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Estimating the effects of seawater intrusion on an estuarine nitrogen cycle by comparative network analysis

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ABSTRACT: Nitrogen (N) removal from estuaries is driven in part by sedimentary microbial processes. The processes of denitrification and anaerobic ammonium oxidation (anammox) remove N from estuaries by producing N_2 gas, and each can be coupled to N recycling pathways such as nitrification and dissimilatory nitrate reduction to ammonium (DNRA). Environmental conditions such as seawater intrusion influence sedimentary estuarine N cycling processes. This study investigated the potential effects of seawater intrusion on N cycling processes and their couplings through a comparative modeling approach. We applied environ analysis, a form of ecosystem network analysis, to 2 N cycling mass-balance network models constructed at oligohaline and polyhaline sites in the Cape Fear River Estuary, North Carolina, USA. We found that nitrification coupled to both denitrification and anammox was 2.5 times greater at the oligohaline site, while DNRA coupled to anammox was 2.7 times greater at the polyhaline site. However, the total amount of N_2 gas produced relative to the N inputs to each network was 4.7 and 4.6% at the oligohaline and polyhaline sites, respectively, as direct removal was greater at the polyhaline site. An uncertainty analysis using linear inverse modeling indicated that our results are relatively robust to the effects of parameterization uncertainty. These results suggest that changes in water chemistry from seawater intrusion may favor direct over coupled N removal, but may not substantially change the N removal capacity of sedimentary microbial processes.

KEY WORDS: Coupling · Seawater intrusion · Ecosystem network analysis · Environ · Uncertainty analysis · Ecosystem services

INTRODUCTION

Estuarine sediments support microbial communities that provide important ecosystem services ranging from the decomposition of organic material to the recycling and removal of nutrients. The microbial communities that provide these ecosystem services can vary with environmental conditions such as temperature, nutrient availability, and salinity (Bouvier & del Giorgio 2002, Bernhard et al. 2007, Baron et al. 2013). However, the environmental conditions in estuarine ecosystems may undergo substantial changes in the future resulting from continued urban development and global climate change. An exemplar of this is the dredging of estuarine channels and the addition of canals to accommodate shipping traffic, which may lead to seawater intrusion into the freshwater portions of estuaries (Newport 1977, Hackney & Yelverton 1990, Zhang et al. 2012). Predicted sea level rise over the next century (IPCC

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2007) may further promote seawater intrusion, leading to greater shifts in the water chemistry of affected areas that may have ecological consequences.

The removal of nutrients from estuarine sediments through microbially mediated processes can have important implications for the health of estuaries (Pinckney et al. 2001, Christian et al. 2010). Primary production in estuaries is typically limited by the availability of nitrogen (N; Ryther & Dunstan 1971, Howarth & Marino 2006). Therefore, N removal processes can help to alleviate the effects of eutrophication caused by anthropogenic nutrient loading (Anderson et al. 2002). The processes of denitrification and anaerobic ammonium oxidation (anammox) convert reactive forms of N to di-nitrogen (N_2) gas in estuaries. Denitrification produces N_2 gas from nitrate (NO_3^-), while anammox combines ammonium (NH_4^+) with nitrite (NO_2^-) to produce N_2. The N_2 gas produced by these processes is freely exported from blackwater estuaries because most organisms cannot use it. Although N fixation, which converts N_2 gas to NH_4^+, can be important in some estuarine systems with ammonium-saturated sediments (Gardner et al. 2006), fixation rates are likely low in ammonium-rich, low-light sediments such as those found in many estuaries, allowing for the export of N_2 gas (Ohmori & Hattori 1974, Cejudo et al. 1984, Boynton & Kemp 2008).

Denitrification and anammox can be either uncoupled (direct) or coupled to N transformation processes such as nitrification or dissimilatory nitrate reduction to ammonium (DNRA). Direct N removal processes produce N_2 from N in the form in which it enters the estuary, while coupled removal processes consume the products of microbial N transformation processes (Jenkins & Kemp 1984). For example, denitrification and anammox can utilize NO_3^- and NO_2^- produced by nitrification. Anammox can also utilize NH_4^+ and NO_2^- produced by DNRA. The strength of coupling between N removal and transformation processes relative to direct removal processes can have important implications for N residence time and the N removal capacity of estuaries (Thamdrup & Dalsgaard 2002, Seitzinger et al. 2006, Santoro 2010).

Alterations in the coupling strength of N removal and transformation processes may exacerbate eutrophication. Seawater intrusion can facilitate desorption of NH_4^+ in estuarine sediments as a result of cation exchange, making it available to planktonic algae (Gardner et al. 1991, Seitzinger et al. 1991, Hou et al. 2003, Giblin et al. 2010, Weston et al. 2010). Further, because denitrification is the dominant N removal pathway, a strong coupling between nitrification and denitrification is necessary to remove the NH_4^+ before it can be assimilated into algal biomass. Salinity increase and other environmental changes derived from seawater intrusion can also repress the rates of N removal and transformation processes (Dong et al. 2000). For example, nitrification rates typically decrease at elevated salinities due to sulfide inhibition (Joye & Hollibaugh 1995, Rysgaard et al. 1999), while DNRA activity tends to increase along a salinity gradient (Giblin et al. 2010). Under elevated salinity conditions, estuaries could experience weakened coupling of microbial N processes that may substantially hinder the N removal services that estuaries provide (Craft et al. 2009).

Ecosystem network analysis (ENA) provides a means to evaluate the strength of coupling between N removal and transformation processes, along with the potential effects of environmental changes on these relationships (Christian et al. 2011, Hines et al. 2012, Small et al. 2014). Ecosystem networks provide a whole-ecosystem perspective in which thermodynamically conserved material and material fluxes comprise network nodes and edges, respectively. ENA is a set of analyses derived from economic input–output analyses that are applied to mass-balanced network models to evaluate the flow of energy and matter through an ecosystem (Hannon 1973, Patten et al. 1976, Fath & Patten 1999, Ulanowicz 2004). Environ analysis, a form of flow analysis in ENA, is used to partition the flow of energy-matter in an ecosystem network to track material moving through the ecosystem (Patten 1978, 1982, Fath & Patten 1999). Comparisons among different parameterizations of ecosystem networks using ENA tools can provide insight into how differences in network organization can affect the system behavior. For example, Christian et al. (2005) compared ecological trophic networks of different estuaries at different seasons to draw conclusions about the effects of stress on the system. We applied environ analysis in ENA to estimate the coupling of N transformation and removal processes in N cycling networks (Hines et al. 2012).

To determine the potential steady-state effect of seawater intrusion on the sedimentary N cycle, we compared 2 N cycling networks parameterized at oligohaline and polyhaline sites, respectively, in the Cape Fear River Estuary (CFRE), North Carolina, USA. We used environ analysis to evaluate the strengths of the coupling of nitrification to denitrification as well as of nitrification and DNRA to anammox at each site. Because of the negative effects of seawater on the process of nitrification, we hypothesized that nitrification coupled to the removal processes of denitrification and anammox would be

lower in the polyhaline network. However, we hypothesized that DNRA coupled to anammox would be higher in the polyhaline network due to the resilience of DNRA to seawater conditions. To evaluate the potential effect of seawater intrusion on the microbial N cycle, we compared the model results between the oligohaline and polyhaline sites and used a space-for-time substitution to make predictions (Pickett 1989). This approach assumes that, at steady-state, the oligohaline site will resemble the polyhaline site after the seawater has replaced the freshwater. Further, we evaluated the robustness of the model results to parameter uncertainty. Thus, this study used network modeling and analyses to (1) synthesize disparate field measurements to estimate the direct and coupled N removal from denitrification and anammox, (2) test hypotheses about the effect of seawater intrusion on process coupling, and (3) evaluate the N removal capacity of estuarine sediments under different salinity conditions. Insight into differences in how N moves through networks at oligohaline and polyhaline sites was used to predict potential changes in the N cycling of the estuary resulting from seawater intrusion, such as that predicted by the IPCC climate change scenarios (IPCC 2007), where it is expected that the polyhaline area of the estuary will increase at the expense of the oligohaline area and all other factors will remain equal.

