Effects of upwelling on short-term variability in microbial and biogeochemical processes in estuarine sediments from Elkhorn Slough, California, USA

Jane M. Caffrey[1],*, James T. Hollibaugh[2], Nasreen Bano[2], John Haskins[3]

[1]Center for Environmental Diagnostics and Bioremediation, University of West Florida, Pensacola, Florida 32514, USA
[2]Department of Marine Sciences, University of Georgia, Athens, Georgia 30602-3636, USA
[3]Elkhorn Slough National Estuarine Research Reserve, 1700 Elkhorn Road, Watsonville, California 95076, USA

ABSTRACT: We examined short-term variability in sediment microbial processes and biogeochemistry in response to decreased irradiance accompanying an upwelling event. Potential nitrification, extractable nutrients, solid phase iron and pore water sulfide concentrations were measured several times at 3 locations in Elkhorn Slough, California, USA. Benthic fluxes of nitrate+nitrite, ammonium, phosphate, sediment oxygen consumption and denitrification from N_2/Ar flux were also measured. Fog that was associated with upwelling in Monterey Bay reduced photosynthetically active radiation in Elkhorn Slough, which led to increased duration of water column hypoxia. Solid phase Fe^{3+} concentrations and potential nitrification rates declined while pore water sulfide increased over this period. While potential nitrification rates from this study are lower than rates from estuaries that do not experience hypoxia or anoxia, nitrifiers in Elkhorn Slough appear to be capable of adjusting to changing environmental conditions, specifically of tolerating low levels of sulfide. The nitrifying assemblage was similar to the community of ammonia-oxidizing bacteria and ammonia-oxidizing archaea that was previously reported from this system. Eutrophic shallow estuaries like Elkhorn Slough are delicately poised such that their sediment biogeochemistry responds quickly to relatively small changes in the light environment.

KEY WORDS: Nitrogen · Oxygen dynamics · Nitrification · Ammonia-oxidizing bacteria · Benthic flux

INTRODUCTION

Microbial processes regulate the availability of nutrients in the estuarine water column by mediating the balance among inputs, recycling and removal to sediments. Nutrient sources to estuaries vary, with inputs associated with freshwater flow (Peierls et al. 1991), atmospheric deposition (Paerl et al. 2006), submarine groundwater discharge (Santos et al. 2008), and nitrogen fixation (Gardner et al. 2006, Fulweiler et al. 2007). Greater recycling of nitrogen by nitrogen remineralization or the predominance of dissimilatory nitrate reduction to ammonium over removal of fixed

nitrogen via denitrification can lead to a positive feedback at the system level, enhancing eutrophication in estuaries (Kemp et al. 1990). Nitrification, which is the oxidation of ammonium to nitrite and then nitrite to nitrate, can either link remineralization to denitrification, leading to nitrogen removal, or result in nitrate fluxes from sediments to the water column. Thus, nitrification has a central role in regulating benthic nitrogen fluxes. Ammonia-oxidizing and nitrite-oxidizing bacteria have been the focus of most research on nitrification in marine and estuarine environments. However, recent research suggests that some Crenarchaeota may also be responsible for nitrification in

these environments (Francis et al. 2005, Konneke et al. 2005, Treusch et al. 2005). Nitrification rates have been correlated with the abundance of ammonia-oxidizing archaea (AOA) in coastal waters (Wuchter et al. 2006, Beman et al. 2008). In a comparison of 6 different estuaries, the best factors predicting potential nitrification rates were sediment chl *a*, salinity, bottom water dissolved oxygen (DO) and abundance of AOA (Caffrey et al. 2007a).

The seasonal variation of benthic microbial processes has been thoroughly studied, with temperature often controlling sediment processes in temperate estuaries (Cowan et al. 1996). Other important factors include seasonality of freshwater flows and nutrient loading, organic matter deposition from spring phytoplankton blooms, and bottom water DO concentrations. Sediment denitrification rates are often enhanced following pulses of nitrate enriched river water (Nedwell & Trimmer 1996, Caffrey et al. 2003). Nutrients also stimulate phytoplankton and macroalgal production that is subsequently deposited in sediments, leading to increased benthic respiration, nutrient regeneration (Jensen et al. 1990, Vidal et al. 1997, Grenz et al. 2000) and hypoxia (DO < 2 mg l^{-1}) or anoxia (DO = 0 mg l^{-1}; Viaroli & Christian 2003).

Less is known about daily variation in benthic microbial processes. In estuarine sediments with a water column depth that is >20 m, daily variation in benthic fluxes (Nakamura 2003) and denitrification rates (Hietanen & Kuparinen 2008) is minimal. However, in shallow subtidal or intertidal sediments, variations in water level, temperature and light affect microphytobenthos productivity (Miles & Sundbäck 2000), which in turn affect microbial processes and benthic nutrient fluxes (Feuillet-Girard et al. 1997, Porubsky et al. 2009).

In isolated eutrophic lagoons and ponds, diel changes in primary production can lead to shifts in DO concentrations from oxic to anoxic (Krause-Jensen et al. 1996, Viaroli & Christian 2003), along with changes in the speciation and redox state of reactive elements and compounds (Beck & Bruland 2000). In addition to being dependent on oxygen as an electron acceptor, nitrification and other aerobic microbial processes are also influenced by reactive iron, which can provide protection from inhibition by sulfide (Dollhopf et al. 2005). Anoxia and hypoxia occur in many estuarine and marine environments (Diaz & Rosenburg 2008). How does the microbial community, particularly nitrifiers, respond to intermittent periods of hypoxia? We hypothesize that extended periods of hypoxia will reduce the activity of nitrifiers in estuarine sediments. This study examines sediment biogeochemistry over a 7 d period before and during hypoxic events in Elkhorn Slough, CA.

