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Denitrification rates across a temperate North Pacific estuary, Yaquina Bay, Oregon

A. C. Sigleo*

Western Ecology Division, US Environmental Protection Agency, 2111 SE Marine Science Drive, Newport, Oregon 97365-5260, USA

Abstract

The extent and temporal variability of denitrification activity was measured in Yaquina Bay, Oregon, over a year using sediment cores collected approximately monthly from August 2003 through August 31, 2004. Denitrification rates in sediments from a marine-dominated intertidal sand flat near the mouth of the estuary averaged $0.181 \pm 0.114 \text{ mmol m}^{-2} \text{ d}^{-1}$ whereas sediments in the estuary (5 stations) and river averaged $0.626 \pm 0.141 \text{ mmol m}^{-2} \text{ d}^{-1}$. Sediment cores from all estuarine sites indicated denitrification activity throughout the year and were within the values reported for other temperate estuaries. Denitrification rates decreased with depth from $0.4 \text{ mmol m}^{-2} \text{ d}^{-1}$ in the upper 2 to 5 cm of sediment to $0.006 \text{ mmol m}^{-2} \text{ d}^{-1}$ at 28 cm sediment depth, indicating denitrification occurred primarily in the upper 5 cm. There was no relationship between denitrification rate and nitrate concentrations in the overlying water column ($r^2 = 0.16$). Denitrification rates were lowest in areas with low sediment carbon content, particularly in the sandy intertidal areas at the mouth of the estuary ($r^2 = 0.78$). The results suggest that denitrification rates in this estuary were influenced primarily by the availability of organic carbon.

The amount of nitrogen removed by denitrification was estimated to be 8.7 percent of the annual Yaquina River load for August 2003 through August 2004. The relatively low percent lost via denitrification may be due to high river discharge when the nitrogen load was greatest during winter storm events and dissolved nitrogen was exported directly from the estuary into the Pacific Ocean. Stable isotopes were used to investigate the carbon source. The carbon isotope data increased from $-27 \delta^{13}\text{C}$ in the freshwater river to $-21.5 \delta^{13}\text{C}$ at the seawater site, reflecting a typical change from terrestrial plant vegetation to phytoplankton carbon sources. Similar values for $\delta^{13}\text{C}$ between suspended and benthic sediments indicated resuspension and mixing occurred during tidal inflow.

Keywords

Denitrification; estuaries; nitrate; nitrogen cycle; Oregon coast

asigleo@yahoo.com.

*Present Address: Visual Plumes Consulting, 1541 NW Spring Street, Newport, Oregon 97365-2344, USA

Introduction

Nitrogen from agricultural runoff, fossil fuel combustion, and domestic waste results in water quality degradation and eutrophication in many aquatic ecosystems worldwide (Goolsby et al., 1999; Boyer et al., 2002; Castro et al., 2003), causing hypoxia and habitat loss (Turner and Rabalais, 1991; Childs et al., 2002). A major pathway for nitrogen removal is denitrification, a microbial-mediated process in which facultative anaerobic bacteria, archaea and fungi reduce dissolved nitrate to dinitrogen (N₂) gas (Wallenstein et al., 2006). Denitrification occurs in sediments, soils, and surface and groundwaters (Rich and Myrold, 2004; Seitzinger et al., 2006; Groffman et al., 2006). Denitrification communities require low oxygen, dissolved nitrate, and a source of organic carbon (Groffman et al., 1999). The rate of denitrification is influenced by available sediment organic matter, water column nitrate concentrations (Nowicki et al., 1997; Barnes and Owens, 1998; Svensson et al., 2000), along with salinity, and seasonality or temperature, (Nowicki et al., 1997; Fear et al., 2005; David et al., 2006). At both local and regional scales, denitrification removes fixed nitrogen that otherwise would be available for primary production and microbial assimilation (Seitzinger et al., 2006).

Along the US West coast, the Yaquina watershed is classified as forested, although the original complex forest now consists predominantly of single species silviculture (Noble and Dirzo, 1997; Wimberely and Ohmann, 2004). Disturbed and recently cleared sites are colonized by pioneer broad-leaved trees, primarily red alder (Ohmann and Gregory, 2002). Red alder (*Alnus* spp.) occurrence is significant because atmospheric nitrogen is converted to bioavailable nitrogen in alder roots via the symbiotic relationship with the actinomycete *Frankia* spp (Vogal and Gower, 1998). As a result, in heavily logged areas of the Pacific Northwest, leaching from decayed alder litter is a major source of nitrate to coastal rivers, including the Yaquina (Compton et al., 2003; Sigleo et al., 2010).

