

Using nitrate dual isotopic composition (δ^{15} N and δ^{18} O) as a tool for exploring sources and cycling of nitrate in an estuarine system: Elkhorn Slough, California

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[1] Nitrate (NO₃⁻) concentrations and dual isotopic composition (δ^{15} N and δ^{18} O) were measured during various seasons and tidal conditions in Elkhorn Slough to evaluate mixing of sources of NO_3^- within this California estuary. We found the isotopic composition of NO_3^- was influenced most heavily by mixing of two primary sources with unique isotopic signatures, a marine (Monterey Bay) and terrestrial agricultural runoff source (Old Salinas River). However, our attempt to use a simple two end-member mixing model to calculate the relative contribution of these two NO_3^- sources to the Slough was complicated by periods of nonconservative behavior and/or the presence of additional sources, particularly during the dry season when NO_3^- concentrations were low. Although multiple linear regression generally yielded good fits to the observed data, deviations from conservative mixing were still evident. After consideration of potential alternative sources, we concluded that deviations from two end-member mixing were most likely derived from interactions with marsh sediments in regions of the Slough where high rates of NO₃ uptake and nitrification result in NO₃ with low δ^{15} N and high δ^{18} O values. A simple steady state dual isotope model is used to illustrate the impact of cycling processes in an estuarine setting which may play a primary role in controlling NO_3^- isotopic composition when and where cycling rates and water residence times are high. This work expands our understanding of nitrogen and oxygen isotopes as biogeochemical tools for investigating NO₃⁻ sources and cycling in estuaries, emphasizing the role that cycling processes may play in altering isotopic composition.

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1. Introduction

1.1. Background

[2] Nitrogen is often the limiting nutrient for primary production in estuarine ecosystems [*Cloern*, 2001; *Nixon*, 1995]. Because humans have more than doubled the rate at which bioavailable N enters terrestrial and aquatic ecosystems [*Galloway et al.*, 2004; *Gruber and Galloway*, 2008; *Seitzinger et al.*, 2005; *Vitousek et al.*, 1997], the problem of eutrophication has become an increasingly common consequence in estuaries worldwide [*Dumont et al.*, 2005; *Nixon*, 1995; *Paerl et al.*, 1998]. More specifically, increased urban development in coastal areas and intensification of agriculture are both considered substantial contributors of nutrients to estuaries.

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[3] Statewide trends of estuarine habitat impacts and alterations are not well characterized in California; however, available data indicate widespread loss of estuarine habitats. Estimates for southern California indicate decreases in saltmarsh and mudflat habitats of 87% and 84%, respectively [Zedler, 1996]. In San Francisco Bay, the largest estuary on the west coast, over 95% of the historical saltmarsh has been lost [Nichols et al., 1986]. Elkhorn Slough, the site of this study, represents one of the approximately 50 west coast estuaries impacted by marsh habitat loss [Van Dyke and Wasson, 2005] and subject to increasingly high loads of N [Los Huertos et al., 2001]. The chronic exposure to high nitrate (NO_3^-) and eutrophic conditions contributes directly to marsh loss through the alteration of indigenous plant communities and indirectly through overgrowth of eutrophic algal species such as Ulva enteromorpha [Fong et al., 1998] which can smother saltmarsh vegetation, leaving the sediments unvegetated and more easily eroded.

[4] Here we use the isotopic composition of NO_3^- ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) to identify specific sources of N and to evaluate their distribution in a dynamic estuarine regime. The use of oxygen isotopic composition of nitrate ($\delta^{18}O_{NO3}$) together with $\delta^{15}N_{NO3}$ has proven useful as a means for constraining multiple sources and evaluating

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cycling that has occurred. Specifically, this approach has been used in a wide variety of systems, including agriculturally impacted areas [Böhlke and Denver, 1995; Böttcher et al., 1990; Cey et al., 1999], forested catchments [Burns and Kendall, 2002; Kendall et al., 1995; Ohte et al., 2004], groundwater [Aravena et al., 1993; Böhlke and Denver, 1995; Cole et al., 2006; McMahon and Böhlke, 2006], and freshwater aquatic ecosystems such as lakes and rivers [McClelland and Valiela, 1998; Ostrom et al., 2002; Rock and Mayer, 2004]. However, the nitrate dual isotopic composition has only recently been applied to marine and estuarine systems [Casciotti et al., 2002; Lehmann et al., 2004; Sigman et al., 2005; Wankel et al., 2006, 2007]. Existing data for NO₃⁻ dual isotopic composition in estuaries are sparse [Wankel et al., 2006]. Work presented here adds to the available knowledge base on the application of dual isotopes of nitrate in marine ecosystems.

1.2. Site Description

[5] Elkhorn Slough (Figure 1) is a small tidally flushed estuary, with a tidal prism amounting to approximately 70% of the mean estuary volume [Broenkow and Breaker, 2005]. During the wet season, estimates of mean water residence time are on the order of ~ 1 day [Caffrey, 2001]. However, for the upper reaches of the Slough and in outlying tidal creeks and marshes, residence times can be on the order of several weeks, particularly during the dry season [Caffrey, 2001]. The estuary geometry is complex (Figure 1), with the mouth opening to Monterey Bay (MB) and a relatively wide main channel that reaches approximately 11 km inland where it terminates at a tide gate at the upper Slough. Two smaller channels (Moss Landing Harbor and Bennett Slough) enter the Slough about 0.3 km from the mouth. The Moss Landing Harbor, located to the south of the Slough, receives considerable terrestrial input from the old Salinas River (OSR) channel. Bennett Slough, located near the estuary mouth, receives almost no freshwater input and is not explicitly considered in this study. The geometry of Elkhorn Slough plays an integral role in the physical forcing of mixing regimes in the Slough, contributing to the unique biogeochemical characteristics of this estuary. Numerous tidal creeks, mudflats, and saltmarsh habitats surround the main channel of the Slough. Recent estimates indicate there are approximately 3.93 km² of saltmarsh habitat (including both tidally restricted and unrestricted areas) and approximately 3.54 km² of mudflat habitat (including both restricted and unrestricted areas) within the Slough watershed (http:// www.elkhornslough.org).

[6] The primary source of freshwater to the Slough is the old Salinas River channel. This channel drains flow from the Salinas River, as well as flow from the Espinosa, Castroville and Tembladero Sloughs, and enters Elkhorn Slough through the Moss Landing Harbor near the mouth of the Slough (Figure 1). An additional freshwater source is Carneros Creek at the head of the Slough; however, due to the Mediterranean climate, discharge from the Carneros Creek typically occurs only between December and April, with average flow rates ranging from 0.08 to 0.16 m³ s⁻¹ [*Pajaro Valley Water Management Agency (PVWMA)*, 2007]. The OSR has a more substantial discharge throughout the year as a result of its larger watershed and irrigation-derived water inputs. Low flows during the driest months of

summer (June–August) are on the order of ~0.10 m³ s⁻¹ (N. Nidzieko, personal communication) containing a large component of local irrigation water [*Wankel*, 2007]. Substantially higher flows during the wetter winter months (December through April) of 15 to 30 m³ s⁻¹ are not uncommon, particularly during storms (N. Nidzieko, personal communication). Relatively little is known about groundwater inputs; however, the regional water table has experienced substantial overdraft [*Vengosh et al.*, 2002] and recent work suggests that advective inputs of groundwater are minor (J. A. Breier et al., Tidally driven chemical fluxes at the sediment-water interface in Elkhorn Slough: Evidence from a coupled geochemical and hydrodynamic approach, submitted to *Limnology and Oceanography*, 2009).

