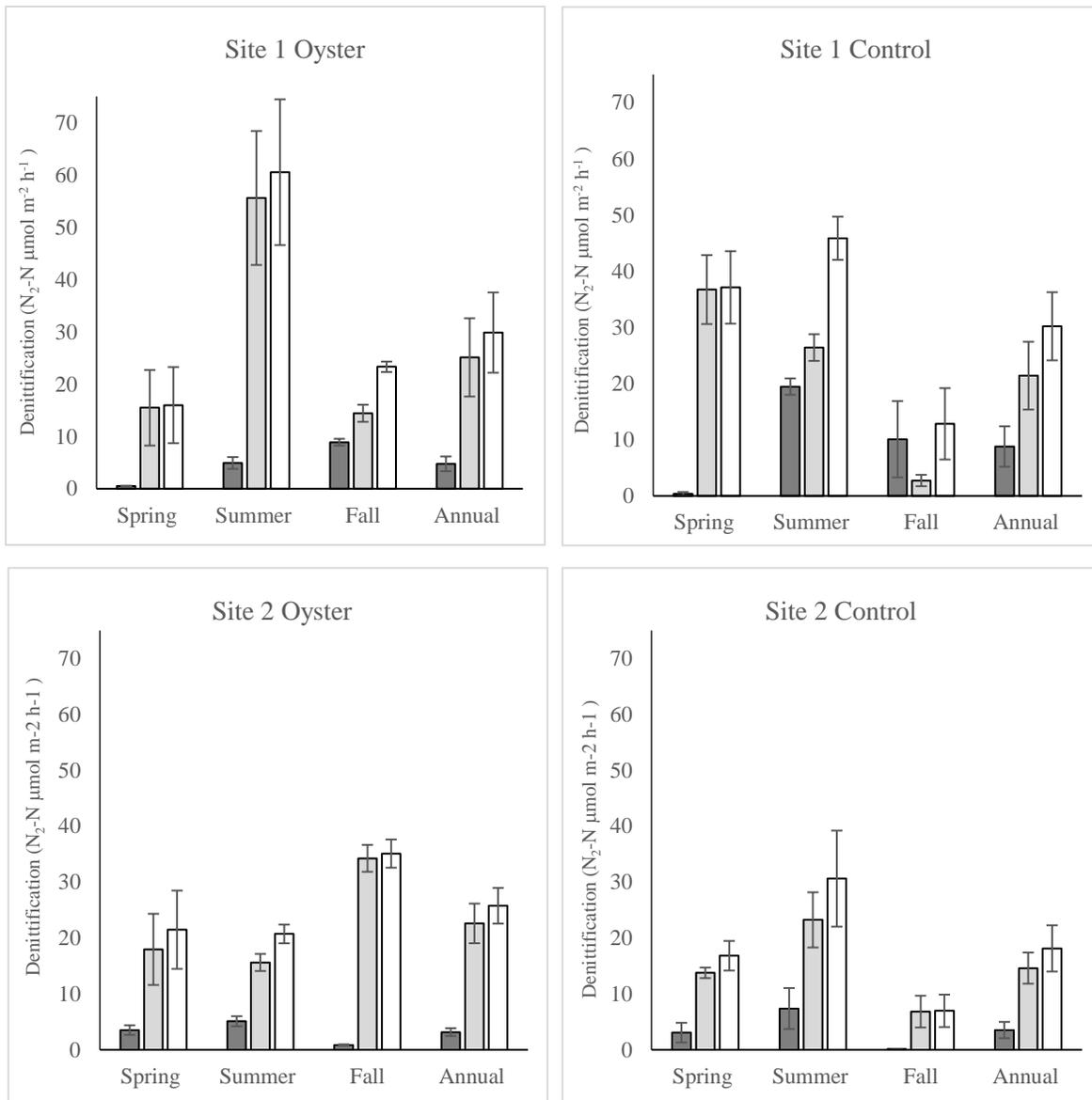


Figure 3. Seasonal and annual averages (\pm SE) of direct (dark grey), coupled (light grey), and total (white) denitrification rates throughout the three years of this study.

and summer ($p < 0.05$, Fig. 3a). Annually, coupled DNF constituted 84% of total DNF in the oyster cores, with rates ranging from 7.6 to 68.4 $\mu\text{mol N m}^{-2} \text{h}^{-1}$. Direct DNF rates in the oyster cores ranged from 0.5 to 9.6 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, and 0.4 to 19.5 $\mu\text{mol N m}^{-2} \text{h}^{-1}$ in the control cores (Fig. 3a, b). As in the oyster cores, coupled was the dominant form of DNF in the controls, comprising 71% of total DNF. Coupled DNF in the control cores ranged from 1.7 to 49.0 $\mu\text{mol N m}^{-2} \text{h}^{-1}$. Overall, total DNF rates ranged from 7.6 to 60.6 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, and while rates were not significantly different between oyster and control cores on annual basis, the oyster cores did have significantly greater total DNF rates during the summer season ($p < 0.05$, Fig. 3a, b).