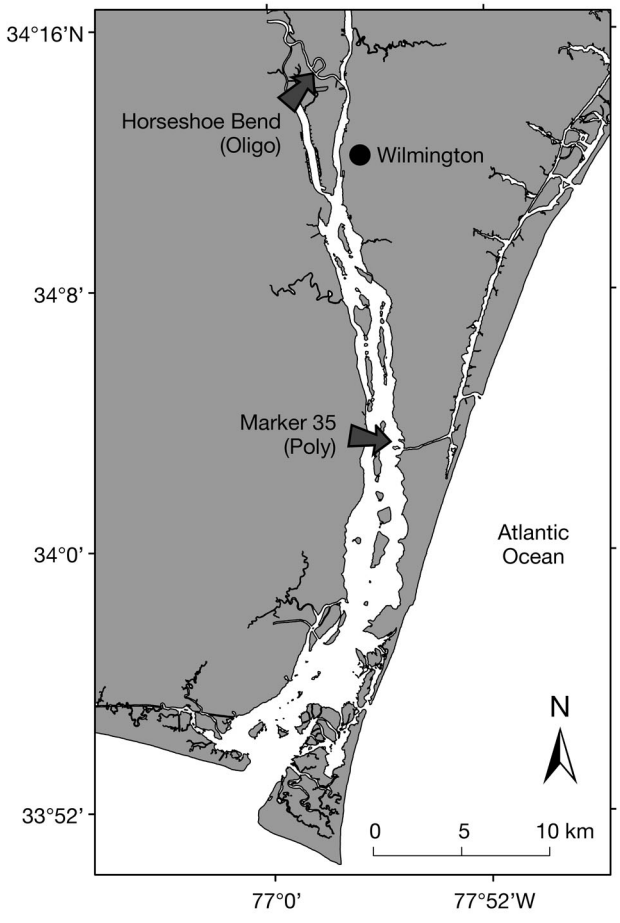

Fig. 1. Cape Fear River Estuary, North Carolina, USA. Horseshoe Bend (oligohaline, Oligo) and Marker 35 (polyhaline, Poly) study sites marked by arrows. The city of Wilmington is shown by a black circle

MATERIALS AND METHODS

Network construction

We compared 2 N cycling networks for the CFRE, one at an oligohaline site (Oligo) and one at a polyhaline site (Poly; Fig. 1). The Oligo network was constructed by Hines et al. (2012) and models a section of river called Horseshoe Bend (34° 14′ 37.464″ N, 77° 58′ 11.280″ W), which typically experiences salinities ranging from 0.1 to 5.0, with occasional salinities as high as 8 (Mallin et al. 2009, 2010). We constructed a second N cycling network for comparison at the Poly site, a section of river at channel marker 35 (34° 2′ 2.688″ N, 77° 56′ 21.948″ W) with a mean salinity consistently above 10 (Mallin et al. 2009, 2010). The modeled sites were similar in depth, tidal range, sediment grain size, dissolved oxygen, total suspended solids, and percent organic matter (Table 1).

Both networks had identical topologies to facilitate comparison (e.g. Baird et al. 1991), but the parameter values representing the magnitudes of N storages and fluxes for each network varied according to differences in the N cycle observed at each site. The means of direct measurements of N storages and transformations at each site were used to parameterize the networks whenever possible; values of fluxes reported in the literature for similar sites were used when direct measurements were not available (Table 2). The models were parameterized to represent the average conditions at the site during a single day in the summer months (June–August, 2008 and 2009).

Each network represented a volume of adjacent 1 cm^3 segments of the water column and sediment at the water–sediment interface at the Oligo and Poly sites, respectively. Like past network analyses of estuarine ecosystems (Baird & Heymans 1996, Christian & Thomas 2003), the modeled volumes were assumed to be at a steady-state to meet the requirements of ENA mathematics. As a prerequisite to conducting ENA, each network should encompass all aspects of the ecosystem of interest through representation as storages or fluxes (Fath et al. 2007). The small scale of

Table 1. Characteristics of the oligohaline and polyhaline sites and network models. Standard deviation indicated by ±, while parentheses indicate 95% confidence intervals from network models. DO: dissolved oxygen; TST: total system throughflow; FCI: Finn's Cycling Index; APL: average path length

	Oligohaline	Polyhaline	Source
Site characteristics			
Depth (m)	11	15	M. McIver pers. comm.
Tidal range (m)	1.2	1.5	M. McIver pers. comm.
Grain size (µm)	180.1 ± 264.9	314.7 ± 229.9	Hirsch (2010)
Salinity (psu)	4.6 ± 2.8	20.5 ± 4.4	Mallin et al. (2009, 2010)
Water DO (mg l^{-1})	4.9 ± 0.8	6.7 ± 0.4	Mallin et al. (2009, 2010)
Total suspended solids (mg l^{-1})	12.5 ± 3.7	10.1 ± 2.4	Mallin et al. (2009, 2010)
Sediment% organic	5.7 ± 7.4	3.0 ± 3.4	Hirsch (2010)
Network characteristics			
Total N input (nmol N cm^{-3} d^{-1})	3802.0 (3045.5–4577.5)	3068.4 (2557.5–3574.0)	Present study
NH_4^+ input (nmol N cm^{-3} d^{-1})	1370.0 (739.0–1947.3)	1047.6 (581.3–1511.6)	Present study
NO_x input (nmol N cm^{-3} d^{-1})	1193.0 (881.3–1504.3)	726.6 (523.5–926.1)	Present study
TST (nmol N cm^{-3} d^{-1})	7088.7 (5852.3–8377.1)	5322.3 (4631.9–6028.8)	Present study
FCI	0.20 (0.12–0.28)	0.17 (0.13–0.21)	Present study
APL	1.9 (1.6–2.2)	1.7 (1.6–2.0)	Present study

these models enabled the networks to focus on the microbial processes involved in N cycling by considering the N contributions of macroorganisms and large detritus as boundary inputs and outputs to the modeled volume (Hines et al. 2012). Specifically, the N contributions of macroorganisms and large detritus to the networks were through dissolved N inputs to the modeled volume, and were accounted for in direct measurements of N inputs to each site (Table 2). The small-scale perspective of these models allows them to meet the requirements of ENA analysis (Fath et al. 2007) while capturing the interactions between microbial processes, and thus they are useful for observing changes in these interactions.

In each network, N was divided into pools of ammonium (NH_4), nitrate and nitrite (NO_X), the N stored in microbial biomass (M), and a combination of dissolved and particulate organic N (ON). Each N pool was assigned a node in the network and pools were repeated in the water column (W-) and sediment (S-), yielding a total of 8 network nodes (Fig. 2). N_2 gas was considered as part of the external environment, and N removal processes were represented as boundary fluxes from nodes to the environment. N fixation was not explicitly represented in this model due to high levels of environmental NH_4^+ (Hines et al. 2012), but is included in the boundary inputs to NH_4 compartments. N transformation processes were measured in units of nmol N cm^{-3} d^{-1} and were used to guide the construction of network links. A detailed description of the storages and internal fluxes in the networks as well as a complete justification for each element of the network design can be found in Hines et al. (2012). The values used for each network flux and storage, and the sources from which these values were obtained, can be found in Table 2.

ENA

ENA is applied to steady-state ecosystem networks to characterize the flow of energy-matter through the system (Patten et al. 1976, Ulanowicz 1986), and consists of several different mathematical analyses including flow and environ analyses. Here, we provide a brief conceptual description of the ENA algorithms used in this work; detailed reviews of ENA can be found in Fath & Patten (1999), Ulanowicz (2004), and Schramski et al. (2011). This study applied ENA to the Oligo and Poly N networks using the *enaR* package for R (Lau et al. 2012, Borrett & Lau 2014). Complete models were formatted for each site according to the Scientific Committee on Ocean Research standards (SCOR files, Ulanowicz & Kay 1991, see Supplement at www.int-res.com/articles/suppl/m524p137_supp.pdf). The difference between the input and output fluxes of each network node were less the 5% following targeted mass-balancing, which modified only the least certain parameters during the model construction (Hines et al. 2012). Thus, the networks were considered to be at steady-state. The results of ENA were used to estimate the coupling of microbial processes at the Oligo and Poly sites, and were compared to make predictions about the potential effects of seawater intrusion on the microbial N cycle in estuarine sediments. The Supplement contains detailed

Table 2. Fluxes, parameter values, and sources for the oligohaline (Oligo) and polyhaline (Poly) networks. Boundary flows represent network inputs and outputs, while internal fluxes represent flows from one compartment to another. Parameter values are in nmol N cm^{-3} d^{-1}. Values for the Oligo model were previously presented in Hines et al. (2012). In each network, N was divided into pools of ammonium (NH_4), nitrate and nitrite (NO_X), the N stored in microbial biomass (M), and a combination of dissolved and particulate organic N (ON). Each N pool was assigned a node in the network and pools were repeated in the water column (W-) and sediment (S-)