[7] In small, wide, and short estuaries such as Elkhorn Slough, which are substantially flushed with each tidal cycle, mixing is rapid and dynamic, often resulting in spatially and temporally complex mixtures of water sources. Geochemical approaches, such as the work reported here, can help resolve the complexity of end-member mixing and controls on nutrient supply and exchange in such a dynamic regime. In this study, we use NO_3^- concentration and isotopic composition as the primary biogeochemical tool to characterize the mixing of known and unknown sources of NO_3^- in the waters of the main channel of Elkhorn Slough.

2. Methods

[8] Two sampling approaches were employed to investigate the distribution of NO_3^- in Elkhorn Slough. The first approach involved samples collected along the longitudinal axis of the Slough, from the head to the mouth, over both ebb and flood tides. By periodically sampling at different locations on both ebb and flood tides, the goal was to both identify and evaluate the spatial contributions of the multiple water sources of NO_3^- (using their NO_3^- isotopic compositions) within the main channel. For the second approach, samples were collected on a transect across the main channel while conducting continuous depth profiles, with the goal of better characterizing the temporal contributions of different sources over a tidal cycle (e.g., changes at one site over time).

2.1. Longitudinal Transects

[9] Samples from the main channel were collected on 6 different dates along a transect of 6 sites (C0-C5) spanning the downstream \sim 7 km length of the \sim 11 km channel (Figure 1), on both ebbing and flooding tides. Three transects were made during the dry season (September 2002, October 2003, and November 2003), and three were made during the wet season (April 2003, May 2003, and May 2004). Physical and chemical parameters (temperature, salinity, dissolved oxygen) were measured during most sampling trips using a Seabird 9000 CTD mounted on a stainless steel frame that was lowered from the side of the boat. Water samples from the main channel were collected at 1.5 m depth intervals using a Van Dorn sampler. Samples were immediately filtered in the field (0.2 μ m) and kept chilled and in the dark until returned to the lab where they were frozen until analysis.



Figure 1. Map showing study area and selected sites in the Elkhorn Slough estuary. Main channel sites are those where samples were collected on longitudinal sampling events (C0–C5), while the cross-channel transects were conducted near the LOBO L01 mooring [*Johnson et al.*, 2007]. NERR sites are long-term water quality monitoring sites operated by the Elkhorn Slough National Estuarine Research Reserve, where ancillary data were collected.

2.2. Cross-Channel Transects

[10] An extensive, high resolution sampling regime, consisting of sampling across the main channel of the Slough over an entire tidal cycle, was conducted between sites 1 and 2 near the LOBO mooring (Figure 1). The channel was continually traversed approximately 9-10 times per hour. During these sampling campaigns, depth profiles were conducted using a standard CTD and an in situ ultraviolet spectrophotometer NO₃⁻ sensor [*Johnson et al.*, 2002] that enabled continuous monitoring of a cross section of the



Figure 2. Long-term nitrate and salinity data from the LOBO-L-01 mooring [Johnson et al., 2007] which is located in the center of the main channel approximately 200 m inland of Moss Landing Harbor (Figure 1). Data are from mooring instruments positioned ~ 1 m below the surface. (a) All data between November 2003 and November 2007. A clear influence by Monterey Bay (MB) seawater is evident, which mixes with at least one freshwater end-member containing high and variable amounts of NO_3^- . (b) An expansion of the top left part of Figure 2a, showing data from three 10-day periods (November 2003, May 2004, and October 2004) for which isotope data are available. Lines are meant to loosely enclose probable end-members. Note the variable NO₃⁻ concentrations of the MB end-member, which sometimes correspond to variations in salinity (gray arrow) and sometimes do not (black arrow). Because the distribution of NO_3^- is largely governed by mixing between seawater and small amounts of freshwater with very high NO₃, salinity is less than ideal for characterizing end-members.

Slough. This approach was employed once during the dry season (October 2004) and once in the wet season (February 2005). Samples were collected at shallow and deep positions along the cross channel transect (3 shallow samples at 1 m below surface; 2 deep samples at 1 m above the bottom) and analyzed for the same constituents as during the longitudinal sampling above.

2.3. Analytical Methods

2.3.1. Isotope Analyses

[11] Isotopic analyses of NO₃⁻ were conducted using the denitrifier method [*Casciotti et al.*, 2002; *Sigman et al.*, 2001] in conjunction with a custom headspace autosampler (AS 1200), using an IsoPrime mass spectrometer. Analytical precision was typically $\pm 0.3\%$ for δ^{15} N_{NO3} and $\pm 0.8\%$ for δ^{18} O_{NO3}. Nitrate isotopic standards USGS 34 (δ^{15} N = -1.8%; δ^{18} O = -27.9%) and USGS 35 (δ^{15} N = +2.7%; δ^{18} O = +57.5%) were used to correct for drift, oxygen isotopic exchange, and blank. Only samples with NO₃⁻

concentrations >1 μ M were analyzed, representing approximately 95% of all samples collected. Because NO₂⁻ interferes with the measurement of the $\delta^{18}O_{NO3}$ using this technique [*Casciotti et al.*, 2007], we do not report $\delta^{18}O_{NO3}$ values for samples in which NO_2^- comprised more than 5% of the total NO_x pool ($NO_2^- + NO_3^-$), which was <1% of all samples. NO_2^- only exceeded 5% of the NO_x on samples from the upper slough during October 2003, November 2003 and May 2004, occurring when NO_3^- concentrations were very low. In contrast, during the October 2004 cross channel transect, contribution of NO_2^- to the NO_x pool ranged from 2% up to 7% at the ebb slack and follows NO_2^- concentrations (which peak at ebb slack) very closely (not shown). However, neither the δ^{15} N nor δ^{18} O exhibit changes relating to the variation in proportion of NO_2^- contributing to the sample and thus we chose to include these data in the plots shown below.

[12] δ^{15} N values are referenced against air N₂, while δ^{18} O values are referenced against Vienna Standard Mean Ocean Water (VSMOW) and are reported in units of ‰. Water samples were also analyzed for the oxygen isotopic composition of water (δ^{18} O_{water}) using the conventional equilibration method [*Epstein and Mayeda*, 1953]. Ammonium nitrogen isotopes (δ^{15} N) were analyzed on a subset of samples using the diffusion method [*Holmes et al.*, 1998] and combusting the samples on an IsoPrime EA-IRMS. Based on incubations of isotope standards alongside field samples, diffusion recovery was estimated to be >98% with an analytical precision for δ^{15} N of ±0.3‰.