Site 2 – As in Site 1, coupled DNF was the dominant process for all cores from Site 2, with significantly higher coupled rates in both sites in all seasons ($p < 0.05$, Fig. 3c, d). Coupled DNF rates ranged from 5.24 to 37.0 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, and represented 88% of total DNF in the oyster cores. In the control cores coupled DNF ranged from 1.27 to 19.4 $\mu\text{mol N m}^{-2} \text{h}^{-1}$, representing 81% of total DNF. Total DNF was 5x greater in the oyster cores compared to controls in the fall ($p < 0.05$ Fig. 3c, d); however total DNF was also 1.8-fold lower than rates at Site 1. Annual averages of total DNF were $25.8 \pm 3.2 \mu\text{mol N m}^{-2} \text{h}^{-1}$ and $18.1 \pm 4.1 \mu\text{mol N m}^{-2} \text{h}^{-1}$ in the oyster and control cores, respectively.

Potential Denitrification

Site 1 - Seasonally averaged potential rates in the oyster cores were 1.8 and 2.7-fold higher in the summer and fall, relative to controls (Fig. 4a, $p < 0.1$), but rates were not significantly different in the spring. Potential DNF rates at Site 1 ranged from 18.2 to 150.0 $\text{N}_2\text{-N } \mu\text{mol m}^{-2} \text{h}^{-1}$, with an annual average of $68.53 \pm 5.1 \mu\text{mol m}^{-2} \text{h}^{-1}$ and $42.08 \pm 15.7 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster and control cores, respectively (Fig 4a). Average annual rates show that potential