MATERIALS AND METHODS

Study area. Elkhorn Slough is a small estuary in central California that connects an intensively farmed watershed with Monterey Bay. Native grasses border the lower slopes of Upper Azevedo Pond (subsequently referred to as Azevedo Pond), while the upper portions of this subwatershed are farmed for strawberries. Both North Marsh and South Marsh sites are in the Elkhorn Slough National Estuarine Research Reserve (NERR). Land use in the upper portions of North and South Marsh subwatersheds beyond the Reserve boundary is primarily residential and agricultural.

Tides in Elkhorn Slough are mixed semidiurnal with 2 unequal high and low tides every 25 h. A railroad bridge restricts tidal exchange between Azevedo Pond and the main channel of Elkhorn Slough. North Marsh has little exchange with the main channel because the tide gate is set so that exchange only occurs on high spring tides. South Marsh has regular tidal exchange with the main channel, although flow is slightly restricted by a railroad bridge.

Nutrient loading to Elkhorn Slough is dominated by runoff during the winter rainy season and by upwelling during the dry season (Chapin et al. 2004, Caffrey et al. 2007b). Pulses of high nitrate following rain events enhance primary production in the upper Slough and in areas with restricted circulation (Caffrey et al. 2007b). Previous studies have shown that sites that are influenced by agricultural runoff have greater recycling of nutrients and decreased removal processes, such as denitrification, than sites in the Reserve (Caffrey et al. 2003).

Sampling design. Water column and sediment samples were collected at or just following low tide (lower low water) from 3 sites: Azevedo Pond (36° 50' 44.64″ N, 121° 45' 13.24″ W), North Marsh (36° 50' 04.75″ N, 121° 44' 18.33″ W), and South Marsh (36° 49' 05.00″ N, 121° 44' 21.83″ W) (Fig. 1). Water depth at each site during lower low water was ~0.5 m. Each site was sampled 3× for potential nitrification rates, extractable nutrients, solid phase Fe, and pore water sulfide. In addition, benthic fluxes were measured once at Azevedo Pond on July 13, 2005 and at South Marsh on July 14, 2005. The sampling sites were adjacent to the NERR System-Wide Monitoring Program water quality monitoring sites where temperature, salinity, DO, pH, turbidity and water level are measured at 0.5 h intervals. Water samples were GF/F filtered for the analysis of nitrate+nitrite ($NO_3^- + NO_2^-$), ammonium (NH_4^+), and dissolved inorganic phosphorus (DIP), and the filters were analyzed for chl *a*.

Sediment analyses. Nine sediment cores (corer i.d. 4.6 cm) were collected for potential nitrification, extractable NH_4^+, $NO_3^- + NO_2^-$, DIP, solid phase Fe^{2+},
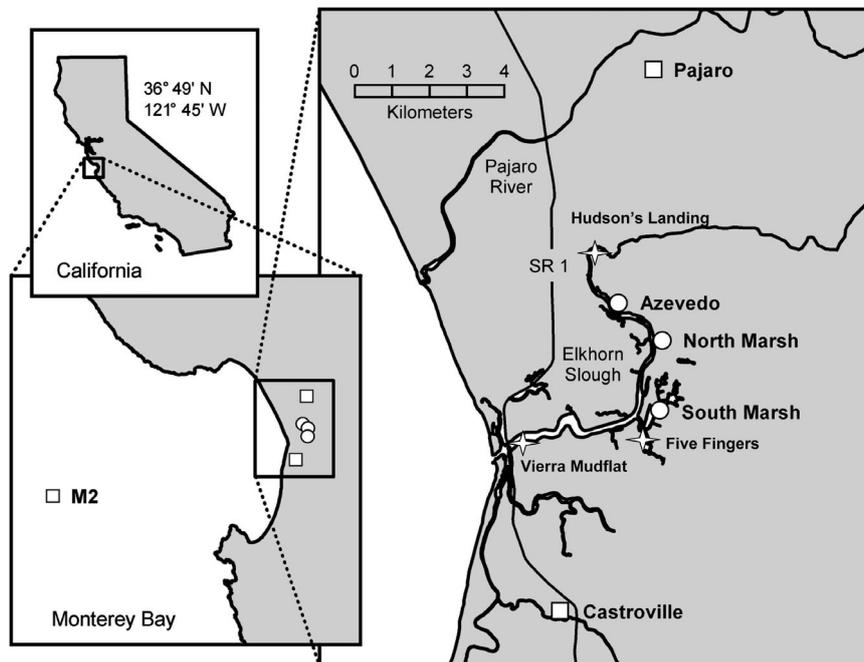
Fig. 1. Locations of (○) sampling sites in Elkhorn Slough, California, USA, (stars) benthic flux sampling sites from previous studies, and (□) CIMIS (California Irrigation Management Information System) climate sites and the MBARI (Monterey Bay Aquarium Research Institute) M2 buoy

total Fe, pore water sulfide, sediment chl *a* concentration, water and organic carbon content. Sediments from 3 cores were sectioned into 0–0.5, 0.5–1, 1–2 and 2–4 cm layers for the measurement of extractable nutrients. A second set of triplicate cores was sectioned into 0–0.5, 0.5–1, 1–2 and 2–4 cm layers for the determination of pore water and organic carbon content. The top 0–0.5 cm layer was also used for potential nitrification rate measurements, sediment chl *a* analyses and nitrifier assemblage composition. Duplicate samples for potential nitrification were taken from the top layer to give 6 replicates. The third set of triplicate cores that were used for Fe and sulfide analyses was sectioned into individual 0–1, 1–2 and 2–4 cm layers in a nitrogen-filled glove bag. Sediment sections were placed in centrifuge tubes and spun for 15 min at 3000 rpm. Overlying water was removed for sulfide analyses and sediment was retained for Fe analysis.