2.3.2. Nutrient Analyses

[13] Filtered (0.2 μ m) samples were analyzed for nitrate (NO₃⁻), nitrite (NO₂⁻), and ammonium (NH₄⁺). NO₃⁻ + NO₂⁻ concentrations were measured using a modification of the colorimetric method of *Armstrong et al.* [1967]. NO₂⁻ was measured separately by omitting the cadmium reduction step, and NO₃⁻ was calculated as the difference between these two measurements. Dissolved NH₄⁺ was analyzed using the indophenol blue method modified from the ALPKEM RFA methodology [*U.S. Environmental Protection Agency (U.S. EPA*), 1983].

3. Results

[14] Samples were collected and analyzed with the goals of (1) identifying and then characterizing the dominant sources of nitrate to the slough and (2) evaluating the spatial and temporal variability in the contributions and mixing of these sources within the slough in this physically dynamic system. In estuaries, where there can be large salinity gradients between freshwater and seawater, plots of salinity versus NO_3^- concentration have been used to describe mixing between two end-members, where conservative mixing of two sources plots as a straight line [Evre and Balls, 1999; Horrigan et al., 1990; Wankel et al., 2006]. Figure 2a shows the salinity and NO_3^- concentrations for the main channel of Elkhorn Slough, using data collected automatically by instruments on the LOBO mooring L-01 [Johnson et al., 2007]. The source of the high salinity, low nitrate water is MB. The other source of water to the Slough is OSR, which has a low salinity but much higher $NO_3^$ concentration than MB. Figure 2b is an expansion of the top left-hand corner of the data cluster in Figure 2a, using only



Figure 3. Wet season transects up the longitudinal axis of the main channel of Elkhorn Slough illustrating variations in nitrate concentrations and δ^{15} N and δ^{18} O compositions as depth contour plots. (a) 7 April 2003 flood, (b) 25 April 2003 ebb, (c) 1 May 2003 flood, (d) 13 May 2004 flood, and (e) 13 May 2004 ebb. Note the nonlinear scale for NO₃⁻ concentration due to isolated high concentration water at the surface near the mouth, where isolated concentrations up to 324 μ M were observed.

data collected during times when discrete water samples were also collected for isotope analysis. Lines are drawn to enclose the data collected from different sampling times, and provide evidence for variable end-member compositions (e.g., Monterey Bay surface and deep water) and/or changing proportions of at least 3 end-members during different sampling times.

[15] The dual isotopic composition of NO₃⁻ (δ^{15} N and δ^{18} O) can offer additional constraint to end-member variability and enable identification of multiple sources and processes [*Kendall*, 1998; *Kendall et al.*, 2007; *Lehmann et al.*, 2004; *Sigman et al.*, 2005; *Wankel et al.*, 2006, 2007]. Longitudinal transects help illustrate the spatial variability in δ^{15} N_{NO3} and δ^{18} O_{NO3} observed during both ebb and flood tides in the main channel. Figure 3 shows NO₃⁻ concentrations, δ^{15} N_{NO3}, and δ^{18} O_{NO3} for samples collected along the transects during different tidal conditions in the

wet season, while Figure 4 shows these parameters for the dry season. Although the results are presented spatially in Figures 3 and 4, the spatial relationships must not be overinterpreted, since the data do not accurately represent one moment in time (e.g., sampling was not Lagrangian and may represent different water masses), particularly in the context of a dynamic system like Elkhorn Slough.

[16] During the wet season, NO_3^- concentrations are generally higher at the mouth and decrease upstream toward the head of the Slough on both ebb and flood tides (Figure 3), indicating that the major source of NO_3^- into the Slough is entering at the mouth, consistent with increased runoff from the OSR [*Johnson et al.*, 2007]. Results of this study show that typical nitrate concentrations are in the range of 5–50 μ M; however, concentrations as high as 300–400 μ M are regularly observed, particularly on flooding tides, observable on the surface at sites C0 and C1



Figure 4. Dry season transects up the longitudinal axis of the main channel of Elkhorn Slough illustrating variations in nitrate concentrations and δ^{15} N and δ^{18} O compositions as depth contour plots. (a) 10 September 2002 ebb, (b) 17 September 2002 flood, (c) 8 October 2003 flood, (d) 14 November 2003 flood, and (e) 14 November 2003 ebb. Note the linear scale for NO₃⁻ concentration (in contrast to Figure 3) and overall lower NO₃⁻ concentrations in the dry season. No δ^{18} O data are available from November 2003 due to analytical error.

(Figure 3). $\delta^{15}N_{NO3}$ values along the main channel generally decrease moving upstream, dropping from values of ~+11 to +15‰ near the mouth to ~+5‰ in the upper Slough, suggesting the possibility of an additional source of NO₃⁻ having low $\delta^{15}N$ values (Figure 3) in the upper Slough. In contrast, while more variable, $\delta^{18}O_{NO3}$ values tended to be lower near the mouth (+6 to +10‰) and higher in the upper Slough (as high as +27‰) again suggesting the potential of a unique source in the upper slough area.

[17] During the end of the dry season (Figure 4), NO₃⁻ concentrations up the main channel of Elkhorn Slough are generally quite low (September 2002: 1.3 to 10.0 μ M; October 2003: 2.4 to 18.9 μ M; November 2003: 2.4 to 9.4 μ M), primarily due to minimal input of nitrate-rich terrestrial freshwater from rivers, groundwater, and/or run-off during the dry, low precipitation season. Notably, while

the water input from the OSR at this time is low, the influence of its high NO_3^- concentration is still periodically evident (data not shown), stemming from high amounts of irrigation during the dry season [*Johnson et al.*, 2007].

[18] Figure 5 shows $\delta^{15}N_{NO3}^{-1}$, $\delta^{18}O_{NO3}^{-1}$, and NO_3^{-1} concentration data during the cross channel transects. Vertical heterogeneity is strong during the wet season sampling (Figures 5c and 5d), with surface layer NO_3^{-1} concentrations up to 250 μ M while deep water concentrations remain low (~20 μ M). In contrast to the variable flood tide chemistry, the more uniform isotopic composition during ebb tide reflects a well mixed water column (Figure 5). The $\delta^{15}N$ and $\delta^{18}O$ of all samples collected during the cross-channel and longitudinal transects in the Slough are shown in Figure 7, along with boxes demarking the isotopic compositions of the two main NO_3^{-1} sources (as the average



Figure 5. Plot of the NO₃⁻ concentration (thin lines) and N and O isotopic composition (heavy lines) over the cross channel transects in (a and b) October 2004 and (c and d) February 2005. Sites on the north (black symbols), middle (gray symbols) and south (white symbols) side of the channel were visited while continuously traversing across the channel over the course of a tidal cycle. Samples were taken from the surface (squares) and deep, 1 m from the bottom (triangles). Note the ebbing tide exhibits a more homogenous composition, while on the flooding tide, there is a dramatic increase in surface NO₃⁻ concentration (small for October 2004, large for February 2005) accompanied by considerable lateral and vertical heterogeneity of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$.

composition of >20 independent samples [*Wankel et al.*, 2007]). While most of the data points fall into the region of mixing between MB and OSR, there are still many points that fall outside this region and cannot be explained by mixing between these two end-members.