Flux	Oligo	Poly	Source
Boundary → W-NH_4	130.0	72.5	Direct measurements (Ensign et al. 2004, Hirsch 2010)
Boundary → W-NO_X	1020.0	381.1	Direct measurements (Ensign et al. 2004, Hirsch 2010)
Boundary → W-M	3.9×10^{-5}	3.9×10^{-5}	Whitman et al. (1998)
Boundary → W-ON	1160.0	1255.1	Direct measurements (Ensign et al. 2004, Mallin et al. 2010)
Boundary → S-NH_4	1238.2	975.1	Mass-balance
Boundary → S-NO_X	173.2	345.5	Direct measurments (Ensign et al. 2004, Hirsch 2010)
Boundary → S-ON	79.0	39.1	Jordan et al. (1983)
W-NH_4 → boundary	276.0	132.4	Direct measurements (Ensign et al. 2004, Hirsch 2010)
W-NO_X → boundary	1008.6	380.9	Direct measurements (Ensign et al. 2004, Hirsch 2010)
W-M → boundary	3.9×10^{-5}	3.9×10^{-5}	Whitman et al. (1998)
W-ON → boundary	1159.6	1246.8	Direct measurements (Ensign et al. 2004, Mallin et al. 2010)
S-NH_4 → boundary	1080.0	1006.9	Tobias et al. (2001)
S-NO_X → boundary	6.0	127.3	Tobias et al. (2001)
S-ON → boundary	104.1	32.7	Jordan et al. (1983)
S-NH_4 anammox	2.5	1.8	Direct measurements (Hirsch 2010)
S-NO_X anammox	2.5	1.8	Direct measurements (Hirsch 2010)
S-NO_X denitrification	172.0	136.7	Direct measurements (Hirsch 2010)
S-NO_X burial	0.3	7.8×10^{-3}	Estimation from sea level rise
S-M burial	3.9×10^{-7}	3.9×10^{-7}	Estimation from sea level rise
S-ON burial	3.9	2.0	Estimation from sea level rise
W-NH_4 → W-NO_X	1.7	0.7	Kemp et al. (1990)[a], Berounsky & Nixon (1993)[a], Whitman et al. (1998)[b]
W-NH_4 → W-M	1.9	1.7	Veuger et al. (2004)
W-NH_4 → S-NH_4	5.5	1.5	Cowan et al. (1996)
W-NO_X → W-M	9.8	0.5	Veuger et al. (2004)
W-NO_X → S-NO_X	14.1	7.7	Cowan et al. (1996)
W-M → W-NH_4	3.1	3.1	Mass-balance
W-M → W-ON	16.0	0.5	Mass-balance
W-M → S-M	119.2	119.2	Cowan et al. (1996)
W-ON → W-NH_4	5.2	5.4	Pujo-Pay et al. (1997)
W-ON → W-M	7.4	1.4	Veuger et al. (2004)
W-ON → S-ON	853.9	425.8	Estimation from sea level rise
S-NH_4 → S-NO_X	144.0	77.5	Hansen et al. (1981)[b], Henriksen & Kemp (1988)[a], Kemp et al. (1990)[a]
S-NH_4 → S-M	212.8	186.2	Whitman et al. (1998)[b], Veuger et al. (2004)[a]
S-NH_4 → W-NH_4	136.5	55.3	Mass-balance
S-NO_X → S-NH_4	39.0	104.4	Direct measurements (Graham 2008)
S-NO_X → S-M	109.0	53.2	Whitman et al. (1998)[b], Veuger et al. (2004)[a]
S-NO_X → W-NO_X	2.1	7.3	Cowan et al. (1996)
S-M → S-NH_4	146.7	146.7	Mass-balance
S-M → S-ON	257.1	253.2	Mass-balance
S-M → W-M	119.2	119.2	Cowan et al. (1996)
S-ON → S-NH_4	150.0	100.0	Blackburn (1988)
S-ON → S-M	82.0	159.6	Whitman et al. (1998)[b], Veuger et al. (2004)[a]
S-ON → W-ON	850.0	423.8	Grant et al. (1997)

[a]used for Oligo network only; [b]used for Poly network only

calculations to replicate the results of all of the ENA subroutines used in this work.

Flow analysis

Flow analysis is used to determine how much material travels across the different pathways and through the different nodes in an ecosystem network (Finn 1980, Hannon 1985). Several network-level indicators are used to characterize the movement of energy or matter through an ecosystem. This study examined 3 of these statistics to compare the movement of N through the Oligo and Poly networks: total system throughflow (TST), Finn's Cycling Index (FCI), and average path length (APL). TST, which is

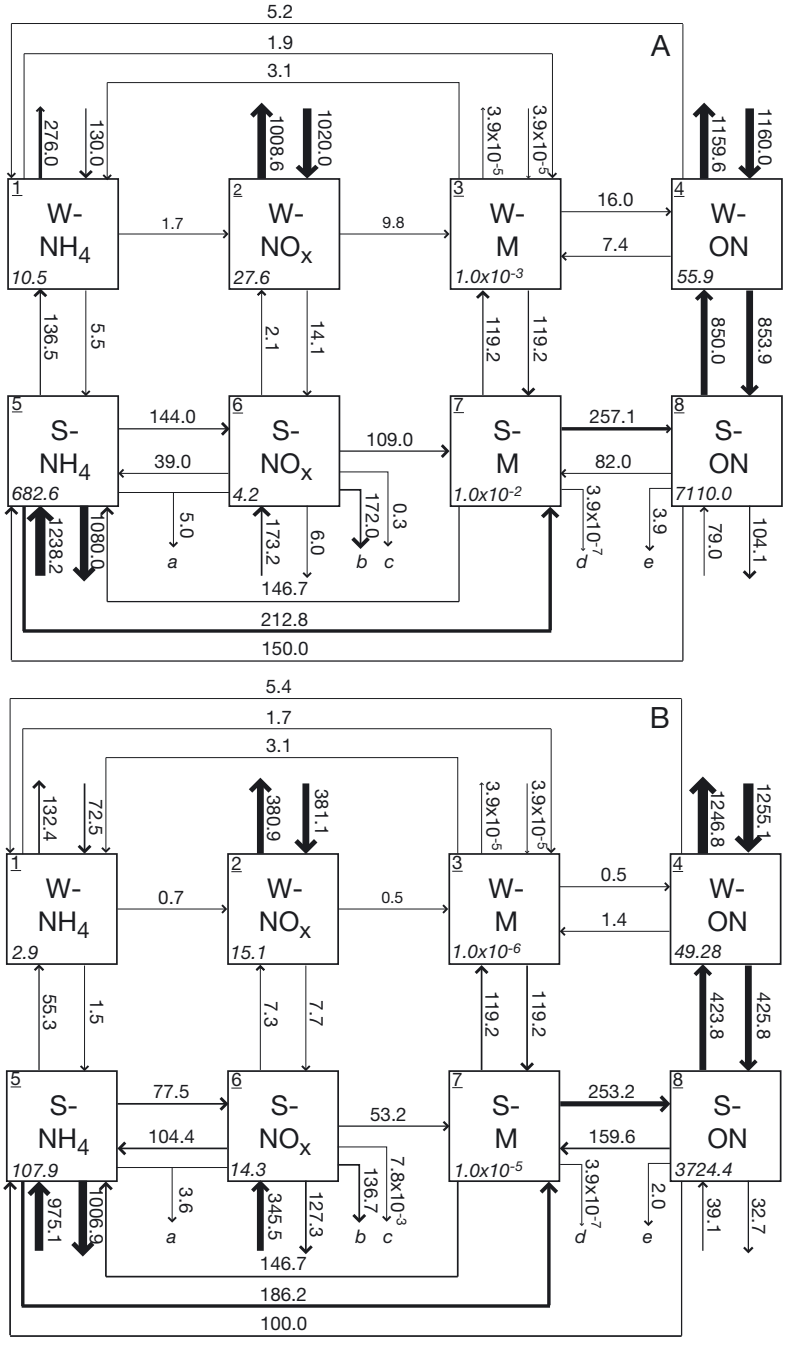

Fig. 2. Network models constructed at the (A) oligohaline and (B) polyhaline sites. Network structure was identical between the 2 sites, while flux magnitudes varied. Arrow widths approximate relative flux magnitudes within each network. Labeled loss arrows represent anammox removal (*a*), denitrification removal (*b*), nitrate and nitrite burial (*c*), microbial burial (*d*), and organic N burial (*e*). Italicized numbers in the bottom left of node boxes represent standing stock concentrations, while underlined numbers in the top left of node boxes show the node label number. See Table 2 for abbreviations

the sum of all activity across all nodes (Fath & Patten 1999), was used to quantify the amount of flow in each network. FCI, which reports the amount of material in a specific network node that leaves that node and returns to it at least once before exiting the network (Finn 1980), was calculated to gain insight into differences in N cycling at each site. APL was used to compare the residence time of N in each network. It is the average number of paths material crossed before exiting each network (Fath & Patten 1999), and is akin to the multiplier effect in economics. We used flow analysis statistics to obtain a broad overview of the differences between the Oligo and Poly networks. A detailed description of flow analysis mathematics can be found in the literature (Patten et al. 1976, Borrett et al. 2010, Schramski et al. 2011).