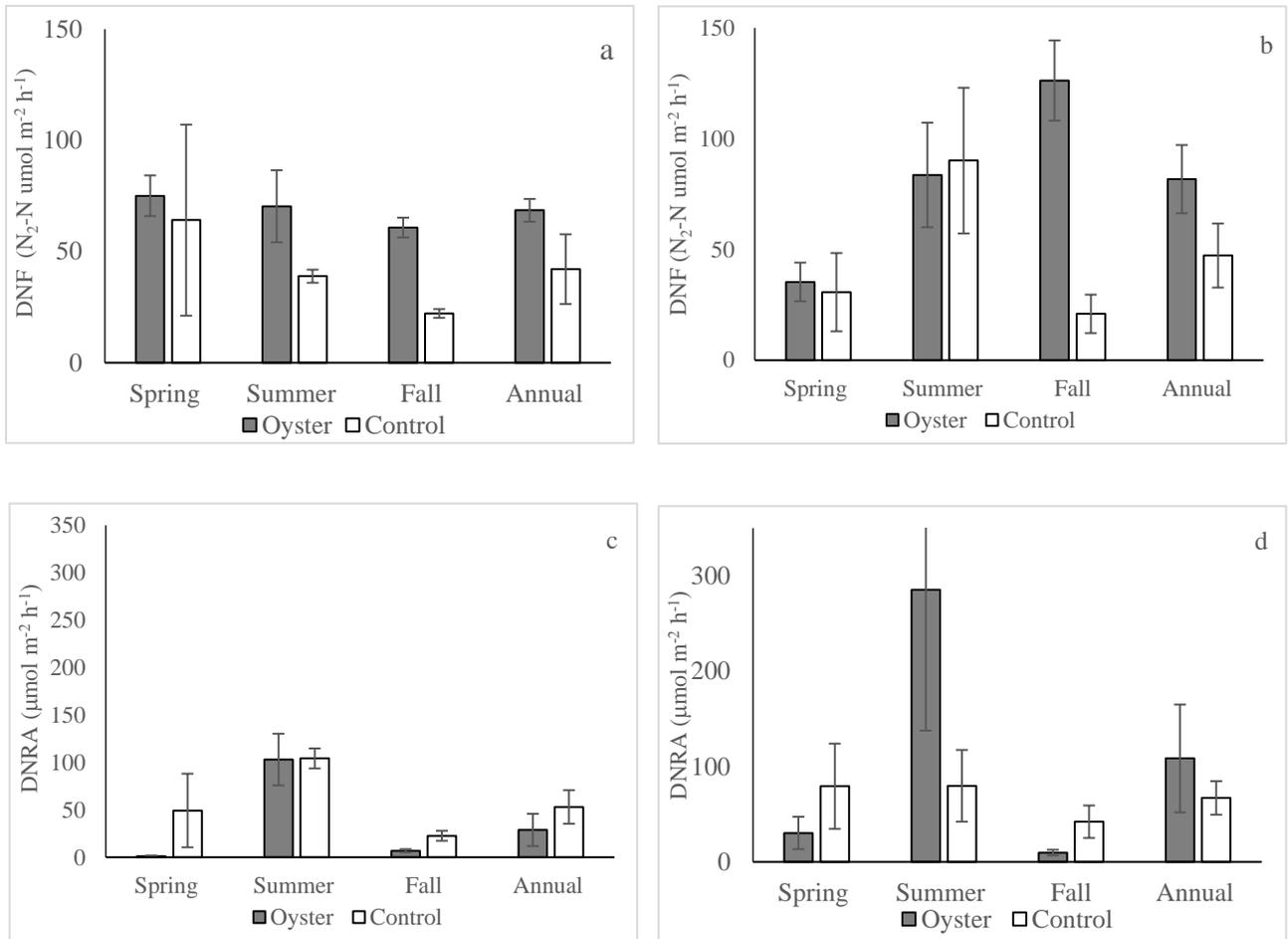


Figure 4. Seasonally and annually averaged (\pm SE) potential denitrification rates from Sites 1 (a) and 2 (b), as well as seasonally averaged DNRA rates in sites 1 and 2 (c and d, respectively) throughout the three years of this study. Shaded bars represent oyster cores while open bars signify control cores.

DNF was 63% higher in the oyster cores, though this difference is only moderately significant.

Site 2 - Overall, potential DNF rates were highest in the fall in the oyster cores and in the summer in the controls. Seasonally averaged potential rates in the oyster cores were 6x higher in fall, relative to controls ((Fig. 4a, $p < 0.05$), with no significant difference between the treatments in the spring and summer. Potential DNF rates scaled annually at this site were slightly greater than those of Site 1, an increase of 17%. Average annual potential DNF rate was 71% higher in the oyster cores compared to controls at Site 2 ($p < 0.1$), with average annual rates of $81.72 \pm 15.4 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster and $47.24 \pm 14.5 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the control cores (Fig. 4b).

Dissimilatory Nitrate Reduction to Ammonium

Site 1 - DNRA rates at Site 1 were highest in the summer, and oyster DNRA rates were 42 and 3.3-fold lower than controls in spring and fall, but not different in the summer ($p < 0.05$, Fig. 4c). DNRA rates ranged from non-detect to $130.18 \mu\text{mol m}^{-2} \text{h}^{-1}$, with annual averages of $28.74 \pm 17.0 \mu\text{mol m}^{-2} \text{h}^{-1}$ and $52.95 \pm 17.7 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster and control cores, respectively (Fig. 4c). Annual DNRA rates were not significantly different between oyster and controls.

Site 2 - Compared to Site 1 there was a larger range of DNRA rates from 5.5 to $517 \mu\text{mol m}^{-2} \text{h}^{-1}$ at Site 2, and DNRA rates were 2.2x higher at Site 2, relative to Site 1, on average ($p < 0.05$, Fig. 4d). Similar to Site 1, DNRA rates were highest during the summer ($p < 0.01$). Oyster DNRA rates were 3.6x higher in the summer and 3.3x lower in the fall relative to controls ($p < 0.05$, Fig. 4d). There was no significant difference in the DNRA between treatments in the spring. Average annual DNRA rates at site 2 ranged between $108.45 \pm 56.6 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the oyster cores and $67.05 \pm 17.5 \mu\text{mol m}^{-2} \text{h}^{-1}$ in the control cores, and were 62% greater in the

oyster cores.

Discussion

Results from this study reveal alterations to N biogeochemistry that were driven both by the presence of oysters, and by differences in oyster grow-out strategy. The data support the following findings: 1) There was no significant oyster effect on nitrification; 2) Sediments under oysters did show enhancement of potential DNF rates which indicated oyster stimulation of DNF under high NO_3^- concentrations; 3) DNRA was only enhanced by oysters in bottom cage grow-out (Site 2) in the summer; 4) DNF/DNRA ratios favored DNF in oysters cores at both sites, showing enhanced DNF over DNRA; 5) DE values indicate that recycling dominates over N removal via DNF at both sites; however, there was a small oyster induced increase in DE under both grow-out scenarios.

Nitrification

Nitrification rates calculated in this study fall in upper end of those reported across a range of marine ecosystems. These rates are consistent with other ^{15}N - NO_3^- isotope dilution derived rates for coastal sediments (Tobias et al. 2003, Drake et al. 2012) as well as in-situ ^{15}N - NH_4^+ tracer derived rates (Gribsholt et al. 2006). Nitrification rates are calculated as gross rates; therefore, we would expect them to be larger than many of the net rates reported.