4. Discussion

4.1. Known End-Members

[19] Based on previous work, it is known that water from Monterey Bay (MB), with NO₃⁻ concentrations ranging from near 0 μ M (surface water) to ~35 μ M (deep water), comprises the largest source of water to the Slough [*Broenkow and Breaker*, 2005; *Chapin et al.*, 2004; *Johnson et al.*, 2007]. Typical δ^{15} N_{NO3} values in MB range from ~+7 to +8‰ in subphotic zone waters up to +12‰ in the euphotic zone, while δ^{18} O_{NO3} values typically range from ~+2 to +3‰ below the euphotic zone up to +10‰ in the surface waters. As a result of NO₃⁻ consuming processes, increases in δ^{15} N_{NO3} are generally coupled with increases in δ^{18} O_{NO3} resulting in a predictable 1:1 increase. However, the coupling between N and O isotopic compositions of nitrate in Monterey Bay may vary with depth (i.e., increases may deviate from 1:1) due to differential effects of cycling (e.g., assimilation, nitrification, etc.) occurring within the euphotic zone [*Wankel et al.*, 2007]. [20] Water from the Old Salinas River channel, which regularly has nitrate in excess of 1000 μ M [Johnson et al., 2007], exiting the Moss Landing Harbor (Figure 1) is a significant source of nitrate to the main channel of Elkhorn Slough. Data from a biogeochemical monitoring mooring (LO-1; Figure 1) show a surface layer of warmer water that regularly enters the Slough on flooding tides [Johnson et al., 2007]. This layer typically contains very high concentrations of nitrate (>400 μ M), consistent with water from the OSR. Nitrate from the OSR has a unique dual isotopic signature with a high mean δ^{15} N value of +14.5‰ (±2.9‰) and a mean δ^{18} O value of +12.6‰ (±2.6‰) (Figure 7).

[21] While these two water sources, with their widely disparate NO₃⁻ loads, represent the major sources to the main channel of the Slough, the relative contribution of NO₃⁻ from these sources to the greater estuary and the spatial and temporal variability in these contributions (e.g., Figures 2 and 7) are not well characterized. Below, we use both $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ as additional tracers to help resolve mixing relations and offer further insight into the nitrogen cycle in the Slough.

4.2. Mixing of Water Sources

[22] Salinity can be a useful geochemical tool for evaluating two end-member mixing, particularly in estuaries where salinity gradients between freshwater and seawater



Figure 6. Plot of NO_3^- isotopic composition against inverse NO_3^- concentration on (a and b) ebbing and (c and d) flooding tides. Linear patterns in the data are indicative of conservative two end-member mixing between OSR (anchored at the lowest values of $[NO_3^-]^{-1}$) and MB. Some sampling dates exhibit strong linear patterns (e.g., 3 April ebb) and suggest that simple two source mixing can adequately explain the distribution. At other times, the spread of data is nonlinear with data tending to spread more at lower concentrations and lower $\delta^{15}N$ and higher $\delta^{18}O$.

inputs are pronounced [Cifuentes et al., 1990; Hager and Schemel, 1992; Horrigan et al., 1990; Middelburg and Nieuwenhuize, 2000]. For example, a conservative two end-member mixing of a solute (e.g., NO_3^-) will be linear when plotted against salinity, with deviations from a line indicative of additional sources or nonconservative behavior (i.e., in situ consumption or production processes). In Elkhorn Slough, however, due to the large proportion of diurnal tidal exchange with MB water which enters the Slough at the mouth (Figure 1) and the fact that the largest source of freshwater, the OSR (which has extremely high $NO_3^$ concentrations), also enters near the mouth, salinity distributions can be quite complex and often appear unrelated to the distribution of NO_3^- . Figure 2 illustrates this complexity, showing the variable NO_3^- concentrations of the MB endmember (due to water originating from various euphotic zone depths), often with little change in salinity. Tidal cycle variations in salinity are typically very small (~ 0.5) and the NO₃⁻ distribution is largely influenced by mixing of seawater with very small amounts of freshwater with very high NO_3^- concentration (i.e., OSR), thus limiting the use of salinity to identify end-members and determine their relative contribution.

[23] During the 10 sampling trips described here, no clear patterns between salinity and NO_3^- emerged. Thus, in contrast to patterns seen in other estuaries (e.g., San Francisco Bay [*Wankel et al.*, 2006]), where two endmember mixing and a relatively long residence time gave rise to strong spatial and geochemical relationships among salinity, NO_3^- concentration, and dual isotopic composition, the rapid mixing and the more complex geometry in Elkhorn Slough result in temporally variable and complex

mixing patterns. In the following three sections, we use the dual isotopic composition of nitrate (δ^{15} N and δ^{18} O) to evaluate end-member mixing, to assess the probability of other sources and to evaluate the potential role of sediment cycling on nitrate dynamics in Elkhorn Slough.

4.3. Isotopic Mixing of Nitrate

[24] Mixing of two isotopically distinct end-members can also be resolved by plotting the isotopic composition against the inverse of the concentration [Kendall, 1998; Mariotti et al., 1988]. In cases where the distribution of isotopes is determined solely by conservative mixing of two endmembers, plotting the isotope ratios against the inverse of concentration will result in a straight line. When a deviation from a straight line is observed, this suggests the influence of additional sources, an end-member with a variable composition, and/or nonconservative behavior (e.g., influence of processes that alter the isotopic signature of the endmembers). Similarly, a relationship between the isotope ratio and the $\ln[NO_3^-]$ would indicate the influence of a single fractionating process (e.g., denitrification). Based on Figure 2 and plotting the isotopic composition against the inverse of the concentration (Figure 6) our data, while showing some strong linear patterns, cannot always simply be explained by mixing of two endmembes with constant composition.

[25] Because our data are not consistent with simple two end-member mixing solution, we use $\delta^{15}N_{NO3}$, $\delta^{18}O_{NO3}$ and $[NO_3^-]^{-1}$ in a multiple linear regression model to determine if conservative mixing of three sources might be responsible for the observed distribution. Data that represent conservative mixing among three NO_3^- end-members, regardless of



Figure 7. Plot of δ^{15} N and δ^{18} O of NO₃⁻ for all of the samples collected. Shaded symbols represent wet season; open symbols represent dry season. Circles are from cross-channel sampling. Boxes represent the average end-member isotopic composition for deep Monterey Bay (MB) (δ^{15} N = +9.0%; δ^{18} O = +2.9% [*Wankel et al.*, 2007]) and Old Salinas River (δ^{15} N = +14.5%; δ^{18} O = +12.6% [*Wankel*, 2007]) \pm one standard deviation. The MB end-member isotopic composition could vary due to seasonal upwelling. Solid lines (slope of 1) represent the expected trend in variability of NO₃⁻ in MB due to the uptake of NO₃⁻ by photosynthetic assimilation in surface waters.

end-member composition, should result in a good linear model fit (i.e., high r² value). The r² values from the multiple regressions ranged from 0.32 to 0.95, with all but two of the events (2 September flood and 4 October cross channel) having significant correlations (p < 0.01), suggesting that mixing among three end-member NO₃⁻ sources may adequately explain the distribution in most cases. However, while significant, these three end-member mixing models cannot always explain all of the variance in the data (i.e., r² sometimes < 0.9) which may instead indicate the influence of nonconservative processes (see below).