The major source of watershed nitrogen for Oregon coastal rivers in the past came from the decay of anadromous salmon carcasses (Bilby et al., 1996; Koyama et al., 2005; Scheuerell et al., 2005). Annual fish returns, however, have decreased to 8% or less of historical numbers (Gresh et al., 2000; Meengs and Lackey, 2005). The combination of decreased fish returns and increased timber harvesting altered the primary source of watershed nitrogen from anadromous fish decay to alder litter decay (Gresh et al., 2000; Compton et al., 2003; Tiegs et al., 2008). Domestic sewage discharge and agricultural runoff presently are minor sources of nitrogen in this system due to low population density and the dominance of silviculture (Wimberely and Ohmann, 2004; Brown and Ozretich, 2009; Sigleo et al., 2010). Beginning in the 1850s Yaquina Bay was exploited for timber and oysters for the San Francisco market. Despite more than a century of exploitation, the bay remains a major commercial fishery and the intertidal areas provide primary habitat for shellfish, oyster aquaculture, and migratory birds (Lamberson et al., 2011). Both adult and juvenile salmon spend time in Yaquina Bay during their migration to and from the ocean, and the estuary is critical for juveniles to increase size and to make the physiological transition between fresh and saltwater environments (Meengs and Lackey, 2005). Commercial fisheries, shellfish aquaculture and recreational activities all require high standards of water quality. The USEPA lists high nutrient concentrations, particularly that of nitrogen, as a major cause of

impairment in rivers and streams in the United States (USEPA, 2006). For these reasons, understanding nitrogen sources and sinks, including the amount denitrified, is essential to the management of nitrogen in estuaries and coastal waters.

The goal of the following study was to determine the spatial and temporal variability of denitrification activity in Yaquina estuary sediments, Oregon (USA) and to evaluate the relative contribution of denitrification to the removal of nitrate. Environmental factors controlling denitrification rates, including water column nitrate, and organic carbon and nitrogen concentrations in suspended and benthic sediments also were measured. The stable isotopic composition of carbon and nitrogen in suspended and benthic sediments were measured to provide information on the marine vs watershed contribution of carbon and nitrogen, and on the trophic level of nitrogen (Fry et al., 2003; Carmichael and Valiela, 2005). Isotopes also integrate nitrogen dynamics at longer scales than nutrient concentrations alone and may provide a historical perspective on sediment nitrogen sources (Fry et al., 2003). The following work expands denitrification studies to include a coastal Oregon estuary.

Methods

Study Area

The Yaquina Bay is a temperate macro-tidal tidal estuary on the west coast of the United States that empties into the Pacific Ocean at Newport, Oregon (44.6°N, 124.0°W, Fig. 1). The Yaquina has a semi-diurnal tidal regime with diurnal inequalities, and a mean tidal range of 2.4 m (NOAA Station 9435380). Within the bay the combined marine and mesohaline zones cover 18 km² of which 40% is subtidal channel and 60% is intertidal sandflat, mudflat, and seagrass habitat covered by *Zostera marina* and *Zostera japonica* (Cortright et al., 1987; Kentula and DeWitt, 2003; Larned, 2003). Seagrasses, macroalgae and benthic diatoms form dense patches in the intertidal zones and provide net sinks for nitrate, particularly during the summer growing season (Larned, 2003; Kentula and DeWitt, 2003; Sin et al., 2007; Weihhoefer et al., 2015).

The region is characterized by a maritime climate with mild wet winters and cool dry summers. Yaquina River discharge averages 6.9 m³ sec⁻¹, although flows can vary from 0.062 m³ sec⁻¹ during late summer low flow conditions to 186 m³ sec⁻¹ during storm events (http://apps.wrd.state.or.us/apps/sw/hydro_near_real_time/display_hydro_graph.aspx?station_nbr=14306030). Typical river discharge is high in late fall, winter and early spring (November to April) and low the remainder of the year. Nitrate export from the river to the estuary also is strongly seasonal with up to 94% of river nitrate export occurring during the winter season when biological productivity is minimal (Sigleo and Frick, 2007). The Yaquina River discharges between 266 and 1210 metric tonnes of dissolved nitrate annually into the Yaquina estuary near the head of tide at Elk City (Sigleo and Frick, 2007). The annual load varies relative to rainfall with greater nitrate being exported in wetter years and less nitrate exported during drought years (Sigleo and Frick, 2007). The amount of both dissolved and particulate carbon exported from Oregon streams also varies relative to rainfall and stream flow (Argerich et al., 2016).