Environ analysis

Environ analysis is a subset of flow analysis that partitions the quantified flows in a network to show where material comes from before it exits the network (time-backward, input orientation) or where material goes after it enters the network (time-forward, output orientation, Patten 1978, 1981, 1982). The environs produced by environ analysis are non-overlapping subnetworks that can be summed to recover the original network (Patten 1978). For this analysis, we used realized input environs, which are scaled by the observed system boundary flows (Whipple et al. 2007, Borrett & Freeze 2011). These techniques were applied to each of the Oligo and Poly networks to facilitate a comparison of process coupling at each site (see Supplement).

Coupling quantification

The realized input environs generated by environ analysis were used to estimate the coupling of (1) nitrification to denitrification, (2) nitrification to anammox, and (3) DNRA to anammox. Coupled N_2 production was defined as N transfer across an internal network pathway immediately prior to export from the network across an N_2 production pathway, while N crossing an

N_2 production pathway without crossing any internal network pathways was considered to be direct N_2 production. Fig. 3 shows an example of how the denitrification environs for each network were used to calculate nitrification coupled to denitrification. N in the S-NO_X node was assumed to have a probability of exiting the network through the denitrification pathway (p_d) equal to the proportion of N involved in the denitrification pathway relative to all N exiting the node in the realized denitrification environ so that

$$p_d = \frac{A}{A+B+C+D} \quad (1)$$

where A is the magnitude of the denitrification flux, B is the uptake of NO_X by microbes in the sediments, C is the movement of NO_X from the sediments to the water column, and D is the conversion of NO_3^- and NO_2^- to NH_4^+ in the sediments through DNRA (Fig. 3). The amount of N involved in nitrification coupled to denitrification ($Coupled_{nd}$) was calculated by multiplying the amount of N crossing the nitrification pathway in the denitrification environ (X) by the probability of N in the S-NO_X node exiting the network through denitrification (p_d) so that

$$Coupled_{nd} = X \times p_d = \frac{XA}{A+B+C+D} \quad (2)$$

The strength of the coupling of nitrification to denitrification (CS_{nd}) was obtained by dividing the coupled nitrification to denitrification by the total denitrification removal, resulting in

$$CS_{nd} = \frac{XA}{A+B+C+D} \times \frac{1}{A} = \frac{X}{A+B+C+D} \quad (3)$$

The strength of the coupling (CS_{nd}) was then multiplied by 100 to determine the percentage of nitrification coupled to denitrification (%CS_{nd}). Similar calculations were used for the S-NH_4 and S-NO_X nodes in the realized anammox environs to determine nitrification coupled to anammox and DNRA coupled to anammox.

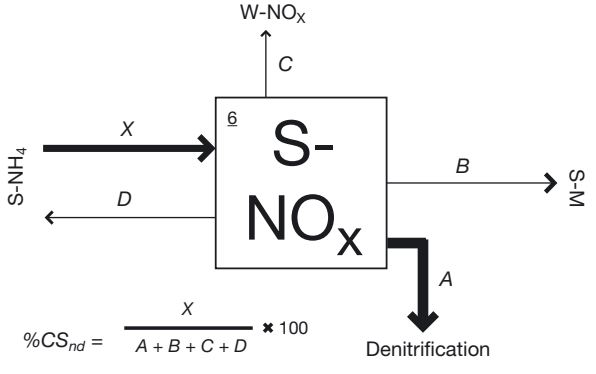

Fig. 3. Example calculation of coupling in the denitrification environ for percent nitrification coupled to denitrification. A: Denitrification, B: microbial uptake of NO_x in the sediments, C: transfer of NO_x from the sediments to the water column, D: dissimilatory nitrate reduction to ammonium, and X: nitrification. %CS_{nd}: percentage of nitrification coupled to denitrification. See Table 2 for abbreviations

Nitrogen removal efficiency

The magnitudes of microbial N removal processes with respect to the N inputs at each site revealed the relative ability of the microbial communities to utilize the available resources. Coupled and direct N removal processes at the Oligo and Poly sites were scaled to the inputs of each network by dividing each removal process (R_p) by the sum of the appropriate input vector (z) such that the relative process magnitudes were $\frac{R_p}{\sum_{i=1}^{n} z}$, where n is the number of nodes in the network.

Model uncertainty

The model quantifications of coupling and N removal efficiency at the Oligo and Poly sites are based on calculations that rely on the network parameterization at each location. However, the data used to assign flow magnitudes to individual fluxes were averaged across multiple summer seasons and, therefore, contained uncertainty that differed among each flux. Further, some model parameters were obtained from literature measurements in similar estuaries or by mass balance, adding to the uncertainty in the parameterization of each network. These uncertainties, which are common in models (Oreskes et al. 1994), imply that a range of plausible networks and associated coupling quantifications exists for each of the Oligo and Poly sites (e.g. Borrett & Osidele 2007).

To evaluate the robustness of our model conclusions to these parameter uncertainties, we performed an uncertainty analysis (Saltelli et al. 2008). This is a Monte Carlo analysis that determines the variation in a model output given the uncertainties in the model input. We used a linear inverse modeling approach based on the techniques presented by Kones et al. (2009) to create 10 000 plausible model parameterizations for each site. We used the *limSolve* package for R (Soetaert et al. 2009) to execute the analysis. Plausible models were considered to (1) be at steady state and (2) contain parameters with values within the range of uncertainty for each network flux.

Table 3. Network fluxes by parameter quality according to the Costanza (1992) rubric; high (H), medium (M), low (L). % disturbance (Dist.) shows the restriction range above and below the original network values used in the whole network uncertainty analysis. Mean and SD are shown for the distributions of parameter values observed in the plausible networks at the Oligo and Poly sites. Parameter values have units of nmol N cm^{-3} d^{-1}