[26] Interestingly, the origin of a third isotopically distinct signature, that has a low δ^{15} N, high δ^{18} O (Figure 6), and is associated with relatively low NO₃⁻, remains difficult to identify. However, data from the longitudinal transects (Figures 3 and 4) seem to suggest that this end-member is associated with the upper half of the Slough where water residence times are longer and mudflats and saltmarsh environments are more prevalent. Thus, we are challenged with the identification of an additional source and/or the processes that cause the deviation from conservative two source mixing. In the following section, several possible NO_3^- sources are explored as explanations for the isotopic signature and distribution of NO_3^- in the Slough. We also present a simple steady state box model demonstrating the influence of N cycling processes on NO₃⁻ isotopic composition in Elkhorn Slough.

4.4. Other Potential Sources

[27] Estuaries can receive nutrient inputs from a variety of point and nonpoint sources. In addition to inputs from both surface water from the surrounding watershed and upwelled nutrient-rich seawater, atmospheric deposition and groundwater can also both play potentially important roles in nutrient delivery to coastal ecosystems.

4.4.1. Upper Slough Riverine Inputs

[28] A freshwater creek draining an agricultural watershed located at the head of the Slough (Carneros Creek) could introduce NO_3^- into the upper Slough. Owing to a Mediterranean climate, however, inputs are very small and seasonal with water only flowing between December and April and average flow rates ranging from 0.08 to 0.16 m³ (http://www.pvwma.dst.ca.gov). Although concentra s^{-} tions of NO_3^- are elevated, because flow is typically very low, high concentrations are not observed in the upper Slough. Furthermore, two long-term monitoring sites at Hudson's Landing (sites 13 and 14, Figure 1, on either side of a tide gate separating Elkhorn Slough from the Carneros Creek watershed) have average NO_3^- concentrations of \sim 325 μ M upstream of the tide gate and \sim 55 μ M downstream [Wankel, 2007]. While the concentrations downstream are higher than ambient main channel waters, $\delta^{15}N$ values from this site are high (+16‰), unlike those observed in the upper Slough. Hence, Carneros Creek, while perhaps locally important, does not seem to contribute substantially to the main channel.

4.4.2. Isotopic Variability of Monterey Bay Inputs

[29] Chapin et al. [2004] noted that a 'tidal bore' of colder and saltier water occasionally enters the main channel of Elkhorn Slough on flooding tides, bringing higher concentrations of NO_3^- from the subeuphotic zone of MB. This NO_3^- is isotopically unique, having lower $\delta^{15}N$ $(\sim +7\%)$ and $\delta^{18}O$ $(\sim +3\%)$ than the more typical MB surface water which enters the Slough. Although this NO₃ isotopic composition is clearly not responsible for the low $\delta^{15}N$ and high $\delta^{18}O$ values seen in the main channel, it contributes to variability in the composition of the MB end-member. This variability, however, is well constrained. Wankel et al. [2007] showed that while deeper MB NO₃⁻ isotopic composition has a consistent composition $(\delta^{15}N \sim +7 \text{ to } +8\%; \delta^{18}O \sim +2 \text{ to } +3\%; [NO_3^-] \sim 30 \text{ to}$ 35 μ M), shallower water (subject to photosynthetic assimilation) will have lower [NO₃] and higher δ^{15} N and δ^{18} O values (approximating a 1:1 relation [Granger et al., 2004]; depicted on Figure 7). Thus, while the NO_3^- input from MB may vary in concentration, its isotopic composition is well constrained and cannot easily explain the deviations from two-source mixing between MB and OSR. 4.4.3. Groundwater

[30] Although groundwater input may be important, data in the Elkhorn Slough region are lacking. Given the high level of agricultural activity in the area, it is not surprising that elevated groundwater NO_3^- levels have been reported in the Salinas River valley, to the south of Elkhorn Slough [Vengosh et al., 2002] and similar levels may be present in groundwater near the Slough. However, recent work by Breier et al. (submitted manuscript, 2009), using Ra isotope budgets, suggests that new advective groundwater input to the Slough is actually quite low, although a very large pool of water is tidally recirculated through shallow sediments. This tidally exchanged saline groundwater (or pore water) represents a substantial component of the water mixing in the upper region of the Slough (where the majority of marsh sediments are located) and this volume is estimated to be as much as 100 times as large as the advective groundwater influx due to regional groundwater table lowering (J. A. Breier et al., submitted manuscript, 2009). In fact, the coastal aquifer has undergone substantial groundwater drawdown, in support of regional agriculture, resulting in saltwater encroachment [Vengosh et al., 2002; PVWMA, 2007], and greatly reducing the likelihood of substantial groundwater inputs to this coastal region.

[31] The low δ^{15} N, high δ^{18} O compositions observed in the upper Slough could be interpreted as advective exchange with groundwater contaminated by NO₃ from KNO₃ fertilizer, which typically has values near 0.0% for $\delta^{15}N$ and +21‰ for δ^{18} O [Kendall, 1998; Kendall et al., 2007]. However, it is rare that KNO₃ is directly applied as fertilizer, since the less soluble forms of N (e.g., urea and/or ammonium) are preferred. Also, when nitrified, these compounds usually produce substantially lower $\delta^{18}O_{NO3}$ values than KNO₃ [Kendall, 1998; Kendall et al., 2007]. Furthermore, except during large storm events (which were not explicitly sampled in our study), the salinity in the Slough is very close to marine, suggesting little influence by a fresh groundwater source. If such a source were important enough to influence the isotopic composition of nitrate in the main channel yet not substantially alter salinity, this would require a high NO₃⁻ concentration. However, the low δ^{15} N (and high δ^{18} O) values we observe are always associated with very low NO₃⁻ concentrations. Hence, a fresh groundwater end-member, or any substantial upper Slough freshwater, seems an unlikely explanation for the observed data. 4.4.4. Atmospheric Deposition