Potential nitrification rate was measured by incubating 1 g of sediment with 50 ml of GF/F filtered bottom water and ammonium chloride (500 µM final concentration) (Henriksen et al. 1981) at room temperature (~22°C) in the dark on a shaker table. Initial (0 h) and final (24 h) samples were collected and GF/F filtered into sample vials and frozen immediately. Samples were analyzed for $NO_3^- + NO_2^-$ and NO_2^-. Extractable nutrient concentrations were determined by adding 1 M NaCl (10 ml [10 g wet sediment]$^{-1}$).

Triplicate cores (corer i.d. 8 cm) were collected at Azevedo Pond and South Marsh sites for use in measuring benthic fluxes. In the laboratory, overlying water was replaced with fresh bottom water that was gently siphoned over the cores to minimize resuspension of sediments. An additional tube containing only water was incubated as a control. Magnetically driven stir bars were used to mix the water at speeds below the resuspension threshold. Cores were incubated in the dark at *in situ* temperatures for 5 h, with samples being removed hourly (with replacement of bottom water) for the determination of the N_2/Ar ratio, DO, NH_4^+, $NO_3^- + NO_2^-$ and DIP concentrations.

Analytical techniques. NH_4^+ and DIP were analyzed on a flow injection autoanalyzer (Lachat Quickchem Series 8500) using the Quikchem methods 31-107-06-1-B (Liao 2003) for NH_4^+ and 31-115-01-1-I (Ammerman 2003) for DIP. $NO_3^- + NO_2^-$ and NO_2^- were analyzed on the same flow injection autoanalyzer using Quikchem method 31-107-04-1-A (Diamond 1997). Total sulfide in pore water was analyzed using a Ag/AgCl electrode. Following the removal of pore water for sulfide analysis, sediments were extracted with 0.5 N HCl for the analysis of solid phase iron concentrations (Kostka et al. 2002). Ferrozine was used to measure Fe^{2+}, hydroxylamine was used to reduce total Fe to Fe^{2+}, and Fe^{3+} was calculated by difference. Sediment chlorophyll samples were extracted in 10 ml of

90% acetone, sonicated and read after 24 h on a fluorometer (Turner Designs) (Welschmeyer 1994). The N_2/Ar ratio in water samples that were preserved with 1% $ZnCl_2$ was analyzed on a membrane inlet mass spectrometer (Kana et al. 1994) at the University of Georgia (S. Joye Laboratory). We used the Winkler method to measure DO concentrations (Parsons et al. 1984). Sediment samples were dried at 80°C for 24 h to determine water content. Organic content was determined on dried sediments by loss on ignition after combustion at 500°C for 1 h. Sediment grain size was determined using the method of Folk (1974), although we only report percent sand.

Nitrifier assemblage composition. We examined the composition of the ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) assemblages in surficial (0–0.5 cm) sediments from the first sampling period. DNA was extracted from sediment samples using a soil DNA kit (UltraClean, MoBio) according to the manufacturer's recommendations. AOA *amoA* genes (635 bp fragment) were amplified using the primers Arch-*amoA*F (5′-STAATGGTCTGGCTTA-GACG-3′) and Arch-*amoA*R (5′-GCGGCCATCCATC TGTATGT-3′) (Francis et al. 2005). AOB 16S rRNA genes (1.1 kb) were amplified with the nitA and nitB primer set (Voytek & Ward 1995). PCR products of appropriate size were recovered from an agarose gel using a gel extraction kit (QiaSpin, Qiagen), and cloned with the TOPO-TA cloning kit (Invitrogen). White colonies were selected randomly and grown in freezing medium in 96-well deep plates (Maniatis et al. 1982). Plasmids were extracted and sequenced at a commercial facility (SeqWright; DNA Technology Services) using the M13F and M13R vector primer. Neighbor-joining phylogenetic trees were constructed from the sequences using Jukes-Cantor distances. Bootstrap analysis was used to estimate the reliability of phylogenetic reconstructions (100 replicates).

Data sources and analysis. We obtained solar radiation and air temperature data for 2 sites (Fig. 1), Castroville and Pajaro, from the California Irrigation Management Information System (CIMIS; www.cimis.water.ca.gov/ cimis/welcome.jsp). Elkhorn Slough NERR collects weather data, including photosynthetically active radiation (PAR), at the South Marsh site. Water temperature data from Monterey Bay at the M2 buoy (36.7° N, 122.39° W) were obtained from the Monterey Bay Aquarium Research Institute (www.mbari.org) (Fig. 1).