Another source of nitrate in Pacific Northwest estuaries comes from ocean upwelling during spring and summer. Coastal upwelling is a seasonal occurrence in response to northerly winds that bring cold, nutrient-rich water to the surface along the Oregon coast (Barth et al., 2000; Colbert and McManus, 2003; Du and Peterson, 2014). Summer upwelling events provide twice daily pulses of nutrient-rich water that supplement nutrient depleted waters in coastal bays and estuaries (Small and Menzies, 1981; Sigleo et al., 2005; Brown and Ozretich, 2009).

Sampling stations in this study (Fig. 1) included a marine intertidal sand flat (IF), estuarine regions with substantial spring and summer *Zostera marina* cover (TD, NS, BS, JS, PS), a saline intertidal station adjacent to oyster aquaculture (OY), a station outside a wetland tide-gate that is closed on the incoming tide to minimize salt water intrusion (BS), and a freshwater station above head of tide at Elk City (EC). The estuarine stations were sampled monthly to bimonthly from August 2003 through August, 2004. On August 31, 2004 eight stations (except NS) were sampled.

Sample Collection

Sediment cores for denitrification rate measurements were collected in triplicate to measure station variability during low tide. Stations PA, OY, JS, BS, and TD were sampled monthly to bimonthly over a year to estimate the temporal variability of denitrification activity. Denitrification rates also were measured across a depth gradient at station JS on 28 April 2005. Three meter cores were subsampled at 7 cm intervals up to 3 m. In addition to measuring habitat (ie across station) spatial variability, within-habitat variability was estimated by taking three cores at each station. Further, within-core variability was estimated by analyzing 3 samples from each of the 3 cores across four stations during September 2003, and analyzed using a nested analysis of variance (Sokal and Rolf, 1981).

Samples were collected by inserting plexiglas core liners (25 cm x 5 cm i.d.) into wet sediment beneath the surface of the water. Approximately 2 to 5 cm of water and 2 to 5 cm of air remained at the top of the core. A cap was secured on the top of the core. The core was removed carefully from the sediment, and a cap was pushed onto the bottom of the core liner while the lower part of the core remained under water. Finally the core was carefully placed upright in ice for transport to the laboratory. Water samples for nutrients and suspended sediments were collected by submersion of 10 liter carboys at the same time the cores were collected. Water samples were filtered and the filtrate frozen until analysis. Suspended sediments for organic carbon and nitrogen concentrations and carbon and nitrogen isotopic analyses were collected by filtration on glass fiber filters, freeze dried and stored in a desiccator until analysis.

Analytical Technique

Denitrification is determined by measuring a small amount of the terminal end product, N_2 . To avoid the interference of atmospheric N_2 , techniques that measure other products often are used (Groffman et al., 2006). The most common method is based on the ability of acetylene (C_2H_2) to block the enzymatic reduction of nitrous oxide (N_2O) to N_2 (Groffman

et al., 2006). Groffman et al. (2006) concluded that in systems with moderate to high nitrate concentrations, the acetylene method provided reasonable results.

Analytical Measurements

The acetylene block technique was used in this study. N₂O was analyzed in the headspace of sealed 125 ml serum vials containing 5 to 10 g wet sediment, 25 ml nitrate media (100 mg N kg⁻¹) and dextrose (40 mg kg⁻¹) at pH 7 (Groffman et al., 1999). The serum vials were evacuated to 700mm Hg and flushed with Ar a minimum of five times to remove oxygen and CO₂. Flasks were finally brought to atmospheric pressure with Ar. Purified acetylene (5 ml) was added by syringe to the serum vials that then were incubated on a rotary shaker table at 125 rpm and 20° C. Gas samples were removed from the serum vial headspace with gas tight glass syringes at 30, 60 and 90 min (Groffman et al., 1999; Holland et al., 1999), and analyzed immediately on a Varian model 3700 gas chromatograph equipped with a Poropak QS column (50° C) and an electron capture detector (350° C). The carrier gas was 5% methane in Ar. A linear calibration of the detector response was performed daily with a N₂O gas standard (Airco Gases, Riverton, NJ, 23 ppm N₂O in Ar). The N₂O peaks were integrated and quantified with a HP 9600 integrator recorder.