Flux	Quality	Oligo			Poly		
		% Dist.	Mean	SD	% Dist.	Mean	SD
Boundary → W-NH_4	H	40	129.8	29.8	41	72.7	16.8
Boundary → W-NO_X	H	32	1020.3	185.2	37	379.1	80.8
Boundary → W-ON	H	47	1158.1	210.9	28	1254.9	114.8
Boundary → S-NO_X	H	16	172.9	15.8	43	345.5	55.0
W-NH_4 → boundary	H	38	276.2	60.5	17	131.7	12.4
W-NO_X → boundary	H	37	1010.3	187.0	38	379.0	80.8
W-ON → boundary	H	28	1158.5	186.4	11	1246.9	77.2
S-NH_4 anammox	H	47	2.5	0.6	36	1.8	0.4
S-NO_X anammox	H	47	2.5	0.6	36	1.8	0.4
S-NO_X denitrification	H	9	171.2	9.5	42	136.6	31.8
S-NO_X → S-NH_4	H	23	38.9	5.1	19	104.4	11.4
Boundary → W-M	M	47	3.9×10^{-5}	1.1×10^{-5}	47	4.0×10^{-5}	1.1×10^{-5}
Boundary → S-ON	M	47	78.9	21.3	47	39.1	9.5
W-M → boundary	M	47	3.0×10^{-5}	1.1×10^{-5}	47	4.0×10^{-5}	1.1×10^{-5}
S-NH_4 → boundary	M	47	1081.1	266.1	47	1013.9	251.8
S-NO_X → boundary	M	47	6.0	1.6	47	127.1	34.6
S-ON → boundary	M	47	104.2	28.1	47	33.0	8.0
W-NH_4 → W-NO_X	M	47	1.7	0.5	47	0.7	0.2
W-NH_4 → W-M	M	47	1.9	0.5	47	1.8	0.5
W-NH_4 → S-NH_4	M	47	5.5	1.5	47	1.5	0.4
W-NO_X → W-M	M	47	9.8	2.7	47	0.5	0.1
W-NO_X → S-NO_X	M	47	14.1	3.7	47	7.7	2.1
W-M → S-M	M	47	118.9	29.4	47	119.5	31.3
W-ON → W-NH_4	M	47	5.2	1.4	47	5.4	1.5
W-ON → W-M	M	47	7.4	2.0	47	1.4	0.4
S-NH_4 → S-NO_X	M	47	143.4	32.6	47	77.1	21.1
S-NH_4 → S-M	M	47	213.2	57.4	47	186.9	47.0
S-NO_X → S-M	M	47	109.0	28.9	47	53.1	14.4
S-NO_X → W-NO_X	M	47	2.2	0.6	47	7.3	1.9
S-M → W-M	M	47	118.9	29.3	47	119.5	31.2
S-ON → S-NH_4	M	47	149.9	40.9	47	99.9	23.8
S-ON → S-M	M	47	82.3	20.0	47	159.2	39.9
S-ON → W-ON	M	47	851.4	230.1	47	422.6	114.8
Boundary → S-NH_4	L	100	1241.5	283.0	100	982.3	260.2
S-NO_X burial	L	100	0.3	0.2	100	7.8×10^{-3}	4.5×10^{-3}
S-M burial	L	100	3.9×10^{-7}	2.2×10^{-7}	100	4.0×10^{-7}	2.6×10^{-7}
S-ON burial	L	100	3.9	2.2	100	2.0	1.2
W-M → W-NH_4	L	100	3.1	1.8	100	3.1	1.8
W-M → W-ON	L	100	15.9	8.2	100	0.5	0.3
W-ON → S-ON	L	100	854.9	254.3	100	424.4	128.3
S-NH_4 → W-NH_4	L	100	137.3	69.2	100	54.5	20.7
S-M → S-NH_4	L	100	146.6	83.5	100	146.0	76.1
S-M → S-ON	L	100	257.9	99.2	100	253.2	89.1

The accuracy and validity of model results are directly related to the quality of the information used to develop the model. As an initial tool to assess quality in a network model, Hines et al. (2012) classified the information used to parameterize the fluxes in the Oligo model using an information-ranking rubric developed by Costanza (1992). According to this rubric, high quality information comes from direct measurement, medium quality data comes from calculations based on direct measurement, and low quality data comes from plausible estimation. We applied the Costanza (1992) rubric in a similar manner to qualify the quality of information used in the construction of the Poly model (Table 3). These quality classifications were used to inform the parameter restrictions applied to the uncertainty analysis, which used a stratified sampling technique.

For the uncertainty analysis, high quality parameters under the Costanza (1992) rubric were restricted to within 1 SD of the mean measured value. Medium quality parameters were restricted to within a percentage of the value in the original networks (Table 2) equal to the largest percent variation observed in the high quality data (±47%), while low quality parameters were allowed to vary by ±100%. The classification of each network flux along with the percentage of disturbance used for plausible model construction can be found in Table 3.

For each model realization in the uncertainty analysis, we performed the ENA and coupling analysis. This let us determine the 95% confidence intervals for the couplings and removal capacities at the Oligo and Poly sites and generally estimate the robustness of the model results to the underlying model uncertainty.

RESULTS

Model evaluation

The Oligo model was constructed from 26% high, 51% medium, and 23% low quality information according to the Costanza (1992) evaluation rubric (Hines et al. 2012). This distribution implies that 77% of the information used in the model construction is based on empirical measurements. The Poly model displayed the same 26%, 51%, and 23% high, medium, and low quality distribution among the ranking categories as the Oligo model. Further, the quality of parameters used for each network flux was identical between the 2 models, facilitating their comparison (Table 3).

Flow analysis

The TST, FCI, and APL statistics did not show strong evidence that the movement of N through the study sites differed in magnitude or organization from a whole-network perspective (Table 1). Although TST showed that more N moved through the Oligo network (7088.7 nmol N cm^{-3} d^{-1}) than the Poly site (5326.8 nmol N cm^{-3} d^{-1}), the uncertainty analysis revealed a 7% overlap in the 95% confidence intervals of TST at these sites. In addition, FCI and APL were greater at the Oligo site, but their values showed 100% and 67% overlaps in their 95% confidence intervals between the Oligo and Poly sites, respectively (Table 1). Despite the similarity between the Oligo and Poly models at the whole-network level, other forms of ENA showed potentially important differences between the 2 sites.

Environ analysis

Environ analysis generated 12 realized input environs for each network, one for every network boundary output. Each environ revealed the amount of N traveling across input and internal pathways that was associated with a specific output boundary flux for a given network. In the denitrification environs, 77.1 nmol N cm^{-3} d^{-1} was involved in sediment nitrification ($S\text{-}NH_4 \rightarrow S\text{-}NO_X$) at the Oligo site, while only 25.1 nmol N cm^{-3} d^{-1} was involved in sediment nitrification at the Poly site (Fig. 4). In the anammox environs, 1.2 nmol N cm^{-3} d^{-1} and 0.1 nmol N cm^{-3} d^{-1} were involved in sediment nitrification and DNRA ($S\text{-}NO_X \rightarrow S\text{-}NH_4$), respectively, at the Oligo site; 0.4 nmol N cm^{-3} d^{-1} and 0.2 nmol N cm^{-3} d^{-1} were involved in the same processes, respectively, at the Poly site (Fig. 5).

Calculation of coupling

Environ results were used to calculate the coupling of microbial N processes at the Oligo and Poly sites. At the Oligo site, an estimated 43.5% (74.8 nmol N cm^{-3} d^{-1}) of denitrification was coupled to nitrification, while the remaining 56.5% (97.2 nmol N cm^{-3} d^{-1}) was a result of direct denitrification. At the Poly site, coupled nitrification to denitrification was responsible for just 18.0% (24.6 nmol N cm^{-3} d^{-1}) of denitrification activity, while 82% (112.1 nmol N cm^{-3} d^{-1}) was attributed to direct denitrification (Fig. 6).

Direct anammox was greater than coupled anammox at both study sites; however, differences in the strength of coupling between the Oligo and Poly sites were observed. Nitrification coupled to anammox and DNRA coupled to anammox at the Oligo site were responsible for 22.7% (1.1 nmol N cm^{-3} d^{-1}) and 1.8% (0.1 nmol N cm^{-3} d^{-1}) of anammox activity, respectively. The remaining 75.4% (3.8 nmol N cm^{-3} d^{-1}) was a result of direct anammox. At the Poly site, however, the strength of nitrification coupled to anammox weakened to 9.6% (0.3 nmol N cm^{-3} d^{-1}), while the strength of DNRA coupled to anammox increased to 4.8% (0.2 nmol N cm^{-3} d^{-1}). The remaining 85.6% (3.1 nmol N cm^{-3} d^{-1}) of anammox activity was a result of direct anammox (Fig. 6).

The process coupling values for the Oligo network presented in this study were not identical to values