ANOVA was performed on potential nitrification, sediment chlorophyll, pore water nutrient and sulfide concentrations and solid phase iron concentrations. For potential nitrification and sediment chlorophyll data, a 2-factor ANOVA testing the effects of site, time and the interaction term was conducted. A 3-factor ANOVA testing the effects of site, time, depth and interaction terms was conducted for the other variables. *Post hoc* least significant difference (LSD) tests were used to determine significant differences in treatment means. Correlation analysis was used to examine relationships among environmental variables. *t*-tests were used to test for differences in benthic fluxes between Azevedo Pond and South Marsh. Data were normalized by log transformation before analysis when necessary. A principal components analysis (PCA) included PAR, water column characteristics (salinity, temperature, overlying water DO and $NO_3^- + NO_2^-$ concentrations) and sediment characteristics (chl *a*, pore water sulfide, extractable NH_4^+ and P).

RESULTS AND DISCUSSION

Physical setting

Elkhorn Slough was affected by upwelling that occurred in Monterey Bay between July 11 and 22, 2005. Upwelling of deep water reduced surface water temperatures in Monterey Bay by 3°C (Fig. 2). The July 12–15 period of most intense upwelling was accompanied by the formation of fog along the coast, lower air temperatures and reduced solar radiation (Fig. 2). Reduction in solar radiation was greatest closest to the mouth of the Slough near the Castroville site and less inland at the Pajaro site (Fig. 2). The average daily water temperatures at Azevedo Pond and South Marsh also declined by ~2°C during this period, while average daily temperature at North Marsh showed little change (Table 1). DO concentrations were highest during mid-day and lowest at night in all 3 sites (Fig. 3). Nighttime DO declined over the sampling period as upwelling intensified, particularly at Azevedo and South Marsh, leading to longer periods of hypoxia (DO < 2 mg l^{-1}; Table 1). Daily integrated PAR was significantly negatively correlated with the duration of hypoxia (r = –0.69, p = 0.04).

Water column characteristics

Azevedo Pond had the greatest salinity range, with values ranging from 31.6 to 40. Salinity ranges in North Marsh and South Marsh were 31.9 to 37.9 and 32.4 to 34.2, respectively. Low values occurred at higher high water in all sites, reflecting the incursion of Monterey Bay water (data not shown), while high values occurred because of evaporation (Largier et al. 1997). Water column nutrients at the sites ranged from 0.6 to 7.1 µM $NO_3^- + NO_2^-$, <0.5 to 6.4 µM NH_4^+ and between 1.3 and 8.1 µM DIP (Table 1).

Sediment characteristics

The North and South Marsh sites were characterized by fine grain sediments (12% or less sand), a high water content (~70%) and an organic content between 9 and 10% (Table 2). In contrast, Azevedo Pond had sandy sediments (97%), a low water content (21%) and low organic matter content (0.7%) (Table 2). Sediment chl *a* ranged from 9.3 to 28.7 µg cm^{-2} (Table 1). The ANOVA revealed that both sampling site and date were significant factors (ANOVA p < 0.001), with the *post hoc* LSD test showing significantly lower concentrations at the South Marsh site than at Azevedo Pond or North Marsh.

Nutrient concentrations that were measured in sediment extracts varied among sites and with date and depth in the sediment (Fig. 4). Extractable NH_4^+ and
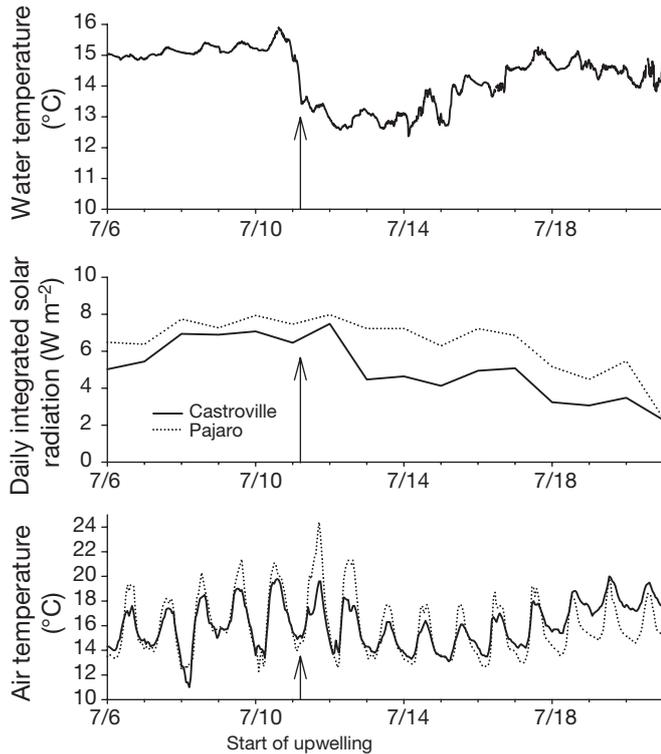