Sediment denitrification rates were determined from the increase in N₂O produced by the sediment in the headspace of the serum vials at 30, 60 and 90 minutes, and corrected for the Bunson coefficient at 20° C (Groffman et al., 1999; Holland et al., 1999). Sediment density was determined as the sediment dry weight (g) divided by volume (ml) in a calibrated container. The porosity was the mass difference between wet and dry sediments. Total organic carbon (TOC) and nitrogen (TON) were measured in the dried sediments using a Carlo Erba EA1108 elemental analyzer with an analytical error of ± 10%. Grain size (± 5%) was analyzed by the sieve-pipette procedure (Buchanan 1984).

Benthic sediment and suspended sediment samples also were analyzed for δ¹⁵N and δ¹³C on an isotope ratio mass spectrometer (Delta 2, Finnigan, Bremen, Germany) interfaced with an elemental analyzer (ECS 4010, Costech, Valencia, CA), located at the Integrated Stable Isotope Research Facility at the Western Ecology Division of the EPA, Corvallis, Oregon. Measurement precision and accuracy were 0.04 ‰ and 0.23 for δ¹³C and 0.12 ‰ and 0.20 for δ¹⁵N as determined by standards run with the study samples (J. Compton, pers. communication).

The nutrients nitrate + nitrite, ammonium, phosphate and silica in the overlying water column at each site were determined colorimetrically using a Lachat autoanalyzer at the MSI Analytical Laboratory, U C Santa Barbara, California. Analytical information, including blank procedures, sample replicate results and other quality assurance details are available at www.msi.ucsb.edu/Analab. Salinity, temperature and dissolved oxygen were measured in the field with a Yellow Springs Instruments (YSI, Yellow Springs, Ohio) Model 85 O₂, conductivity, and temperature meter during sample collection. The watershed nitrate load for August 2003 through August 2004 was calculated using USGS river discharge data and equations from Sigleo et al., 2010. Statistical analyses and graphics were performed in Microsoft Excel and Sigma Plot 10. Within site variability was estimated by analyzing three cores from each station. Within-core variability was estimated by analyzing 3 samples from

each of the 3 cores across four stations during September 2003 and examined using a nested analysis of variance (Sokal and Rolf, 1981).

Results

Water Column Characteristics

Dissolved nitrate values varied seasonally with concentrations increasing rapidly with the first major Fall storm and continuing to increase with increasing river discharge through the winter (Fig. 2). Because sampling began at the seaward-most station at low tide and traveled upstream with the incoming tide, salinity and the dissolved nitrate concentrations were virtually identical for stations PA, OY, JS, BS, NS and TD. In other words, the strong, rapid tidal influx caused dynamic mixing that equaled or exceeded the sampling progress. For this reason data for these sampling dates were nearly identical, and the nutrient concentrations were averaged (Fig. 2). Dissolved nitrate varied from 1 to 70 $\mu\text{mol l}^{-1}$ with largest values coinciding with high river discharge during the winter months. The lowest nitrate values occurred in late summer/early fall prior to the winter rains (Fig. 2).

Dissolved silica varied from 40 $\mu\text{mol l}^{-1}$ in the summer to over 200 $\mu\text{mol l}^{-1}$ during the winter (Fig. 2). Silica concentrations, greater than those of nitrate, were sufficient to promote healthy diatom growth. Ammonium and phosphate mean concentrations varied from 1.7 to 7.9 $\mu\text{mol l}^{-1}$ and 0.5 to 1.2 $\mu\text{mol l}^{-1}$ respectively (Fig. 2). Water column temperature varied from 8 to 10° C during the winter, and increased to 22° C in July and August. Salinity ranged from 2 during high river flow in January 2004 to 28 in August and September 2003. In spring and early summer, salinity ranges in the estuarine mixing zone were in the intermediate range of 16 to 20.