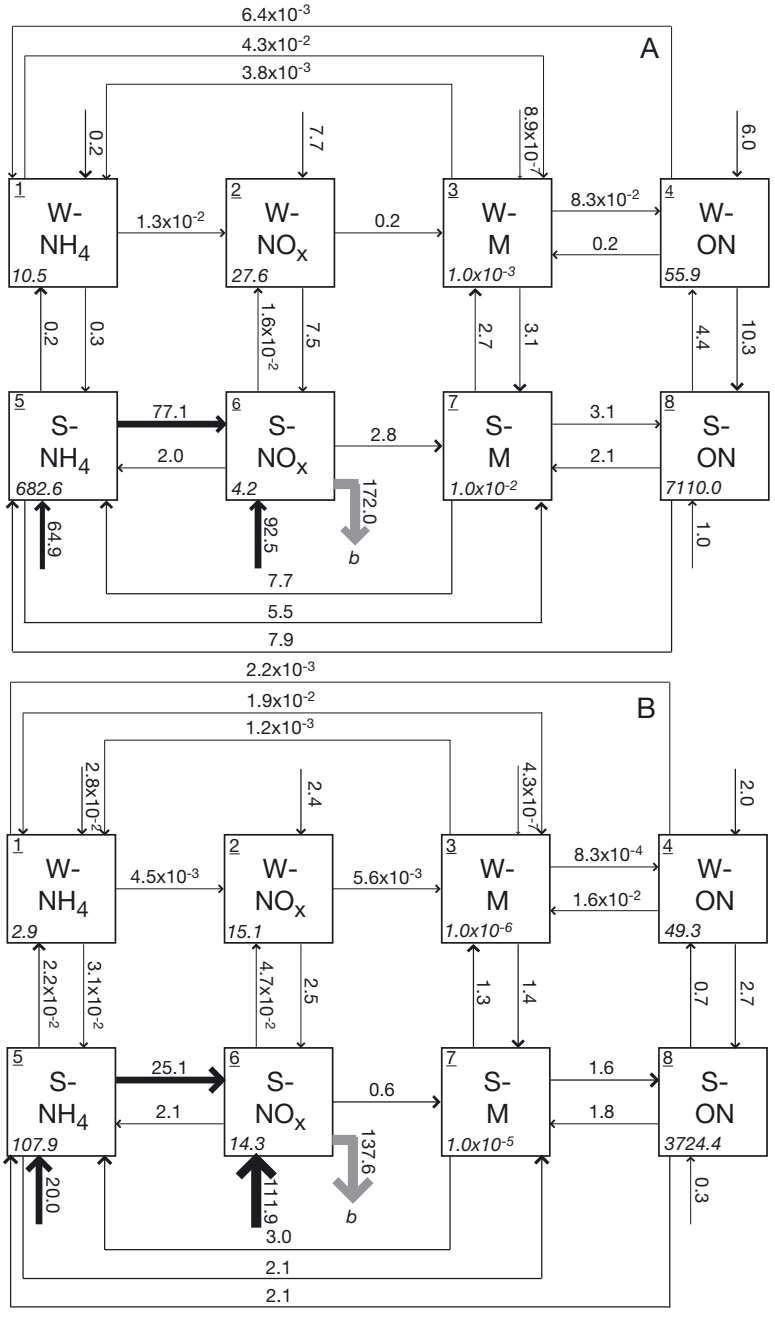

Fig. 4. Realized input environ subnetworks for the denitrification pathway at (A) oligohaline and (B) polyhaline sites. Network outputs highlighted in gray. Arrow widths approximate magnitudes of fluxes in each realized environ. Italicized numbers in the bottom left of node boxes represent standing stock concentrations, while underlined numbers in the top left of node boxes show the node label number. *b*: loss through denitrification. See Table 2 for abbreviations

published for the same network in previous work (Hines et al. 2012) as a result of differences in the software used to conduct the analyses. However, all of the differences were less than 2% of the flux magnitudes and do not affect the conclusions of this study.

Nitrogen removal efficiency

Little change was seen in the ability of the microbial communities to remove N relative to the N inputs at each site. The amount of N removed by denitrification and anammox was higher at the Oligo site (177.0 nmol N cm^{-3} d^{-1}) than the Poly site (140.2 nmol N cm^{-3} d^{-1}). However, relative to the total N inputs into the Oligo and Poly networks, total N_2 production from these 2 processes was 4.7% and 4.6%, respectively (Fig. 7).

Coupling uncertainty analysis

Table 3 shows the mean and SD of each network flux in the 10 000 plausible networks generated for our uncertainty analysis. The 95% confidence intervals of nitrification coupled to denitrification and DNRA coupled to anammox did not overlap between the Oligo and Poly sites (Fig. 8). The 95% confidence intervals of nitrification coupled to anammox, however, overlapped by 16% between the 2 sites. There was little difference between the 95% confidence intervals of N removal capacity at the 2 sites, which ranged from 3.8 to 5.8% and 2.8 to 6.7% of N input at the Oligo and Poly sites, respectively.

DISCUSSION

Network model comparison

The network models for the Oligo and Poly sites had comparable characteristics. The uncertainty analysis showed that the NH_4^+, NO_x and total N inputs to each network, as well as the TST, FCI, and APL network statistics generated by flow analysis, were not significantly different between sites (Table 1). The similarity in the various boundary inputs of N to these models suggests that the differences observed in their internal flows may be a result of differences in the ability of the microbial community to conduct various N transformations under the environmental conditions at each site. The lack of difference be-

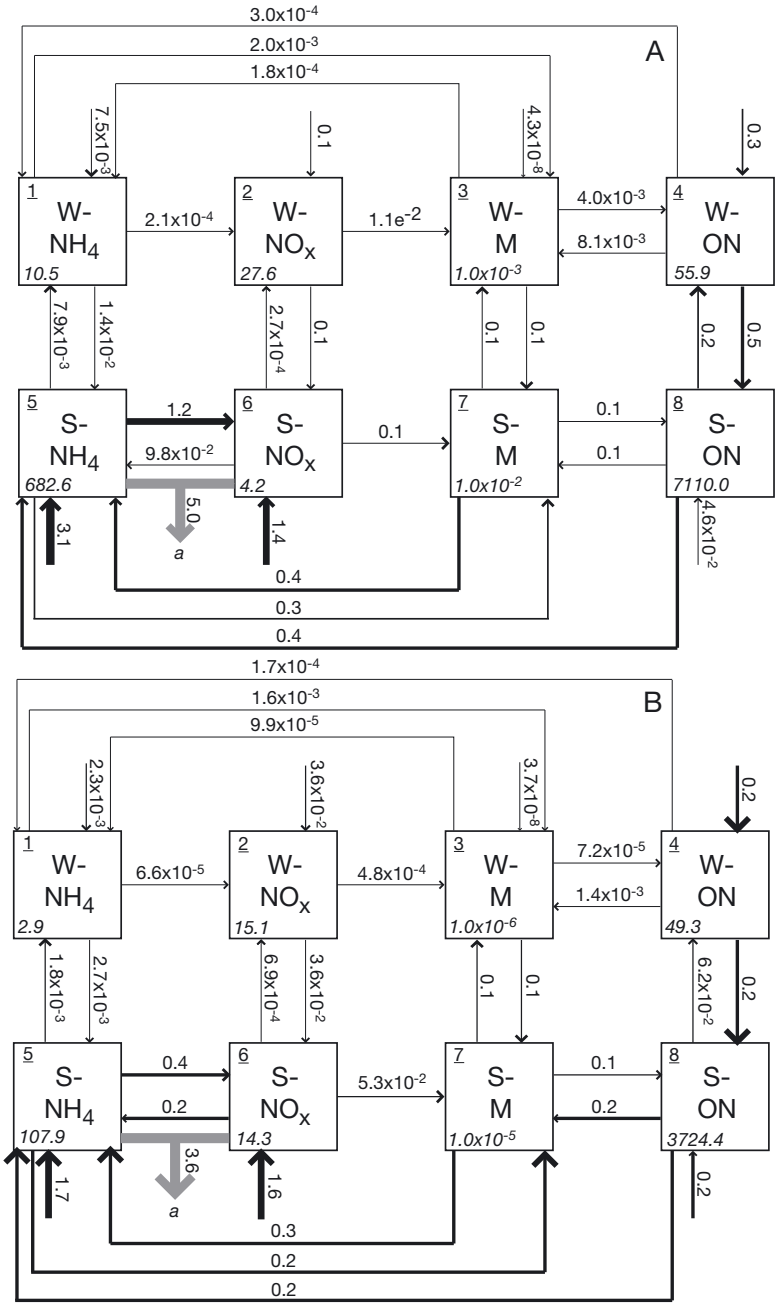

Fig. 5. Realized input environ subnetworks for anammox N_2 production at (A) oligohaline and (B) polyhaline sites. Network outputs highlighted in gray. Arrow widths approximate magnitudes of fluxes in each realized environ. Italicized numbers in the bottom left of node boxes represent standing stock concentrations, while underlined numbers in the top left of node boxes show the node label number. *a*: loss through anammox. See Table 2 for abbreviations

tween TST and FCI metrics at each site suggests that overall activity and movement of N through these networks was similar, and the fact that APL was greater than one for each network helps to demonstrate that coupling is present at both sites (Hines et al. 2012).

The similarities between the network models used in this study facilitate their comparison, as the identical topologies and similar flow regimes meet the criteria for network comparison (Baird et al. 1991, Fath et al. 2007). Furthermore, little difference was reported in depth, tidal height, sediment grain size, water column dissolved oxygen, or percent organic matter (Table 1). The only major difference observed between sites was the degree of influence from seawater, facilitating a space-for-time comparison to estimate the effects of seawater intrusion.