It has been suggested that oysters may increase nitrification indirectly by increasing available NH_4^+ via excretion of NH_4^+ and remineralization of biodeposits (Dame et al. 1984, Lavrentyev et al. 2000, Newell et al. 2005). As a result, we might expect to see an increased nitrification rates in oyster cores; however, the first step in nitrification (ammonia oxidation) is an aerobic process, and requires molecular oxygen. As a result, higher rates of aerobic

respiration resulting from oyster biodeposits can decrease oxygen concentrations, creating a more reducing environment, and limiting nitrification (Smith et al. 2013). The balance between O_2 and NH_4^+ limitation of nitrification ultimately dictates whether oyster biodeposition increases the amount of N being nitrified and subsequently denitrified. Given the high NH_4^+ fluxes, it is likely that nitrification rates in this study are O_2 limited. At both sites, there was no statistically significant evidence of an oyster effect on gross nitrification and conclude that the presence of oysters did not accelerate the transformations of sediment DIN in a way that would ultimately promote nitrification. These cores however did not contain any live oysters or intact oyster shells, which have been shown to contribute to nitrification ((Lavrentyev et al. 2000, Welsh & Castadelli 2004). It is therefore possible that I may not have captured all direct influences of oysters on nitrification.

Nevertheless, nitrification rates were sufficient to supply enough NO_2^- and NO_3^- to support the coupled DNF rates measured throughout the study. In all cores, nitrification supplied 2 to 5x as much nitrate as was coupled to DNF. Additionally, the nitrification rate was equivalent to at least 90% of the nitrate required to support both the potential DNF and DNRA rates measured in all incubations.

Denitrification

Overall, total DNF rates in this study were on the lower end of the range of DNF rates from other studies of DNF and oysters (e.g. Higgins et al.2013, Kellogg et al. 2013, Smyth et al. 2013). However, the majority of these studies were done in the mid-Atlantic, or Southeastern USA in ecosystems, which experience higher temperatures and longer oyster growing seasons than that of the Northeast. As a result, lower temperatures are likely a driver for the differences we see in DNF rates, as these rates are comparable to another Rhode Island study (Humphries et

al. 2016). An additional explanation for these differences may be that of methodology. Most prior studies did not use IPT, which can yield slightly lower rates than the N₂:Ar method (Eyre et al. 2003). These rates may also be conservative estimates given the high degree of isotopic dilution observed in the ¹⁵N-NO₃⁻ pool as a result of nitrification. High nitrification rates, combined with a short ¹⁵N-NO₃⁻ incubation period, and a relatively high NO₃⁻ concentration all contribute to a potential disequilibrium between the ¹⁵N-NO₃⁻ added and the ¹⁵N enrichment at the reaction site. This disequilibrium, then, results in more ²⁸N₂ produced than is predicted by the IPT calculations, as they do not directly account for ²⁸N₂ produced (Nielsen 1992). Further, although NO₂⁻ was produced in excess, we do not have direct ¹⁵N-NO₂⁻ measurements which would represent the effective substrate enrichment for denitrification. These factors may well lead to an underestimation of coupled DNF in this study (Nielsen 1992). If this were the case, the net NO₃⁻ mass balance in the incubations would achieve better closure, and would suggest that the any underestimation of the coupled DNF rate would be approximately a factor of 2. Nevertheless, my coupled and direct rates fall well within the rates found in other studies using IPT in temperate ecosystems (e.g. Eyre et al. 2002, Deutsch et al. 2010, Gongol and Savage 2016) and a conservative DNF rate estimate does not hinder my interpretation of how oyster aquaculture influences DNF rates.

In general, nitrification rates supported coupled-dominated DNF. Coupled DNF is generally thought to be the dominant DNF pathway in sediments with low ambient nitrate, as is the case in both these sites (Koop-Jakobsen & Giblin 2010, Seitzinger et al. 2006). While the higher rates of total DNF in oyster cores compared to control cores were statistically significant in the summer in Site 1 and the fall in Site 2 but, there was no significant difference in total DNF on an annual basis (Fig. 3). The potential DNF in this study, however, was anywhere from 1.2 to

6x higher in the presence of oysters relative to control sediments during periods when oysters were most active. These results agree with several studies which have found elevated DNF associated with either oyster aquaculture, or natural oyster grow-out (e.g. Kellogg et al. 2013, Smyth et al. 2013, Humphries et al. 2016).

There are two different mechanisms by which oysters might increase DNF in sediments. The first is via an enhancement of N transformation from high N biodeposits through increased nitrification rates, which ultimately stimulate coupled DNF. This mechanism increases the supply of the terminal electron acceptor for DNF, but can only happen if the necessary conditions for increased nitrification are met, i.e. nitrification can be neither ammonium nor oxygen limited. The second method by which oysters might stimulate DNF is by providing more OC for this heterotrophic process. Increased OC in sediments from oyster biodeposits can serve as an increased electron donor pool for DNF in this case, but only if there is enough ambient nitrate to keep DNF from becoming NO_3^- limited.