Fig. 2. Surface water temperature (°C) from Monterey Bay M2 buoy, daily integrated solar radiation (W m^{-2}) and hourly air temperature (°C) at Castroville and Pajaro. Arrow: start of upwelling. Dates are mo/d
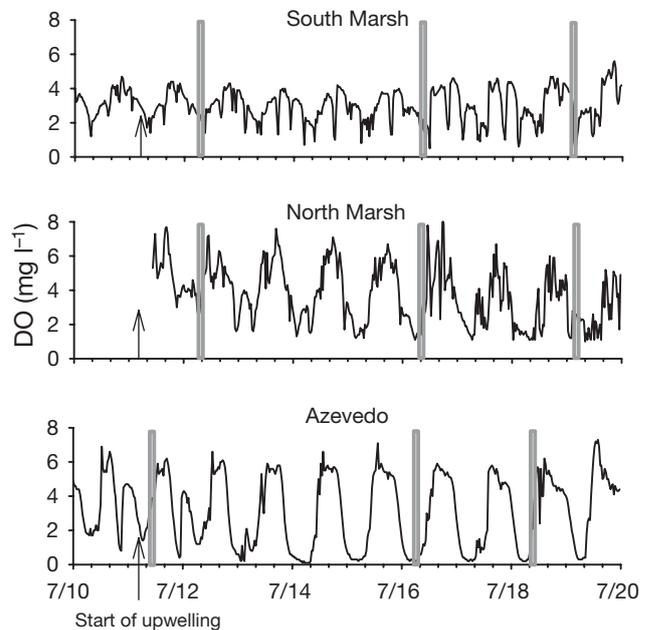

Fig. 3. Dissolved oxygen (DO; mg l^{-1}) in South Marsh, North Marsh and Azevedo Pond. Arrow: start of upwelling. Vertical boxes: time of sampling. Dates are mo/d

Table 1. Duration of water column hypoxia ($[O_2]$ < 2 mg l^{-1}) during the 24 h immediately prior to sampling. Temperature, nutrient concentration, and sediment chl *a* concentrations on each sampling date are also shown. Upwelling began on July 11, 2005. Dates are mo/d

Site/sampling date	Hypoxia duration (h)	Temperature (°C)	$NO_3^- + NO_2^-$ (µM)	NH_4^+ (µM)	DIP (µM)	Sediment chl *a* (µg cm^{-2})
Azevedo Pond						
7/11	4	22.2	0.9	6.0	4.1	28.7 ± 4.1
7/16	10.5	20.8	0.6	3.2	8.1	26.8 ± 2.7
7/18	11	20.7	2.2	<0.5	1.3	24.3 ± 3.9
North Marsh						
7/12	0	22.7	1.0	2.5	2.3	28.1 ± 3.1
7/16	4	22.4	0.6	6.4	3.1	26.7 ± 3.4
7/19	7	20.0	0.6	0.8	1.9	11.3 ± 0.2
South Marsh						
7/12	1	20.9	2.0	3.9	2.2	11.3 ± 0.7
7/16	3.5	19.7	1.4	6.0	3.4	9.3 ± 0.6
7/19	4.5	18.1	7.1	3.8	2.3	13.5 ± 1.6

DIP concentrations were significantly correlated (r = 0.77, p = 0.02). Site, date, depth, site × date and site × depth were all significant terms (ANOVA p < 0.001) for NH_4^+, while site, site × date and site × depth were the significant terms for DIP. Both nutrients had significantly higher concentrations in Azevedo Pond and at North Marsh than at South Marsh. Both nutrients sig-

nificantly increased with depth at the North Marsh site, where NH_4^+ concentration in the 2–4 cm depth interval exceeded 600 nmol cm^{-3} and DIP concentrations exceeded 60 nmol cm^{-3} (Fig. 4). Site and depth were significant terms (ANOVA p < 0.001) for NO_3^- + NO_2^-, with significantly higher concentrations above 1 cm than below it. Concentrations were highest at Azevedo, intermediate at South Marsh and lowest at North Marsh.