Sediment Characteristics

The sediments at Stations PA, OY, JS, BS, NS, TD, and EC consisted of silt and clay whereas the intertidal sediments at Idaho Flat (IF) were predominantly sand (>95% sand) (Table 2). The TOC content ranged from 3 to 4 % in the silt/clay stations to < 0.3% in the sandy intertidal area. The TON content in the silt/clay estuarine sediments varied between 0.2 to 0.3 %, whereas the intertidal sand flat had <0.1 % TON (Table 2). The C/N ratio averaged 11.7 ± 1.96 for suspended sediments and 17.1 ± 2.5 for benthic sediments indicating decreased nitrogen in benthic sediments (Fig. 3). Stable carbon isotope values for $\delta^{13}\text{C}$ were -26.0 ± 0.77 in suspended sediments and -25.9 ± 0.37 in benthic sediments (Fig. 4). The similar values for $\delta^{13}\text{C}$ between suspended and benthic sediments suggest resuspension and mixing occurred during tidal inflow. The carbon isotopic data showed an increase from $-27 \delta^{13}\text{C}$ in the freshwater river to $-21.5 \delta^{13}\text{C}$ at the seawater site (Fig. 4). The nitrogen stable isotope values were $4.0 \pm 0.81 \delta^{15}\text{N}$ in benthic sediments and $3.72 \pm 0.31 \delta^{15}\text{N}$ in overlying suspended sediments (Fig. 4). The nitrogen isotopic values from the freshwater to the ocean influenced intertidal area increased from 3.5 to 4 $\delta^{15}\text{N}$ in the benthic sediments and from 3.8 to 5.4 $\delta^{15}\text{N}$ in suspended sediments.

Sediment Denitrification Activity

Denitrification rates in Yaquina sediments averaged $0.626 \pm 0.141 \text{ mmol m}^{-2} \text{ d}^{-1}$ and ranged from 0.15 to $1.35 \text{ mmol m}^{-2} \text{ d}^{-1}$ (6.25 to $56 \text{ } \mu\text{mol m}^{-2} \text{ hr}^{-1}$) in river and estuarine sediments (Table 3). In the intertidal sandy areas near the mouth of the estuary the mean denitrification rate was $0.181 \pm 0.114 \text{ mmol m}^{-2} \text{ d}^{-1}$. Sediment cores from all sites within the estuary indicated denitrification activity at all times of the year (Fig. 5). Although there was considerable variation in values within the estuarine silt/clay sediments, there was no significant spatial pattern in denitrification rates across the estuarine transect (Table 3).

Denitrification rates within cores, between cores and between sites were examined using a nested analysis of variance (Sokal and Rolf, 1981) in which three samples were taken from each of three cores at 4 sites. The data for sites PA, OY, JS and BS in September 2003 indicated that 4.4 % of the variance occurred between the measurements within cores, 3.95 % was among cores from the same site, and 91.6 % of the variance occurred among sites.

In a 30 cm core denitrification rates decreased with depth from $0.4 \pm 0.07 \text{ mmol m}^{-2} \text{ d}^{-1}$ in the top 2 to 5 cm of sediment to $0.006 \text{ mmol m}^{-2} \text{ d}^{-1}$ at 28 cm depth, indicating that denitrification occurred primarily within the upper 5 cm (Fig. 6).

Sediment organic carbon content was associated positively with mean denitrification rates in that the greater carbon concentrations ($2.6 \pm 1.2\%$) in estuarine sediments had greater denitrification activity relative to the lower carbon ($0.28 \pm 0.12\%$) sandy intertidal sediments that had lower denitrification rates (Fig. 7, $r^2 = 0.78$). The amount of nitrate exported from the watershed for 2003 to 2004 was 16.5×10^6 moles nitrogen (Table 1). The amount of nitrogen removed by denitrification was estimated by summing the annual denitrification rates for the subtidal and intertidal areas (Table 1) following the examples of Nowicki et al., (1997), Smyth et al., (2013) and Cornwell et al. (2014). The estimate indicated that 1.21×10^6 moles N yr^{-1} and 0.113×10^6 moles N yr^{-1} were removed from the subtidal and intertidal areas respectively.