Microbial N process coupling

The model comparison supported the hypothesis that the coupling of nitrification to denitrification would be higher at the Oligo site than the Poly site. Nitrification coupled to denitrification was responsible for 43.5 and 18.0% of N_2 production through denitrification at the Oligo and Poly sites, respectively, and the 95% confidence intervals of these estimations generated by the uncertainty analysis did not overlap (Fig. 8). These predicted values fell within the range of reported values for measurements of nitrification coupled to denitrification in estuarine sediments (Nishio et al. 1983, Jenkins & Kemp 1984, Rysgaard et al. 1993, Koop-Jakobsen & Giblin 2010), suggesting that these models are producing plausible results. The fact that differences in the movement of N through these networks were observed despite similarities in the summary statistics generated by flow analysis (Table 1) highlights that network summary statistics, while useful, can be too broad to capture potentially important network features (Hines & Borrett 2014). Furthermore, the fact that the topology of both networks was identical implies that the difference in coupling of nitrification to denitrification was a result of differences in the amount of material moving across internal and boundary N fluxes at each site.

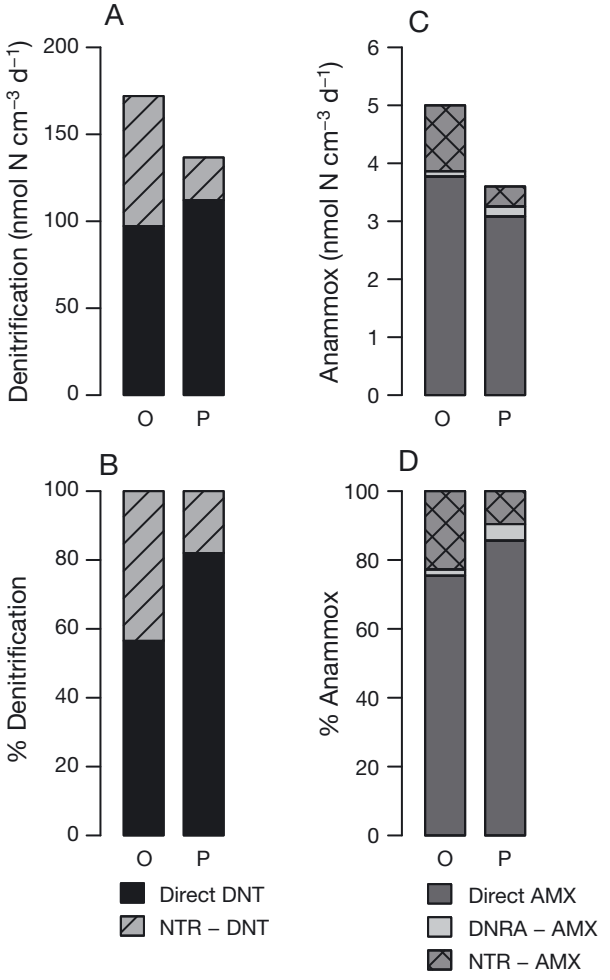

Fig. 6. Estimated coupling of N transformation and removal processes at oligohaline (O) and polyhaline (P) sites for (A,B) denitrification and (C,D) anaerobic ammonium oxidation (anammox) in (A,C) absolute units and (B,D) as a percentage of each process. Direct DNT: direct denitrification, NTR–DNT: nitrification coupled to denitrification, direct AMX: direct anammox, DNRA–AMX: dissimilatory nitrate reduction to ammonium (DNRA) coupled to AMX, NTR–AMX: NTR coupled to AMX

The majority of the reduction in denitrification between the Oligo and Poly sites was a result of decreased coupling to nitrification (Fig 6). This finding is consistent with literature observations, which suggest that denitrification rates in estuaries can be greatly reduced when nitrification is inhibited (An & Joye 2001, Kemp et al. 2005). These results suggest that if seawater intrusion causes the N cycling processes at the Oligo site to more closely resemble those at the Poly site, a decoupling of nitrification to denitrification may be observed at the Oligo site.

The model results also suggested that direct anammox was responsible for the majority of anammox N_2 production at both sites. This finding suggests that

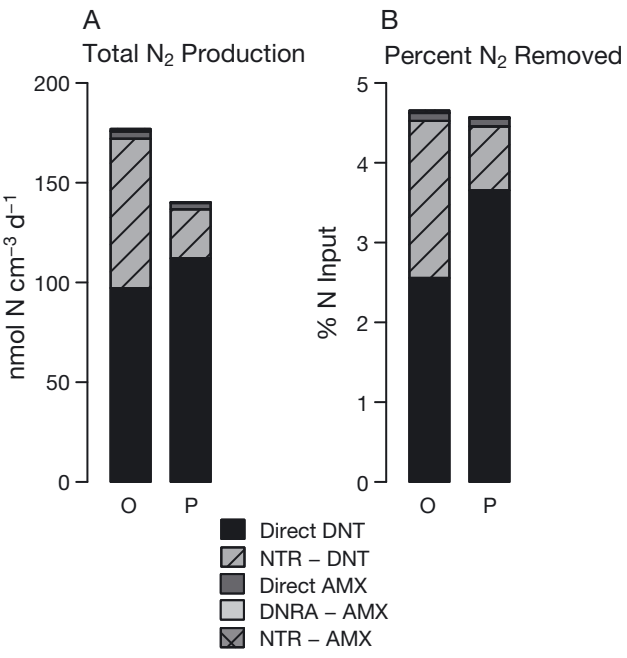

Fig. 7. Total amount of direct and coupled microbial processes involved in (A) N_2 production and (B) the proportion of N involved in removal processes relative to the N input at each site. See Fig. 6 for abbreviations

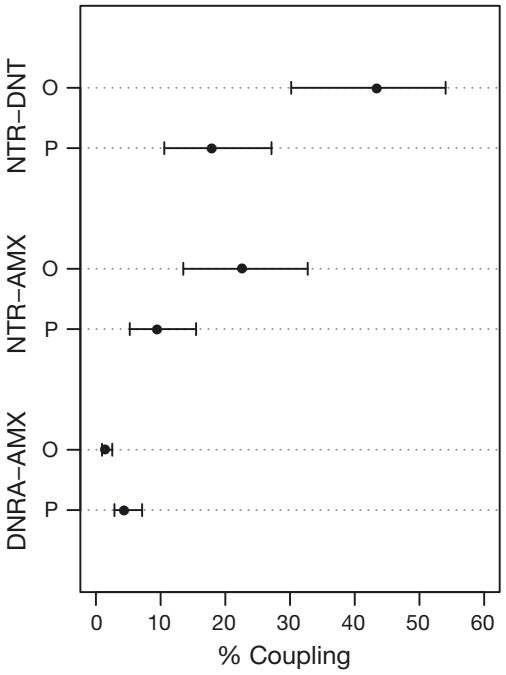

Fig. 8. Uncertainty analysis showing the 95% confidence intervals of estimations for nitrification coupled to denitrification, nitrification coupled to anammox, and DNRA coupled to anammox based on 10000 plausible network parameterizations. Large dots indicate the values calculated by the original network parameterizations, while horizontal error bars indicate the 95% range of each coupling calculation distribution. See Fig. 6 for abbreviations

anammox organisms capable of reducing NO_3^- may play an important role in the CFRE (Kartal et al. 2007, 2012). Nitrification coupled to anammox was greater at the Oligo site. However, the uncertainty analysis revealed that the 95% confidence intervals of plausible estimations for this coupling overlapped by 16% and therefore did not support the hypothesis that nitrification coupled to anammox would be greater at the Oligo site. While these models estimate that nitrification coupled to anammox makes up less than 25% of anammox N_2 production at each site, other research has suggested that this coupling may be the principal form of N_2 production in some environments (Lam et al. 2007), and more empirical work is required to investigate its role in estuaries.

Despite lower total anammox rates at the Poly site, possibly driven by lower nitrification coupled to anammox, the percentage of N_2 production resulting from DNRA coupled to anammox was nearly 3 times higher at the Poly site; a finding that was robust to the uncertainty analysis (Fig. 8). Therefore, the model results suggest that DNRA may play an increasingly important role in estuarine N removal as a result of seawater intrusion, although this coupling was responsible for less than 5% of anammox N_2 production at each site. Previous research corroborates the observation that the coupling of DNRA to anammox was weaker at the Oligo site than the Poly site by suggesting that DNRA plays a relatively minor role in freshwater sediments (Scott et al. 2008).