The latter scenario is likely the case in this study where potential DNF was stimulated more than total DNF in the oyster cores. These data show that oysters increased DNF more when there was excess NO_3^- available (potential DNF), than in ambient NO_3^- conditions (total DNF). Additionally, increased potential DNF coincided with a slight decrease in C/N. The decreased C/N seen in the oyster cores can be an indication of increased sediment lability for heterotrophs including denitrifying bacteria. Interestingly, potential DNF rates were comparable, if not slightly greater in Site 2 where organic carbon content was lower but likely of better 'quality'. Site 2 had a lower C/N and the $\delta^{13}\text{C}$ was -18‰, suggesting a marine algal source (Fry and Sherr 1989, Caésar et al. 2008; Table 1). However, Site 1 had a higher C/N and an average $\delta^{13}\text{C}$ of approximately -25‰ (Table 1). This $\delta^{13}\text{C}$ value is indicative of a contribution of

terrestrially-derived carbon, and was evidenced further by the presence of leaf litter in Site 1 sediments. This terrestrial carbon source is considered generally less labile than marine algal sources (Fry and Sherr 1989, Wu et al. 2007). . These site differences in OC composition further supports a mechanism whereby increase the availability of a more labile OC electron donor for DNF, and may explain the higher rates of both DNRA and DNF in Site 2, as the OC at site 2 and may have been easier for sediment heterotrophs (including denitrifiers) to utilize. Recently, Plummer et al. 2015 reported that a combination of OC content, as well as OC source, were important determinants of denitrification in the nearby Niantic, River Estuary. Additionally, studies of sub and intertidal sediments have found that DNF rates can be greater in sandy sediments with high permeability, characteristic of Site 2, than in low permeability mud provided C and N are not limited (Patel 2008).

In this study, I observed no significant stimulation of nitrification by oysters, that potential DNF rates coincide with increased carbon lability, and that oysters stimulate DNF more when NO_3^- is not limiting. These results indicate that the existing geochemical conditions in a particular site may influence how much, or little oysters can affect DNF rates. In photic environments where N cycling is very tight and NO_3^- does not accumulate, oysters may have a lesser impact on DNF rates, because even if there is more labile carbon available, there may not be enough NO_3^- to fuel increased DNF. Conversely, oysters may be able to have a more marked effect on DNF in systems with high NO_3^- loading or high turbidity that leads to persistence of higher NO_3^- in the water column. If NO_3^- can accumulate in the system, the oyster derived increase in labile carbon can fuel increased DNF rates because DNF will not be NO_3^- limited. This suggests that oysters may serve the most benefit to the most highly nitrate impacted ecosystems.

It also follows then, that some of the highest rates of oyster stimulated DNF have been measured in systems where NO_3^- has been allowed to accumulate. For example, Kellogg et al. 2013 conducted *in situ* incubations in the dark, and saw a large NO_3^- efflux that would ordinarily be consumed by photosynthesis during the day. This accumulation of NO_3^- as well as increased OC availability by oysters, resulted in some of the largest DNF rates ever measured in a temperate estuary (Kellogg et al. 2013). Though there was net NO_3^- uptake by the sediment in this current study, the $85\mu\text{M}$ NO_3^- added ensured that DNF was not NO_3^- limited, thus satisfying the conditions of the IPT equations, but also resulting in stimulated potential DNF in the oyster cores.

Dissimilatory Nitrate Reduction to Ammonium

The only time DNRA was enhanced by oysters was at Site 2 in the summer when the water was warmest. Though there are not many studies which have examined DNRA rates in sediments influenced by bivalves, most have seen an increase in DNRA rates in the presence of bivalves, presumably due to the increased labile carbon load from biodeposits (Chutivisut et al. 2014, Murphy et al. 2016). However, one study did find that DNRA only represented 11% of the total nitrate uptake in a natural oyster reef system (Smyth et al. 2013). However, there was no detectable oyster alteration of total C, or $\delta^{13}\text{C}$ by the oysters. This lack of detectable influence from biodeposits on the sediment OC pool suggests that the biodeposit additions are either quickly mineralized, as in Site 2 (Dame et al. 1989), or as in the case of Site 1, bulk OC from oysters is small against a large OC background. As a result, these mechanisms did not translate into higher DNRA between oyster and control except for the summer at Site 2 where bottom cages sit directly on sediments.