Discussion

The rate of denitrification at the mouth of the estuary ($0.181 \pm 0.114 \text{ mmol m}^{-2} \text{ d}^{-1}$) to that within the estuary and river ($0.626 \pm 0.141 \text{ mmol m}^{-2} \text{ d}^{-1}$; Table 3) was within the range found elsewhere in temperate estuarine sediments (Nowicki et al., 1997; Cornwell et al., 2014 and tables therein). The amount of available water column nitrate frequently correlates with denitrification rate, with increased nitrate supporting an increase in denitrification rate (Svensson et al., 2000; Mulholland et al., 2008). In the Yaquina estuary, however, there was no correspondence between nitrate concentrations in the overlying water column and measured denitrification rates ($r^2=0.16$), probably because high dissolved nitrate concentrations during winter runoff from freshwater regions of the watershed was supplemented in summer by tidally-entrained oceanic upwelled nitrate (Sigleo et al., 2005; Brown and Ozretich, 2009; Du and Peterson, 2014). In other words, the potentially limiting component of nitrate was adequate for continuous year-round denitrification activity, and seasonal denitrification differences were not observed.

Denitrification in other areas

Sediments in San Francisco Bay, south of the study area, also showed no seasonal differences in denitrification rates, although the authors did observe a seasonal shift in denitrification processes (Cornwell et al., 2014). Specifically, coupled nitrification-denitrification was the primary process in September, whereas greater dissolved nitrate concentrations in the overlying water in March indicated that denitrification was driven by dissolved nitrate from the water column into the sediments in Spring (Cornwell et al., 2014).

Rivers worldwide export only a portion of the nitrate received from surrounding runoff indicating that large percentages are stored in vegetation and soils, or lost via denitrification within the catchment (Boyer et al., 2002). Smyth et al., 2013 found that the amount of nitrogen removed by denitrification depended upon the habitat type and its extent within the estuary since each habitat removed different amounts of nitrogen. Specifically, marsh habitat removed $146 \text{ mmole N m}^{-2} \text{ yr}^{-1}$ whereas the oyster reef and SAV habitats removed up to $510 \text{ mmole N m}^{-2} \text{ yr}^{-1}$ (Smyth et al., 2013). The authors estimated that the total removed from all habitats accounted for 76% of the annual watershed nitrogen load (Smyth et al., 2013).

Residence time and nitrogen removal

The extent to which organic material is mineralized and nutrients are modified by denitrification depends on the amount of time that the organic matter spends within the estuary, or the residence time (Dettmann, 2001). Because the Pacific seaboard along the Oregon coast typically has steep mountain slopes, cliff-dominated shorelines, and rivers that flow rapidly to the sea, residence times tend to be short relative to other areas where residence times can exceed a year (Dettmann, 2001; Uncles and Smith 2005). In the Yaquina estuary, during late summer low flow conditions, the total water exchange time from Elk City to the mouth of the estuary was calculated at 18 tidal cycles, or a residence time of 9 days (Choi, 1975). During winter storms when fresh water overrides the saline tidal wedge, the residence time decreases to one to five tidal cycles (Choi, 1975; Lemagie and Lerczak, 2015). Maximum dissolved nitrate concentrations also occurred during the winter, when biological productivity was minimal and nitrogen was effectively exported out of the estuary (Sigleo and Frick, 2007). For these reasons, Yaquina estuary nitrogen removal by denitrification was relative low. Specifically, Yaquina estuary denitrification removed an estimated 1.43×10^6 moles N from the subtidal and intertidal areas, an amount equivalent to 8.7 percent of the estimated river load for August 2003 through August 2004. In San Francisco Bay, denitrification represented an estimated 10% of the net river nitrate export (Cornwell et al., 2014), an amount comparable to Yaquina sediments.

Elemental factors

TOC and DON both were lower in benthic sediments relative to suspended sediments indicating both heterotrophic consumption of labile compounds and admixture with inorganic sediment. Denitrification activity was lowest in sandy sediments that also contained the lowest amount of sediment organic carbon (Fig. 7). The correspondence of low carbon concentration and low denitrification activity suggest that organic carbon was the limiting factor in this estuary. Organic carbon also was the limiting nutrient in Boston

Harbor (Nowicki et al., 1997). Specifically, in Boston Harbor, where mean denitrification rates were correlated linearly with sediment organic carbon, the highest rates occurred at stations with high sediment organic content relative to lower rates in low carbon sandy sediments (Nowicki et al., 1997), similar to results found in the Yaquina estuary.