[32] Inputs of atmospheric NO_3^- have been shown to play large roles in many watersheds [Burns and Kendall, 2002; Campbell et al., 2002; Kendall, 1998; Ohte et al., 2004]. Since atmospherically derived nitrate has a very high δ^{18} O (+65 to +95‰) and low $\delta^{15}N$ (-10 to +5‰) [Hastings et al., 2004; Kendall, 1998], this would be a plausible explanation for the third signature observed in the upper Slough. Atmospheric N fluxes (dry plus wet) reported by the National Atmospheric Deposition Program site 66 in central California (approximately 39 miles to the southeast) range from 2.3 to 3.9 μ mol m⁻² d⁻¹. However, by comparison, estuarine sediment nitrogen fluxes are typically on the order of 100 to 10,000 μ mol m⁻² d⁻¹ [Cornwell et al., 1999; Kana et al., 1994; Seitzinger, 1988; Trimmer et al., 1998]. Indeed, sediment core incubation experiments showed that nitrification rates in Elkhorn Slough sediments ranged from 4 to 44 mmol $m^{-2} d^{-1}$. Thus, as a direct flux, atmospheric deposition to Elkhorn Slough is roughly 3-4 orders of magnitude less than N fluxes operating in estuarine sediments [Wankel, 2007]. Considering a depth of 1 m, a direct atmospheric flux would contribute on average 4.4 nM d^{-1} . Accordingly, accumulation of atmospheric nitrate over a week or a month (which bracket the approximate residence times in the upper slough) could lead to contributions of up to 23 nM d^{-1} and 99 nM d^{-1} , respectively, still far below likely controls by sediment cycling. Indirect atmospheric deposition is also expected to be small because the watershed draining into upper Elkhorn Slough is quite small (<15-20 km²) and therefore would not result in a substantial watershed focusing of atmospheric inputs. Furthermore, according to the NADP, most of this deposition occurs as wet deposition during the winter months when increased surface runoff and generally higher slough-wide concentrations would likely overwhelm an atmospheric signal. During the dry months atmospheric deposition is even lower. Thus, while there is certainly some amount of atmospheric N deposited into Elkhorn Slough, we feel that it represents only a very minor component in comparison to the other sources and processes at hand.

4.5. Sediment Nitrogen Cycling

[33] Since there is little evidence that any of the potential sources described above is a significant contributor to the upper Slough, or can explain the observed isotopic compositions, we now consider whether nitrogen cycling processes could be responsible for altering the isotopic compositions of the end-members. The co-occurrence of nitrification and denitrification (and assimilation) is a common characteristic of organic-rich estuarine sediments with sharp redox gradients; in such environments, oxygen diffusion downward aids in nitrification of NH⁺₄ and the production of NO_3^- that supports a community of denitrifying bacteria living just below the oxycline [Jenkins and Kemp, 1984; Jensen et al., 1994; Revsbech et al., 2006; Rysgaard et al., 1993]. Here we suggest that these processes may lead to the appearance of a third nitrate isotopic signature which is periodically mixed into the main channel.

4.5.1. Steady State Box Model

[34] To illustrate the influence of cycling on NO_3^- isotopic composition, we construct a simple steady state box model (Figure 8) similar to those described by others [Lehmann et al., 2004, 2007]. While the use of a steady state model is admittedly simplistic in nature, it is meant only to illustrate how cycling processes can substantially influence NO₃⁻ isotopic composition and thus, should be considered when deconvoluting mixing of multiple sources. Figure 8 illustrates the conceptual model used for constraining the dual isotopic composition of water column NO_3^- in the upper estuary, similar to that outlined by Lehmann et al. [2004] for continental shelf sediments. Water enters and exits the box via exchange with the lower Slough, where NO_3^- comes from a mixture of OSR and MB water having a concentration and isotopic composition representative of this mixture. Since the residence time of water in the upper Slough is on the order of weeks (much longer than a tidal cycle), the influence of cycling can be pronounced.

4.5.2. Influence of Cycling Pathways on δ^{15} N and δ^{18} O

[35] While the isotopic composition of dominant $NO_3^$ sources to Elkhorn Slough can vary substantially, the most likely mechanism for decreasing the δ^{15} N and increasing the δ^{18} O values in waters composed of a mixture of MB and OSR is biological nitrogen cycling including denitrification and nitrification. Denitrification occurring within the sediments is most likely the dominant sink for nitrate from the water column, leading to lower concentrations in the upper Slough. The intrinsic isotope effect (e.g., the kinetic discrimination by the rate limiting enzymatic step) for denitrification (ε_{dnf}) is quite large and we adopt a value of 25‰ [Barford et al., 1999; Brandes et al., 1998; Mariotti et al., 1981]. However, expression of the apparent isotope effect (e.g., the observed change in isotope value) for denitrification ($\varepsilon_{dnf-app}$) will depend partly on the mechanism of delivery of NO_3^- to the denitrifying zone within the sediments. Given that the net flux of nitrate is always into the sediment in the upper Slough [Caffrey et al., 2002; Wankel,



Figure 8. Conceptual schematic for sediment-water nitrogen cycling steady state box model. Q_{NH4} refers to the amount of NH_4^+ diffusing upward out of the sediment that escapes oxidation at the sediment surface or in the water column. Isotope effects for each cycling process are given (ε), where ¹⁵ ε and ¹⁸ ε refer to nitrogen and oxygen isotope effects for nitrate consuming processes, respectively. Q_{CND} refers to the degree of coupling between nitrification and denitrification [*Brandes and Devol*, 1997; *Lehmann et al.*, 2004] and has a strong influence on the composition of the NO₃⁻ escaping and influencing the overlying water column. While the intrinsic isotope effect for denitrification is high ($\varepsilon_{dnf} = \sim 25\%$), NO₃⁻ from the overlying water may be subject to a lower apparent isotope effect ($\varepsilon_{dnf-app} \sim 0\%$) due to diffusion limitation of the sediment denitrifying community [*Brandes and Devol*, 1997; *Lehmann et al.*, 2004]. A higher degree of tidal pumping or irrigation of overlying water through these sediments, will cause the $\varepsilon_{dnf-app}$ to approach ε_{dnf} .

2007], without advection of nitrate out of the sediment, $\varepsilon_{dnf-app}$ will be ~0 [Brandes and Devol, 1997; Lehmann et al., 2004, 2007; Sebilo et al., 2003]. However, if there is an advective flux returning NO_3^- to the overlying water (whether through tidal pumping or bioirrigation), δ^{15} N and δ^{18} O values of this pore water NO₃⁻ are expected to be substantially higher, reflecting more closely the intrinsic value of ε_{dnf} [Lehmann et al., 2004, 2007]. Additionally, conditions at the zone of denitrification including the availability of electron donors (e.g., organic C) may play an important role in the expression of fractionation. For example, if supply of the electron donor is high, the expression of the intrinsic fractionation may be minimal [*Ostrom et al.*, 2002]. Thus, the $\varepsilon_{dnf-app}$ for nitrate consumption could range from ~0 up to 25% depending on the balance of pore water exchange with electron donor availability. In reality, both diffusive and advective processes probably deliver nitrate to the sediments, leading to the expression of a moderate value for $\varepsilon_{\rm dnf-app}$ expressed in overlying water. Importantly, however, all of these mechanisms will operate to increase both $\delta^{15}N$ and $\delta^{18}O$ values in a coupled 1:1 manner [Granger et al., 2004].