As with DNF, higher rates of DNRA were found in Site 2, which had generally less available sediment carbon that was, perhaps, more labile carbon as a marine derived $\delta^{13}\text{C}$. It is also possible that differences in sulfide concentrations further influence on DNRA rates as a function of grow-out method. DNRA has been shown to be more dependent on OC and sulfide content and less influenced by OC source than DNF (Plummer et al. 2015). While we did not measure sulfide concentrations in these cores, but H_2S off-gassing from sediments collected from this site was evident, particularly in the summer. Additionally, the higher salinity in Site 2 relative to Site 1 indicates a higher availability of sulfate to support sulfate reduction under the cages, resulting in increased sulfide reduction and higher sulfide concentrations, which have been shown to enhance DNRA, suppress DNF, and increase N retention (An et al. 2002, Giblin et al. 2013).

The DNRA rate was equivalent to 8% and 23% of the net NH_4^+ flux to the overlying water, on average in Site 1 and 2, respectively. While this percentage suggests that DNRA may be a contributor to the NH_4^+ efflux, the DNRA rate measured in this study is a potential rate due to the high NO_3^- addition relative to ambient concentrations that are required for IPT. DNRA, like DNF, in coastal sediments is expected to be a function of NO_3^- concentration, so the DNRA contribution to NH_4^+ efflux is likely to be substantially smaller in-situ where NO_3^- concentrations are an order of magnitude lower than in these incubations (Jørgensen 1989).

Nitrate Removal vs. Retention (DNF/DNRA and DE)

Though the rates of DNRA are not significantly different in the oyster and control cores, DNRA comprised a significantly lower percentage of the nitrate uptake than DNF in oyster cores

Table 3. Annual averages \pm SE of the ratio of DNF to DNRA, percentage of the net nitrate flux represented by DNF and DNRA, and denitrification efficiencies.

Site	Core	DNF/DNRA	DNF/NO ₃ ⁻	DNRA/NO ₃ ⁻	DE	DE*
Site 1	Oyster	25.4 \pm 11	1.12 \pm 0.2	0.24 \pm 0.1	20% \pm 3	12% \pm 2
	Control	1.27 \pm 0.4	1.04 \pm 0.2	0.98 \pm 0.1	16% \pm 5	7% \pm 2
Site 2	Oyster	5.72 \pm 2.4	0.87 \pm 0.3	0.40 \pm 0.1	29% \pm 5	14% \pm 4
	Control	0.90 \pm 0.4	0.54 \pm 0.1	0.82 \pm 0.2	22% \pm 12	7% \pm 2

at Site 1 ($p < 0.05$; Table 3). On an annual basis, the ratio of DNF/DNRA was significantly higher in the oyster cores compared to the controls in both sites ($p < 0.05$, Table 3). These data show that DNF was favored over DNRA by 6 and 25-fold in the oyster cores of Sites 2 and 1, respectively. The oyster cores from both sites also had DNF as a significantly greater percentage of the total NO_3^- uptake compared to DNRA ($p < 0.05$, Table 3). These results show that oysters cause DNF to be dominant NO_3^- transformation process over DNRA, and therefore favor NO_3^- removal over recycling.

The oyster effect on this balance was particularly notable at the Site 1 where oysters were growing suspended from the surface. At Site 2, we saw a similar trend of DNRA comprising a smaller percentage of nitrate uptake in the oyster cores at Site 2, although the differences are only moderately significant ($p < 0.1$). We propose that the DNF/DNRA ratio is smaller (but still greater than 1) at Site 2 because of the bottom cage grow-out. Having oysters growing directly on the sediment concentrates biodeposits, reducing oxygen and creating a more reducing environment, which is more favorable for DNRA (Burgin & Hamilton 2007). This affect is particularly strong in summer when oyster respiration, filtration, and biodeposition are highest. At Site 2, there is a shift in the summer where oyster cores transition from being DNF dominated, to DNRA dominated.

Alternatively, Site 2 is located in one of many seasonally active coastal communities along the coast of southern New England, with many summer homes, summer-only residents. With increased summertime population comes increased N loading from the many septic systems still in use in the state. Increased N loading in the summer is evidenced by large scale eutrophication in this and other coastal lagoons via large algal blooms. We suggest that the shift

from an DNF dominated to a DNRA dominated regime is a result the deposition of N rich OM to the sediment (low summer C:N in control and oyster; Table 1). High OC loads and low C:N, microalgal derived OM are conditions under which DNRA is favored relative to DNF (e.g. Burgin and Hamilton 2007; Plummer et al. 2015). This suggests that, though DNF is favored over DNRA for most of the year in the oyster cores, increased eutrophication in the summer at Site 2, may overpower any oyster effect on this ratio and force the system to favor DNRA in the summer months.