Stable Isotopic Indicators of Nutrient Sources

Carbon isotopic values that increased from $-27 \delta^{13}\text{C}$ in the freshwater river at Elk City to $-21.5 \delta^{13}\text{C}$ downstream at the marine intertidal site indicated a greater proportion of upland C-3 plants (-31 to $-27.5 \delta^{13}\text{C}$) in freshwater suspended sediments relative to a greater amount of phytoplankton (-22.5 to $-20 \delta^{13}\text{C}$) in suspended sediments at the mouth of the estuary (Coffin et al., 1989, Fry et al., 2003). Intermediate isotopic values of $-25 \delta^{13}\text{C}$ within the estuary indicated combined C-3 plant and algal carbon sources (Fig. 4). The similar stable carbon isotope values between suspended ($-26.0 \pm 0.77 \delta^{13}\text{C}$) and benthic ($-25.99 \pm 0.37 \delta^{13}\text{C}$) sediments, suggested that constant resuspension and mixing occurred frequently during dynamic tidal inflow.

An isotopic study of two indigenous burrowing shrimp species in Yaquina Bay found that both the shrimp and their food sources varied by location (Bosley et al., 2017). Specifically, carbon isotope values exhibited a strong spatial trend with shrimp located near the mouth of the estuary being more enriched in ^{13}C than those from sites from upriver. The authors found that shrimp diets also generally reflected the estuarine gradient with shrimp in the lower estuary feeding on diets rich in marine sourced carbon while upriver shrimp fed on riverine and terrigenous sources (Bosley et al., 2017).

Nitrogen isotopic values of $4.0 \pm 0.81 \delta^{15}\text{N}$ in benthic sediments and $3.72 \pm 0.31 \delta^{15}\text{N}$ in overlying suspended sediments also suggest resuspension and mixing. The nitrogen isotopic value increases from 3.5 to 4 $\delta^{15}\text{N}$ in the benthic sediments and from 3.8 to 5.4 $\delta^{15}\text{N}$ in the suspended sediment from the freshwater to ocean areas suggest biological utilization of organic matter. Biological processes, including denitrification, discriminate for molecules containing the lighter isotope ^{14}N relative to the heavier isotope ^{15}N so that increasing trophic levels show greater ^{15}N levels (Mariotti et al., 1981). Because the lighter isotope is utilized first, the isotopic composition of the remaining nitrate becomes enriched in ^{15}N . Owens (1985) noted that the ^{15}N content of suspended particles in estuaries reflects the effects of both hydrodynamic mixing of freshwater and ocean sources as well as biological transformations within the estuary (Owens, 1985). Increased residence time increases microbial degradation leading to higher ^{15}N values in the refractory nitrogen (Carmichael and Valiela, 2005). An additional consideration is that secondary sewage treatment utilizes alternating nitrification-denitrification to remove nitrogen from sewage effluent worldwide (David et al., 2006). For this reason sewage effluent typically is enriched in ^{15}N from the secondary treatment (Hadwen and Arthington, 2007). This effect may have caused the slight increase in suspended sediment ^{15}N at station TD where samples were collected below the Toledo City sewage outfall, a secondary treatment facility.

State of the Estuary

Weilhoefer et al., (2015) found high nitrogen adapted diatom species present in coastal Oregon estuaries and side creeks, and concluded that the area was eutrophic. In fact historically, decaying salmon carcasses provided high levels of estuarine nitrogen that would have acclimated native diatoms to a relatively high nitrogen diet (Bilby et al., 1996; Koyama et al., 2005; Scheuerell et al., 2005). At the present time, returning salmon comprise approximately 8% of historical returns and dissolved nitrate is provided by decayed alder litter (Gresh et al., 2000; Compton et al., 2003; Meengs and Lackey, 2005; Tiegs et al., 2008). Because diatom communities are determined by annual temperature, moisture and substrate availability, the actual community composition integrates long term sediment conditions, including resource availability (Wallenstein et al, 2006).

Previously Sin et al., (2007) concluded that there was sufficient biological activity, including the microalgal community, to consume nitrate and prevent eutrophication, despite considerable nitrate loading from river and oceanic sources. Chemical measures using nutrient concentrations and isotopic values for eutrophication in West Coast estuarine sediments found $\delta^{15}\text{N}$ values of 9 to 11‰ in eutrophic estuaries, values considerably greater than those of 3.7 to 4‰ $\delta^{15}\text{N}$ for Yaquina Bay sediments (Fry et al., 2003). Fry et al. (2003) concluded that a strong tidal exchange and short residence times for estuaries of the U. S. West Coast decreased the impacts of watershed nitrate, a conclusion in agreement with the results in this study.