Solid phase Fe^{2+} and Fe^{3+} concentrations generally showed an inverse depth distribution, with higher Fe^{3+} in the 0–1 cm layer compared to deeper layers (1–4 cm) and higher Fe^{2+} in the 2–4 cm layer compared to the 0–1 cm layer (Fig. 5). Site, date and depth were significant terms for Fe^{3+} (ANOVA p < 0.001). Fe^{3+} was significantly higher at the South Marsh site than at North Marsh or Azevedo Pond, the latter 2 sites being
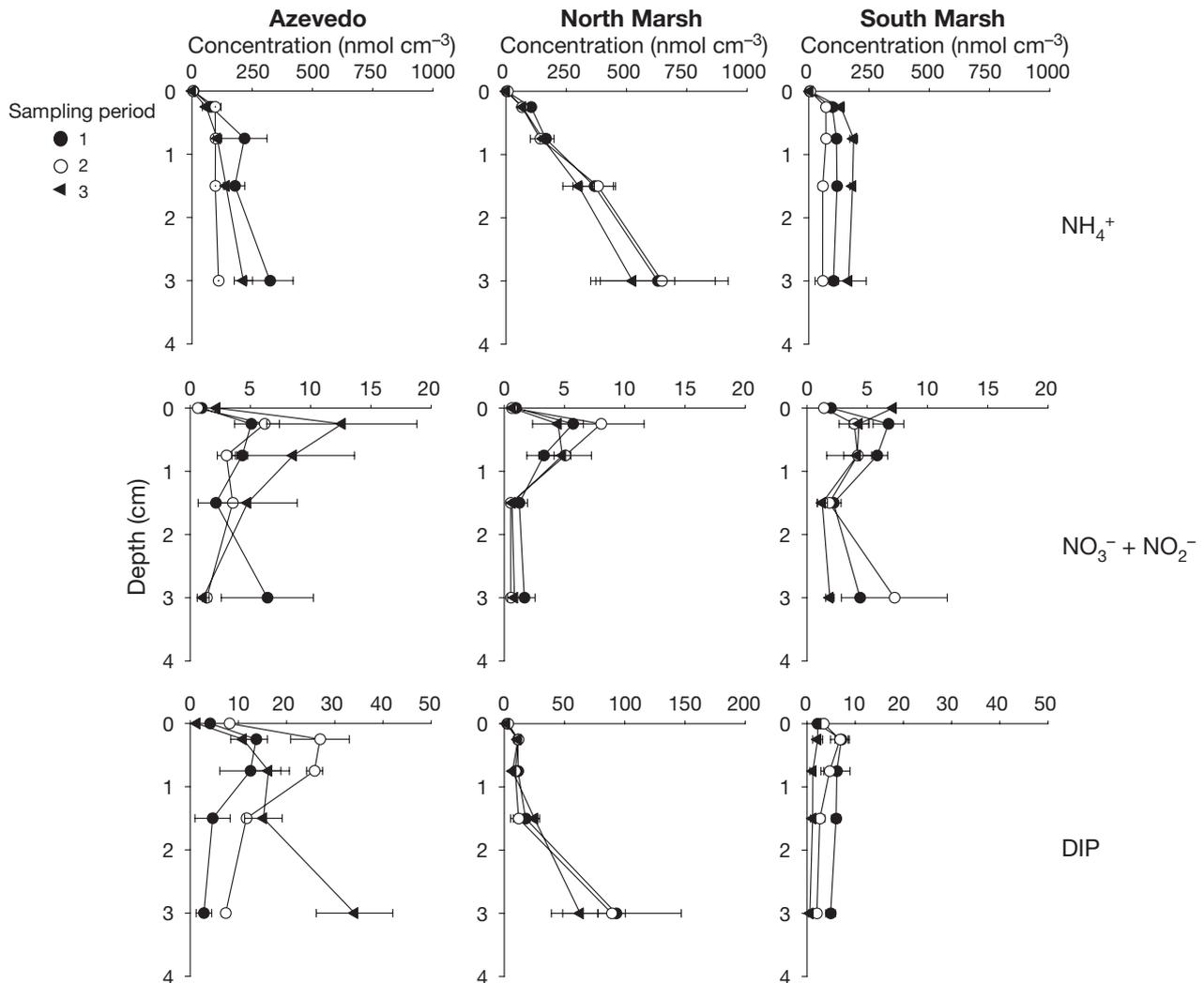
Table 2. Sediment characteristics: percent sand, water, and organic matter content at 0–1 cm depth from the 3 sites. Mean ± SE for water and organic matter content

Site	% Sand	% Water	% Organic matter
Azevedo Pond	97	21 ± 0.4	0.8 ± 0.1
North Marsh	12	74 ± 2.4	10.2 ± 1.8
South Marsh	2	68 ± 1.4	9.1 ± 0.2

Fig. 4. Extractable NH_4^+, NO_3^-+NO_2^- and DIP concentrations (means ± SE) in Elkhorn Slough sediments. Overlying water concentrations are plotted at 0 cm

more hypoxic than South Marsh (Table 1). Fe^{3+} was significantly lower on the third sampling date after upwelling was well established, compared to the first 2 sampling dates. Site, date, depth and site × day were all significant terms for Fe^{2+} (ANOVA $p < 0.001$). Solid phase Fe^{2+} concentrations were highest at North Marsh, intermediate at South Marsh and lowest at Azevedo Pond (Fig. 5). Site, date, and all interaction terms were significant for sulfide (ANOVA $p < 0.001$). Pore water sulfide concentrations were highest at North Marsh, intermediate at Azevedo Pond and lowest at South Marsh (Fig. 5). Pore water sulfide was negatively correlated with PAR ($r = -0.69$, $p = 0.04$) and significantly increased over the sampling period as upwelling intensified.

The first 3 components of the PCA explained 77% of the variation in the samples. The sites were separated along the first component, which explained 38% of the variation. This axis was dominated by salinity, sediment chl *a*, sediment water content and extractable DIP. Samples from the Azevedo Pond site were characterized by high salinity, sediment chl *a* and extractable P and lower water content than samples from South Marsh, while North Marsh samples were intermediate. The second axis, which explained 26% of the variation, was dominated by PAR, temperature, DO and pore water sulfide and showed how conditions in the Slough changed from a more oxidized to a less oxidized water column and sediment over the period of upwelling. The third axis, which explained 13% of the variation, was dominated by overlying water $NO_3^- + NO_2^-$ concentration, extractable NH_4^+, and solid phase Fe^{3+}.