Despite the DNF/DNRA ratios in favor of N removal at both sites, with the exception of Site 2 summer, the efficiency of denitrification relative to sediment water fluxes of DIN is small. Denitrification efficiency ($DE = N_2-N / (DIN + N_2-N) * 100$) is a measure which indicates the proportion inorganic N that is converted into N_2 through DNF, relative to DIN efflux to the overlying water (Eyre and Ferguson 2002). Across sites and seasons the highest DE was on the order of just $\frac{1}{4}$ of the DIN flux back to overlying water, though there was a modest but significant oyster effect on DE. DE was 26 to 35% greater in the oyster cores of Site 1 and 2, respectively, compared to controls (Table 3). Though DE values were well below 50% at all sites and far below those reported from some natural oyster reefs (Piehler & Smyth 2011), they are comparable to DE values reported by other studies conducted in natural and restored reefs (e.g. Eyre and Ferguson 2009, Kellogg et al. 2013). The higher DE in the oyster cores shows that more reactive N is being removed in the oyster compared to the control cores. We calculated a second efficiency which includes the production of NO_3^- via nitrification ($DE^* = N_2-N / (DIN + NITR + N_2-N) * 100$). DE^* gives us an indication of how much N is recycled to the overlying water vs. removed via DNF. DE^* values were generally lower than DE as a result of adding nitrification to the calculation; however, DE^* was still 2 to 2x greater in the oyster

compared to control cores (Table 3). These values indicate that, overall these systems leak 81% to 93% more N to the overlying water than they remove via DNF.

Even when including the gross nitrification term, which has not been measured in past studies and which lowers the calculated DE, we found that DNF was more efficient in the oyster cores at both sites ($p < 0.1$, Table 3). This is further evidence that the oyster cores are supporting relatively more N removal than the controls. Finally, DNF represented a greater proportion of the net nitrate flux into the sediment than did DNRA in the oyster cores annually, however, the difference was only significant at Site 2 ($p < 0.05$, Table 3). DNF comprising a larger proportion of the NO_3^- flux than DNRA is an indication that DNF is the dominant NO_3^- transformation process in both systems.

Conclusion

In this three-year, seasonal study of sediment N biogeochemistry, I found that cores taken amongst oysters exhibited significantly higher rates of total denitrification compared to control cores in the summer and fall months when oysters are most active at Site 1 and in the fall at Site 2. Gross nitrification rates, though not significantly different in oyster and control cores, were sufficiently high to support coupled-dominated DNF in all cores. DNRA rates were highest in the summer at both sites, but, contrary to my hypothesis, did not appear to be directly affected by oysters. Additionally, I observed higher rates of both DNF and DNRA at the lower OC, higher salinity Site 2 overall. Carbon quality and organic matter source appear to account for these differences in DNF rates between the sites, while increased salinity and therefore sulfate reduction is the most likely driver of the increased DNRA rates at Site 2. Additionally, larger net DIN fluxes, lower total DNF, and lower DNF/DNRA at Site 2 indicate that the bottom cage grow-out may have fostered an environment that was more favorable to DNRA and N retention

compared to Site 1.

While both sites are N retaining systems over all, the ratio of DNF/DNRA, denitrification efficiency, and the percentage of the net nitrate uptake represented by both DNF and DNRA in the oyster cores indicate that oysters may be fostering an environment that favors DNF over DNRA and decreases N retention compared to control sediment. These results suggest that oysters are performing an additional ecosystem service of boosting N removal at both sites. Overall this study has demonstrated the benefits of oysters for favoring N removal using two different grow-out strategies.

Appendix A

Table 4. Seasonal DIN, coupled (Dn) and direct (Dw) DNF, and DNRA rates in $\mu\text{mol N m}^{-2} \text{h}^{-1}$ with root mean square error (RMSE).