In summary the present study measured denitrification activity in a coastal Oregon estuary across a gradient from marine intertidal to freshwater sediments over a year. The Yaquina range in denitrification rates ($0.15 - 1.35 \text{ mmol m}^{-2} \text{ d}^{-1}$) was comparable to that of temperate estuaries elsewhere in the world. It differed, however, from other areas in that a primary source of nitrogen was from decayed alder litter, the result of extensive logging that replaced the original mixed species forest with pioneer nitrogen-fixing species in river riparian zones. This data set showed no temporal or seasonal pattern due to a continuous nitrate supply and a temperate climate in which denitrification progresses throughout the year.

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Figure 1.
Locations where samples for denitrification were collected at Idaho Flat (IF), Parker Slough (PA), Oregon Oyster (OY), Johnson Slough (JS), Boone Slough (BS), Neale Slough (NS), Toledo dock (TD) and Elk City (EC).

Figure 2. Yaquina water column nutrients concentrations at Parker Slough (PA), Oregon Oyster (OY), Johnson Slough (JS), Boone Slough (BS), Neale Slough (NS) and Toledo dock (TD).

Figure 3.
Total organic carbon (TOC) and nitrogen (TON) concentrations in suspended and benthic sediments

Figure 4. Total organic carbon and nitrogen isotopic values in Yaquina suspended and benthic sediments

Figure 5.
Denitrification rates 2003-2004 at Parker Slough (PA), Oregon Oyster (OY), Johnson Slough (JS), Boone Slough (BS), Neale Slough (NS) and Toledo dock (TD).

Figure 6.
Denitrification activity relative to depth at Johnson Slough (JS) April 2005.

Figure 7.
Denitrification rates relative to benthic sediment organic carbon content ($r^2 = 0.84$)

Table 1.

Yaquina Watershed and estuary characteristics

Drainage area at river mouth	655 km ²
Estuary subtidal area ^a	5.28 km ²
Idaho Flat intertidal	1.64 km ²
River export ^b moles N	16.5 ×10 ⁶

^a one to six meters depth

^b Flow weighted nitrogen load at Elk City 8/2003 through 8/2004.

Table 2.**Yaquina Estuary Sediment Characteristics**

Sample Station	Sediment Type	Organic C %	Organic N %	C/N	Notes
1. Elk City (EC)	Clay/Silt	2.4 ± 0.9	0.161 ± 0.13	17.4	Fresh water
2. Toledo Dock (TD)	Clay/Silt	2.1 ± 1.1	0.188 ± 0.09	13.1	<i>a, b</i>
3. Neale Slough (NS)	Clay/Silt	1.89 ± 1.1	0.4 ± 0.15	5.5	<i>a, b</i>
4. Boone Slough (BS)	Clay/Silt	3.1 ± 1.2	0.254 ± 0.12	14.3	<i>a, b</i>
5. Johnson Slough (JS)	Clay/Silt	3.7 ± 1.2	0.278 ± 0.11	15.5	<i>a, b</i>
6. Oregon Oyster (OY)	Clay/Silt	2.2 ± 1.2	0.161 ± 0.15	15.9	<i>a, b</i>
7. Parker Slough (PS)	Clay/Silt	3.2 ± 1.2	0.199 ± 0.12	18.8	<i>a, b</i>
8. Idaho Flat (IF)	Sand	0.28 ± 0.12	< 0.1	2.8	Marine, <i>c</i>

a. Eel grass

b. Eel grass with epiphytes June, July and August

c. Benthic diatoms, mud shrimp, ghost shrimp, macro algae (*Ulva spp.*)

Table 3.

Yaquina Estuary Denitrification Rates

Sample Station	Denitrification Rate (mmol N m ⁻² d ⁻¹)	Denitrification (mmol N m ⁻² yr ⁻¹)
1. Elk City (EC)	0.745 ± 0.4	
2. Toledo Dock (TD)	0.676 ± 0.175	
3. Neale Slough (NS)	0.502 ± 0.09	
4. Boone Slough (BS)	0.655 ± 0.175	
5. Johnson Slough (JS)	0.687 ± 0.13	
6. Oregon Oyster (OY)	0.591 ± 0.156	
7. Parker Slough (PS)	0.633 ± 0.12	
8. Idaho Flat (IF)	0.181 ± 0.114	0.113 × 10 ⁶
Estuarine average	0.626 ± 0.141	1.21 × 10 ⁶