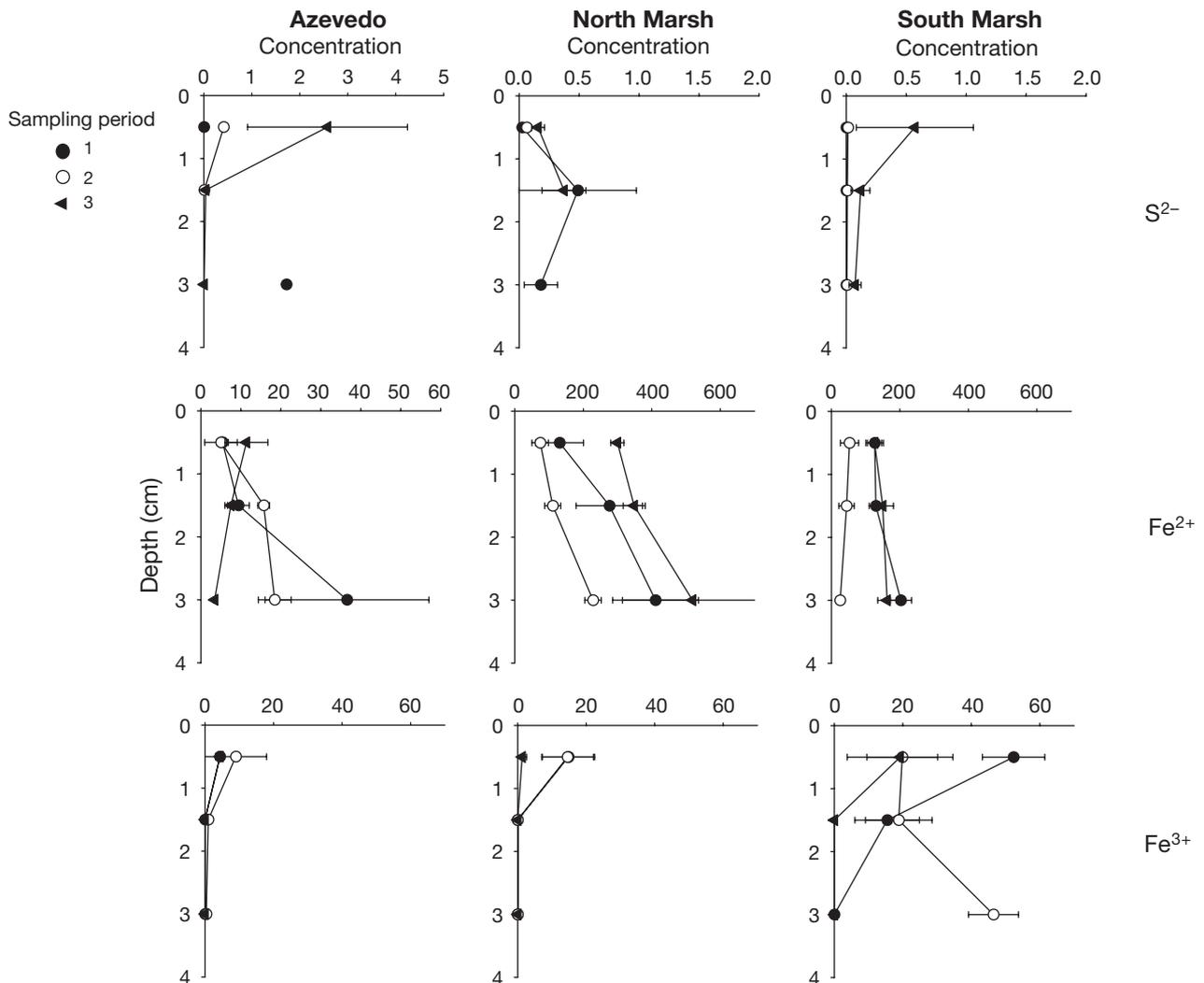Fig. 5. Solid phase Fe^{2+} and Fe^{3+} (means ± SE, μmol g^{-1} dry wt) and pore water S^{2-} concentrations (mM) in sediments from Elkhorn Slough study sites
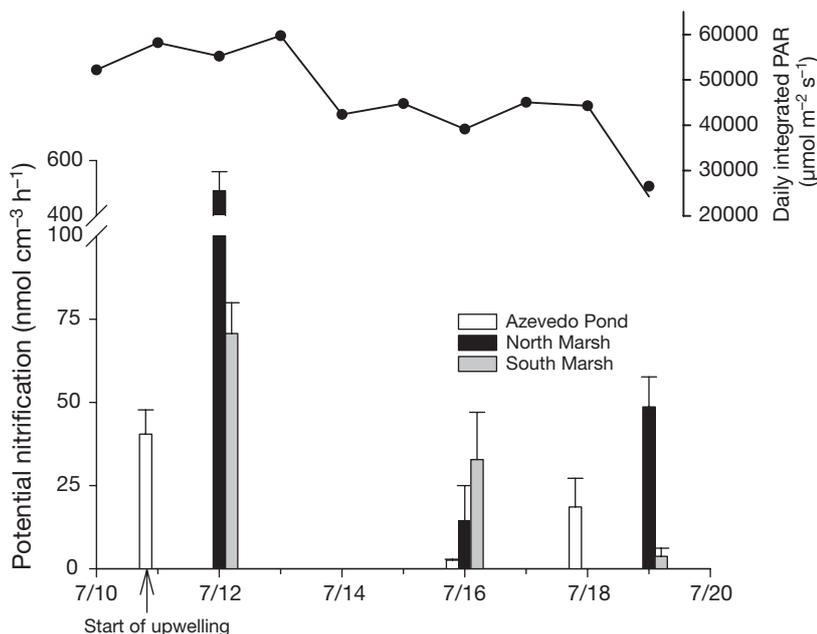
Fig. 6. Daily integrated photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) and potential nitrification rates (means ± SE, nmol cm^{-3} h^{-1}). Arrow: start of upwelling. Dates are m/dd

Microbial processes

Potential nitrification rates ranged from 2.6 to 490 nmol cm^{-3} h^{-1}, with the highest rates being in North Marsh during the initial sampling just when upwelling began to affect the Slough (Fig. 6). Rates were significantly different among the 3 sites, with the highest rates being at North Marsh and the lowest being at Azevedo Pond (ANOVA p < 0.001). Potential nitrification was lowest at both Azevedo Pond and North Marsh on July 16, 2005, and increased slightly several days later, while rates at South Marsh declined throughout the 8 d period (Fig. 6). Potential nitrification was negatively correlated with PCA component 2 (Fig. 7; r = –0.73, p < 0.02), suggesting that nitrifiers were less active at low DO and high pore water sulfide.