The fact that DNRA coupled to anammox was responsible for only a minor part of anammox N_2 production at both sites suggests that this coupling may not increase dramatically as a result of seawater intrusion in the CFRE. However, the models presented in this study aggregated NO_3^- and NO_2^- pools into NO_x, and likely underestimated the contribution of DNRA coupled to anammox by only reporting anammox coupled to complete reduction to NH_4^+ (Kartal et al. 2007). Anammox coupled to incomplete reduction of NO_3^- to NO_2^- was excluded from coupling estimations in this study because NO_3^- and NO_2^- were aggregated into the NO_x nodes. Bacterial processes including both incomplete DNRA and incomplete denitrification produce NO_2^-, and the higher sulfide concentrations associated with seawater intrusion may enhance these processes (An & Gardner 2002, Laverman et al. 2007). In some ecosystems, DNRA can provide the main NO_2^- input for anammox (Jensen et al. 2011), and the ephemeral nature of NO_2^- in estuaries suggests that strong coupling may exist between anammox and NO_3^- reduction pathways. Therefore, the effects of seawater intrusion on DNRA coupled to anammox may be greater than suggested by these models.

The total increase of N_2 produced by DNRA coupled to anammox at the Poly site compared to the Oligo site (0.1 nmol N cm^{-3} d^{-1}) was 2 orders of magnitude smaller than the decrease in N_2 produced by nitrification coupled to denitrification and anammox at the same 2 locations (51.0 nmol N cm^{-3} d^{-1}). Despite the potential for underestimating the proportion of DNRA coupled to anammox in these models, it is clear that the reduction in nitrification coupled to denitrification is greater than all of anammox N_2 production, which is often a small fraction of total N_2 production in estuaries (Koop-Jakobsen & Giblin 2009). This result implies that, even if DNRA were completely coupled to anammox, this process would not be able to compensate for a reduction in N_2 production by nitrification coupled to denitrification and anammox caused by saltwater intrusion.

Seawater intrusion and the microbial N cycle

As seawater intrusion from dredging and sea level rise continues to progress, the environmental conditions for the microbial communities at the Oligo site may shift to more closely resemble the conditions at the Poly site. While the model analysis predicts a decoupling of nitrification to denitrification and enhancement of DNRA coupled to anammox, the relative amount of N removed at both the Oligo and Poly sites was similar (4.7 and 4.6% of total N input, respectively; Fig 7). These findings were robust to the uncertainty analysis and are consistent with reported percentages of denitrification removal in estuaries with similar flushing times, which ranged from 2 to 8% (Nielsen et al. 1995, Nowicki et al. 1997). Estuaries with longer flushing times will likely have higher percentages of N inputs removed through microbial processes (Joye & Anderson 2008). For example, the nearby New River Estuary, North Carolina, which has a flushing time approximately 10 times longer than the CFRE (Ensign et al. 2004), likely converts a higher percentage of its N inputs to N_2 gas.

Although nitrification coupled to denitrification and anammox decreased substantially from the Oligo to the Poly site, direct denitrification increased from 56.5 to 82.0% of denitrification removal. Relative to the N inputs at each site, direct denitrification was able to compensate for reductions in nitrification coupled to denitrification and anammox in these models. The similarity in the percentage of N input converted to N_2 gas in each network suggests that

seawater intrusion may alter which biogeochemical pathways contribute to N removal, but have little effect on the total amount of N_2 produced. These findings corroborate the results of Fear et al. (2005), who observed little change in relative N_2 production despite highly variable denitrification rates across a range of salinity conditions in the Neuse River Estuary, North Carolina. Furthermore, these findings imply that little change in N_2 production may be observed as a result of seawater intrusion despite the sulfide inhibition of nitrification (Joye & Hollibaugh 1995, Rysgaard et al. 1999), the release of ammonium from sediments (Gardner et al. 1991, Seitzinger et al. 1991), and the enhancement of DNRA (Brunet & Garcia-Gil 1996, Giblin et al. 2010) associated with higher salinities, and suggest that microbial communities will be able to adapt to these changes in environmental conditions.

Alternatively, the similarity in relative N_2 production between the Oligo and Poly sites may be an indication that factors other than salinity, such as carbon availability and substrate concentration, play an important role in regulating the biological removal of N from these systems. For example, the ratio of carbon to NO_3^- can influence which NO_3^- reduction process, DNRA or denitrification, is dominant in the sediments (Tiedje et al. 1982, King & Nedwell 1985). In addition, a recent model by Algar & Vallino (2014) provides a theoretical basis for the importance of carbon to NO_3^- ratios for N_2 production pathways. Others have shown that salinity may not directly inhibit some N transformations, but the nutrient conditions that can be affected by seawater intrusion can regulate these processes (Magalhães et al. 2005, Weston et al. 2006). The extent to which salinity versus nutrient conditions and carbon availability influence the sedimentary N cycle in estuaries is an area of active research and this study cannot comment directly on this relationship. Further investigations into this area will provide insight as to how these factors interact to influence N_2 production in estuaries.

If the N cycle at the Oligo site more resembles that of the Poly site as seawater intrusion progresses, these shifts in which biogeochemical pathways contribute to N_2 production may have important implications for the health of estuaries. NH_4^+ is converted to N_2 gas primarily through nitrification coupled to denitrification. The decoupling of these biogeochemical processes and increased importance of direct denitrification resulting from seawater intrusion, in combination with the decreased adsorption of NH_4^+ to sediments (Hou et al. 2003, Giblin et al. 2010, Weston et al. 2010), may decrease NO_3^- pools available to algae while increasing available NH_4^+ pools. Under these conditions, the availability of NO_3^- could limit denitrification and anammox N_2 production. Because phytoplankton preferentially take up NH_4^+ over other forms of inorganic N (McCarthy et al. 1977, Carpenter & Dunham 1985), increased NH_4^+ pools may lead to larger phytoplankton populations, exacerbating eutrophication.

Limitations

There are several limitations to the techniques used in this study to estimate the effects of seawater intrusion on the N cycle. First, the comparison made in this work assumed that the Oligo and Poly sites will behave similarly under equivalent conditions, and cannot address the dynamic processes and transient effects of seawater intrusion (Pickett 1989). The transition from oligohaline to polyhaline conditions may not occur as a smooth interpolation between the Oligo and Poly sites, and may instead pass through alternative transient states that this modeling technique cannot predict. Further, the rate of change in water chemistry may influence how microbial N cycling communities respond to seawater intrusion. Second, this substitution assumed that the Oligo system will not reach an alternative stable state, different from either the Oligo or Poly networks, as a result of the transient dynamics mentioned above or hysteresis. Third, this study presents a comparison of 2 sites during the summer in a single estuary. While the CFRE is considered to represent a typical coastal plain estuary in the south-eastern USA (Dame et al. 2000), the conclusions of this work may not generalize well to estuaries with different environmental conditions, or to other seasons. However, despite the drawbacks inherent in this work, it is a useful first approximation to understanding the potential effect of seawater intrusion on the sedimentary N cycle and provides a basis for future research.

CONCLUSIONS

The model comparison presented here makes 4 contributions to the scientific understanding of the effects of seawater intrusion on sedimentary microbial N cycling processes. (1) This work synthesizes disparate measures of nutrient concentrations and microbial transformation processes to generate an N budget for the Poly site that can be used in further

analyses. (2) This study provides evidence that the lower observed rates of N_2 production in more saline estuarine sediments are likely a result of decreased coupling between nitrification and the removal processes. Modeled DNRA coupled to anammox was strongest at the high salinity site, but accounted for less the 5% of anammox N_2 production, and therefore did not compensate for reductions in nitrification coupled to denitrification and anammox. (3) The models suggest that seawater intrusion may lead to a higher contribution of direct denitrification as a result of nitrification inhibition, limiting the abilities of estuaries to produce N_2 gas from NH_4^+. The similarities in modeled N_2 production relative to N inputs suggests that the total amount of N_2 produced may change little as a result of seawater intrusion in estuaries with an abundance of inorganic N. However, in estuaries where NO_3^- is not abundant, N_2 production could become NO_3^- limited as a result of this shift toward a greater reliance on direct denitrification. (4) Our findings imply that seawater intrusion into the freshwater portions of estuaries may exacerbate the effects of nutrient loading and eutrophication through the decreased couplings of nitrification and N removal pathways. A lessened capacity of estuaries to remove N in the form of NH_4^+ could result in longer N residence times and, therefore, in greater resource availability for phytoplankton communities.

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