[36] In contrast, nitrification has disparate effects on the δ^{15} N and δ^{18} O of the resulting NO₃⁻. Given the abundance of NH⁴₄ in the sediment pore water (up to 500 μ M) [*Caffrey et al.*, 2002; *Wankel*, 2007], the δ^{15} N_{NO3} produced by nitrification (Figure 8) will depend on (1) the δ^{15} N of the source NH⁴₄ being oxidized, (2) the isotope effect for nitrification (ε_{ntr}), (3) the fraction of NH⁴₄ diffusing out of the sediment that escapes nitrification (Q_{NH4}), and (4) the fraction of NO₃⁻ produced that is directly coupled to denitrification within the sediments (Q_{CND}) [*Lehmann et al.*, 2004]. Based on our measurements of sediment organic matter δ^{15} N [*Wankel*, 2007], NH⁴₄ produced from sediment organic matter will have a δ^{15} N value near +5.5‰,. High values of δ^{15} N_{NH4} in the upper Slough water column (+17‰) imply the expression of a high value for ε_{ntr} . We adopt a range of values from 14 to 38‰ for ε_{ntr} represen-

tative of Nitrosomonas and Nitrosospira [Casciotti et al., 2003] which are typical of ammonia oxidizing bacteria found in Elkhorn Slough sediments and water column [Caffrey et al., 2003; S. Wankel et al., manuscript in preparation, 2009]. We estimate the fraction of NH_4^+ diffusing out of the sediments that is consumed by nitrification (in the sediments or water column) using our $\delta^{15}N_{NH4}$ measurements (in the water column of the upper Slough) together with a range of values for ε_{ntr} and +5.5‰ for pore water $\delta^{15}N_{NH4}$. This partial consumption of effluxing NH₄⁺ represents the first of two important constraints on the composition of nitrate resulting from nitrification. The fraction of NH₄⁺ consumed during nitrification under steady state conditions is calculated as: $(\delta^{15}N_{NH4} - \delta^{15}N_{orgN})/\varepsilon_{ntr}$. Thus, we estimate the fraction of NH₄⁺ consumed by nitrification to be approximately 0.68 (although values ranged from as low as 0.17 to as high as 0.82 depending on the value used for $\varepsilon_{\rm ntr}$).

[37] Based on these conditions, NO₃⁻ from nitrification will have a low δ^{15} N of ~0‰. Thus, increases in δ^{15} N caused by denitrification are counteracted by input of low δ^{15} N_{NO3} from nitrification. The coupling of nitrification to denitrification in the sediments is the second important constraint on the composition of NO₃⁻ from nitrification (Figure 8). Depending again on the balance of flow and electron donor supply, the value for ε_{dnf} coupled to nitrification may range from near 0 up to 25‰ (ε_{dnf}). Higher expression of the fractionation will lead to a greater increase in the δ^{15} N and δ^{18} O (coupled in a 1:1 fashion [*Granger*, 2006]). Hence, the more coupled these two processes are, the higher the δ^{15} N and δ^{18} O inputs from nitrification will be.

[38] While δ^{15} N reflects the balance between the influence of denitrification (acting to increase δ^{15} N) and nitrification (acting to decrease δ^{15} N), the δ^{18} O is immune to these counteracting effects. If we assume that the δ^{18} O_{NO3} produced by nitrification is slightly higher than ambient water (δ^{18} O water ~ -2‰; data not shown) as has been suggested by recent marine studies [*Casciotti et al.*, 2002;



Figure 9. Summary of NO_3^- dual isotopic composition for each sampling date along with selected model results. Large symbols represent concentration-weighted average nitrate isotopic composition for that sampling date/tidal condition. Boxes as in Figure 2. The solid line represents conservative mixing between MB and OSR. Note that wet season values (triangles) closely follow conservative mixing between Monterey Bay (MB) and the old Salinas River (OSR) with OSR tending to dominate NO_3 delivery. Dry season values (circles) show more influence by sediment N cycling process, which tend to lower δ^{15} N values and can increase δ^{18} O values. Concentration-weighted mean value for a 24-h sampling in South Marsh (see Figure 1) showing a NO₃⁻ dual isotopic composition with a lower δ^{15} N than in the main channel (and higher δ^{18} O than MB NO₃) due to intensive N cycling and higher water residence time (i.e., prolonged contact with marsh sediments). Dotted lines represent modeled NO_3^- dual isotopic compositions affected by the combined fractionating processes of nitrification and denitrification in marsh sediments (see Figure 8). Each line (lines a-d) spans the range of resulting values for 0 to 100% coupling of nitrification to denitrification, with bottom left ends of the line representing less coupling and the top right end more coupling. Notably, changing ε_{dnf} (from 0 to 25%) for coupled nitrificationdenitrification has the same effect, moving the resulting values along a line with slope 1:1, with higher ε_{dnf} causing higher δ^{15} N and δ^{18} O of the modeled NO₃. All dotted lines include the following parameters unless otherwise noted: starting NO₃⁻ isotopic composition is a ~50/50 mixture of MB and \overrightarrow{OSR} ($\delta^{15}N =$ +11.8%; $\delta^{18}O = +7.8\%$), $\delta^{18}O$ of NO₃⁻⁻ from nitrification is equal to 0%, N cycling rates are 20 times as fast as water turnover, $\varepsilon_{dnf-app} = 0$, and $\delta^{15}NH_4$ (which was measured) is equal to +17% and controls the value for Q_{NH4} along with ε_{ntr} . Note that changing the value of ε_{ntr} has no net effect on the resulting NO₃⁻⁻ isotopic composition; see text for more complete discussion. Line a uses the parameters above, while line b illustrates an increase in the δ^{18} O of NO₃⁻ from nitrification to 10%; line c illustrates a hypothetical decrease in the measured δ^{15} NH₄, and line d (terminating with open squares) illustrates a hypothetical increase in the ε_{dnf} to 12‰ due to tidal pumping and/or bioirrigation.

Sigman et al., 2005; Wankel et al., 2007], then the $\delta^{18}O_{NO3}$ would tend to have a relatively constant (fixed) value near 0‰. However, the $\delta^{18}O_{NO3}$ could also be affected by the close proximity of the oxic/anoxic interface in the water column, where O₂ has high $\delta^{18}O$ due to respiration [*Hendry* et al., 2002; Quay et al., 1995; Roberts et al., 2000]. If some proportion (~1/6 [*Casciotti et al.*, 2002]) of oxygen atoms from dissolved O₂ are incorporated into NO₃⁻ during nitrification, this would also lead to higher $\delta^{18}O_{NO3}$ [*Kendall*, 1998]. In summary, as outlined above, the combined effects of nitrification and denitrification in Slough sediments act to increase both $\delta^{18}O$ and $\delta^{15}N$, though increases in $\delta^{15}N$ will be smaller than that for $\delta^{18}O$ leading to the appearance of a lower $\delta^{15}N$, higher $\delta^{18}O$ signature in the main channel of the Slough.

[39] Finally, as further evidence for the influence of cycling occurring within marsh and mudflat areas (where water residence time is higher and, hence, reaction times of NO₃⁻ with sediments is prolonged), water samples collected hourly over a 24-h period on 1 July 2003 in a region of South Marsh (residence time > 30 days) (Figure 1) indeed exhibit low δ^{15} N values (+1.8 to +8.8%; concentration

weighted mean = +5.4‰) and moderate δ^{18} O values (+4.0 to +16.6‰; concentration weighted mean +7.2‰). While these values only represent one day during the dry season, they nonetheless support the interpretation that prolonged contact with marsh sediments can lead to lower δ^{15} N (as low as +1.8‰) and higher δ^{18} O (as high as +16.6‰) than MB. Figure 9 shows the concentration weighted average nitrate isotopic composition for each date sampled (wet season, triangles; dry season, circles), highlighting the unique composition that the South Marsh samples exhibit in contrast to the main channel mixtures of nitrate from MB and OSR (solid line).