Location	Season	NO ₃ ⁻ Flux	RMSE	NO ₄ ⁺ Flux	RMSE	NO ₂ ⁻ Flux	RMSE	D _n	RMSE	D _w	RMSE	DNRA	RMSE
Site 1 Oyster	Fall 2013	-90.43	2.16	158.94	2.48	5.34	2.84	12.26	5.40E-15	9.60	7.40E-17	3.39	4.17E-03
	Spring 2014	-49.65	5.68	543.85	1.28	19.17	6.15	29.92	1.30E-15	0.62	0.00E+00	0.00	
	Fall 2014	-81.36	5.07	398.30	4.14	4.80	2.59	13.37	2.00E-03	9.49	8.00E-03	8.49	1.88E-03
	Spring 2015	-31.52	8.95	128.29	14.47	1.35	3.60	7.56	6.22E-15	0.40	7.42E-17	1.76	9.86E-04
	Summer 2015	-111.02	2.12	445.11	1.18	25.53	2.69	42.78	5.23E-15	3.79	2.75E-17	75.75	1.53E-03
	Fall 2015	-72.35	2.78	314.26	5.89	4.27	2.64	17.63	3.61E-15	7.60	7.88E-10	8.61	6.16E-04
	Spring 2016	-39.41	9.74	188.06	5.21	1.69	6.95	8.97	6.05E-13	0.50	7.16E-17	1.72	8.53E-04
	Summer 2016	-148.79	3.95	344.99	16.49	34.22	7.91	68.39	8.01E-15	6.06	3.55E-15	130.18	7.82E-03
	Fall 2013	-28.50	2.52	368.53	23.00	5.12	4.53	4.78	1.30E-15	3.32	7.00E-02	32.37	7.92E-04
	Spring 2014	93.75	0.00	145.52	13.40	-1.57	5.89	49.00	2.20E-16	1.01	4.20E-17	126.69	1.73E-03
Site 1 Control	Fall 2014	-27.72	2.65	162.76	3.25	4.98	3.48	1.73	4.00E-16	3.27	5.00E-18	14.30	5.59E-03
	Spring 2015	-12.19	8.34	170.32	7.67	-1.22	3.67	30.59	2.10E-15	0.14	1.29E-05	14.83	2.00E-04
	Summer 2015	-89.32	3.67	393.02	18.75	-1.88	2.83	24.03	4.87E-15	1.80	8.51E-17	93.58	2.24E-03
	Fall 2015	-21.98	3.77	238.45	2.76	3.95	2.17	1.73	9.98E-15	23.67	2.12E-17	20.94	3.57E-03
	Spring 2016	-13.21	8.07	78.92	3.85	-1.32	5.01	30.59	7.65E-15	-9.75	3.47E-15	6.25	9.89E-04
	Summer 2016	-99.68	4.09	341.29	14.34	-2.09	1.59	28.79	7.74E-16	187.52	7.33E-17	114.68	1.75E-03
Site 2	Fall	-503.98	6.23	98.42	6.53	40.72	3.50	29.44	1.76E+00	0.61	0.00E+00	5.54	5.42E-03

Oyster	2013 Spring												
	2014	-649.65	8.90	567.33	16.00	92.99	3.18	5.24	4.00E-16	2.23	3.10E-18	64.32	8.60E-04
	Summer			-									
	2014	1487.40	24.04	1318.46	34.03	57.25	2.01	13.20	1.80E-02	5.63	4.90E-02	517.65	3.95E-03
	Fall												
	2014	-81.98	4.01	143.09	1.78	14.76	2.84	36.27	0.00E+00	0.99	4.20E-17	8.05	9.50E-04
	Spring												
	2015	-33.00	5.51	121.07	6.72	3.30	1.32	23.82	1.49E-12	5.17	1.38E-18	13.73	7.38E-03
	Summer												
	2015	-150.53	6.64	287.52	19.72	10.54	5.93	16.31	5.32E-15	3.66	8.57E-17	113.20	8.07E-03
	Fall												
	2015	-84.31	1.71	276.26	9.24	15.17	1.14	37.01	4.96E-16	0.96	4.53E-17	15.54	3.95E-04
	Spring												
	2016	-21.61	2.86	114.50	8.13	2.16	4.42	24.81	3.46E-15	3.14	5.80E-17	12.98	2.90E-03
Summer													
2016	-204.76	9.16	346.62	12.49	14.33	3.24	17.34	2.17E-15	6.02	3.42E-17	225.08	4.43E-03	
Fall													
2013	-54.26	16.50	887.18	13.16	4.89	3.30	1.27	7.20E-16	0.03	9.00E-03	11.22	8.86E-04	
Spring													
2014	-676.48	7.63	632.75	6.78	16.31	5.26	15.41	2.70E-01	6.57	3.00E-02	168.55	8.30E-03	
Summer													
2014	-119.83	6.11	-13.68	66.78	49.36	6.91	31.29	1.30E-15	13.34	4.30E-17	128.87	6.15E-03	
Fall													
2014	-65.88	1.28	614.83	9.49	6.59	1.75	10.64	4.78E-15	0.22	9.00E-18	70.00	8.66E-03	
Spring													
2015	-23.29	7.11	121.67	6.27	0.56	7.12	12.15	5.40E-15	1.04	7.04E-17	32.41	4.70E-04	
Summer													
2015	-90.41	6.03	245.27	14.67	22.60	3.00	19.37	8.50E-15	4.74	1.59E-17	23.37	9.83E-04	
Fall													
2015	-58.30	6.51	396.21	8.65	5.83	3.79	8.57	3.34E-15	0.18	1.24E-18	45.11	7.82E-03	
Spring													
2016	-27.22	1.49	138.26	1.50	0.66	5.70	13.71	3.33E-14	1.57	5.93E-17	36.83	8.28E-04	
Summer													
2016	-80.32	6.96	217.37	20.74	20.08	2.52	19.04	7.78E-16	4.06	4.62E-17	87.12	6.86E-03	

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