4.5.3. Insights Into NO₃⁻ Isotope Dynamics From the N Cycling Model

[40] Based on measured concentration and experimental rate data [*Wankel*, 2007] and assuming residence times on the order of \geq 20 days, the contribution of NO₃⁻ by nitrification can be as much as 100 times higher than inputs of NO₃⁻ from advective exchange with the lower Slough. With the assumptions outlined above, we can calculate NO₃⁻ dual isotopic compositions (Figure 9) that could result from prolonged contact with sediment and N cycling. Dotted

lines on Figure 9 represent hypothetical modeled $NO_3^$ isotopic composition resulting from influence by sediment cycling. Each line spans a range of nitrate dual isotopic compositions over varying degrees (0 to 100%) of coupling between nitrification and denitrification (Q_{end}), with the bottom left end of the line representing values for low Q_{end} and the top right representing those with high Q_{cnd}. Note that increasing the value for $\varepsilon_{dnf-app}$ coupled to nitrification has the same general effect as increasing Q_{cnd} : a coupled increase in $\delta^{15}N$ and $\delta^{18}O$ along a 1:1 slope. In all four cases (Figure 9, lines a-d) the following parameters are assumed unless otherwise noted: starting NO₃⁻ isotopic composition is a \sim 50/50 mixture of MB and OSR ($\delta^{15}N =$ +11.8‰; δ^{18} O = +7.8‰), δ^{15} N of sediment organic N +5.5‰, δ^{18} O of NO₃⁻ from nitrification is equal to 0‰ (except in line b = 10%), N cycling rates are 20 times as fast as water turnover, the expressed fractionation by denitrification ($\varepsilon_{dnf-app}$) = 0% (except in line d = 12‰), and $\delta^{15}NH_4$ (which was measured) is equal to +17‰ and controls the value for Q_{NH4}^+ along with ε_{ntr} . Notably, the choice of values for $\varepsilon_{\rm ntr}$ (14 to 38‰) has no net impact on the N isotopic flux of NO_3^- stemming from nitrification, owing to the fact that we use measured values for $\delta^{15}N_{NH4}$ to constrain Q_{NH4} . For example, higher values of ε_{ntr} require Q_{NH4} to be smaller (to force $\delta^{15}N_{NH4} = 17\%$) and hence the $\delta^{15}N$ of NO₃⁻ from nitrification to be lower. However, lower values of $\varepsilon_{\rm ntr}$ require Q_{NH4} to be larger and the $\delta^{15}N$ of NO_3^- from nitrification to be higher. In either case, the isotopic mass flux of N into the NO_3^- pool will have the same leverage on the mixture of NO_3^- in the overlying water. It follows then that using lower values for δ^{15} NH⁴₄ (for example 12‰ instead of 17‰; shown as line c in Figure 9) would require lower values for Q_{NH4} and hence a smaller flux of low $\delta^{15}N$ from nitrification and an ultimately smaller effect on the overlying NO_3^- pool. Hence, the isotopic composition resulting from sediment cycling can be quite variable, since both the N and O isotopic composition will be influenced by spatial heterogeneity in sediment composition (high/low organic mater) and temperature, as well as by variability in rates of remineralization, respiration, benthic photosynthesis, nitrification and water exchange rates. In summary, nitrogen cycling in estuaries, particularly in areas with high residence times, should lower δ^{15} N values (as dictated by the δ^{15} N of the sediment organic matter relative to exogenous NO₃⁻ sources) and possibly increase δ^{18} O values (as dictated by denitrification and the incorporation of high δ^{18} O by nitrification) and complicate simple source partitioning.

[41] While the influence of sediment N cycling processes was apparent during both the wet and dry season (e.g., data points falling outside of mixing between MB and OSB in Figure 7), effects were more prevalent during the dry season when NO₃⁻ concentrations were low ($<5 \mu$ M) (Figure 9, circles). Spatially, the influence of cycling tended to be most dominant in the upper Slough, consistent with the abundance of marshes and mudflats, which contain organic rich sediments known to harbor abundant microbial populations, and longer water residence times, which allow the processes of nitrification and denitrification more leverage over tidal exchange of water containing 'fresh' nitrate. Although this recycled nitrate from nitrification may not be an important source of NO₃⁻ to the main channel on an annual timescale, the influence of sediment cycling is doubtlessly important to

the local mud flat and salt marsh communities; hence, its influence on NO_3^- isotope dynamics should be accounted for, particularly when estuary-wide concentrations are low. We note that the primary limitations on the use of NO_3^- dual isotopic composition for resolving contributions of different nitrate sources in the Slough are the unknown rates of and fractionation effects by N cycling processes. Better estimates of these factors will help to better constrain mixing and elucidate both sources and cycling of NO_3^- in estuaries. Furthermore, to appropriately deconvolute the influence of multiple fractionating processes, knowledge of water residence times is critical. For example, rapid tidal exchange will likely overwhelm any cycling signature, while a longer residence time will lead to a much greater influence by sediment microbial processes. Hence, simple three endmember mixing models may be inappropriate for accurate source partitioning in such a hydrologically dynamic system, in which residence times vary spatially. Finally, the coupling of an isotope biogeochemical model with a hydrodynamic model would represent a great step toward the goal of truly estimating the significance of different nitrate sources to any estuarine system.

5. Conclusions

[42] While delivery of NO_3^- into the main channel of Elkhorn Slough is dominated by a mixture of OSR and MB water during most of the year, processes of NO_3^- consumption (leading to higher $\delta^{15}N$ and $\delta^{18}O$) and nitrification (leading to lower δ^{15} N and a fixed or higher δ^{18} O) offer the most reasonable explanation for observed deviations of the isotopic compositions of NO₃⁻ from two end-member mixing. Although marsh sediments represent a net NO_3^- sink, we emphasize that simultaneous N cycling processes cannot be estimated accurately from nutrient fluxes alone [e.g., *Caffrey et al.*, 2003] and that NO_3^- dual isotopes add an important dimension to our understanding of sediment N cycling in estuaries by aiding in the constraint of the relative importance of these cycling processes. The influence of sediment nitrification on the isotopic composition of $NO_3^$ in the Slough underscores the need for understanding the controls on dual isotopic composition, specifically δ^{18} O, as there is still debate surrounding exactly what dictates the oxygen isotopic composition stemming from nitrification in marine systems [Casciotti et al., 2002; Sigman et al., 2005; Wankel et al., 2007]. In estuarine systems, even less is known. Finally, we stress that nitrogen cycling at the sediment-water interface plays a primary role in controlling NO_3^- isotopic composition when and where cycling rates and water residence times are high.

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