Sediment oxygen consumption, denitrification and benthic nutrient fluxes were similar between Azevedo and South Marsh sites (Table 3). Sediment oxygen consumption was between 28 and 29 mmol m^{-2} d^{-1}. NO$_3^-$+NO$_2^-$ fluxes were negative, indicating uptake by sediments, while NH$_4^+$ fluxes were positive. DIP fluxes in Azevedo were not significantly different from 0, while fluxes in South Marsh were negative (net uptake by the sediment). The differences between the 2 sites were not significantly different for any of the flux measurements (*t*-test p > 0.20). Diffusive NH$_4^+$ fluxes that were calculated based on pore water gradients were similar to fluxes measured in core incubations (data not shown). Site was a significant term (ANOVA p = 0.01),

with significantly higher diffusive NH$_4^+$ fluxes in North Marsh than in South Marsh or Azevedo. Denitrification rates were 2.0 and 5.7 mmol m^{-2} d^{-1} in South Marsh and Azevedo, respectively, but were not significantly different (*t*-test p > 0.20).

Upwelling effects on microbial processes

Coastal upwelling has a significant impact on estuaries. Upwelling water with high NO$_3^-$+NO$_2^-$ concentrations can be the dominant source of nutrients to Pacific coast estuaries during the summer dry season when river inputs are minimal (Colbert & McManus 2003, Hickey & Banas 2003, Chapin et al. 2004). Upwelling and transport of organic carbon from offshore can also fuel respiration within estuaries (Smith & Hollibaugh 1997). In Elkhorn Slough, we observed decreasing DO concentrations, which we hypothesize to be a community-level response by the microphytobenthos to lower irradiance during a period of summer upwelling. High chl *a* concentrations in surface sediments indicate the presence of an active microphytobenthic community that is capable of enhancing DO levels in surficial sediments. Thick mats of *Ulva lactuca* and *Enteromorpha intestinalis* are also common on Elkhorn Slough mudflats (Zimmerman & Caffrey 2002). However, as PAR decreases, so do photosynthesis and the thickness of the oxic zone in sediments (Krause-Jensen et al. 1996, Porubsky et al. 2009). Declines in PAR during this upwelling period in Elkhorn Slough led to increased
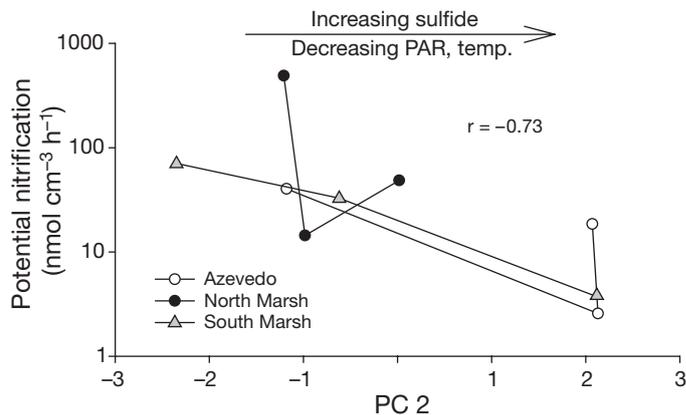

Fig. 7. Potential nitrification rates (nmol cm^{-3} h^{-1}) versus PC axis 2. PAR: photosynthetically active radiation

Table 3. Sediment oxygen consumption (SOC), denitrification and benthic fluxes of $NO_3^-+NO_2^-$, NH_4^+ and DIP (means ± SE) in mmol m^{-2} d^{-1} at Azevedo Pond and South Marsh from this study and previous dry season measurements in Elkhorn Slough. Negative values are fluxes into the sediments. nd: not determined

Site	Month	SOC	Denitrification	$NO_3^-+NO_2^-$	NH_4^+	DIP	Source
Azevedo Pond	7/2005	28.1 ± 5.36	5.74 ± 2.52	−1.63 ± 0.31	2.10 ± 1.58	0.39 ± 0.60	This study
South Marsh	7/2005	29.1 ± 6.63	1.96 ± 0.63	−1.22 ± 0.46	2.52 ± 1.52	−0.25 ± 0.11	This study
Azevedo Pond	9/1994	73 ± 15	nd	−1.1 ± 0.3	4.8 ± 1.1	−1.0 ± 0.3	Caffrey (2002)
Five Fingers	9/1994	24 ± 4	nd	1.1 ± 0.1	1.0 ± 0.5	−0.8 ± 0.3	Caffrey (2002)
Whistle Stop	9/1994	51 ± 6	nd	0.5 ± 0.2	2.1 ± 0.3	0.1 ± 0.3	Caffrey (2002)
Hudson's Landing	7/1999	49.5 ± 29.6	nd	4.30 ± 16.01	0.1 ± 4.1	−5.55 ± 2.75	Caffrey et al. (2002)
Vierra Mudflat	7/1999	7.3	nd	−0.55 ± 1.0	1.4 ± 1.3	−0.11 ± 0.1	Caffrey et al. (2002)

hypoxia in the water column, a decline in aerobic sediment processes such as nitrification, and reduced levels of oxidized constituents such as solid phase Fe^{3+} at the same time that pore water sulfide concentrations were increasing. This decline in solid phase Fe^{3+} concentrations could be the result of enhanced iron and sulfate reduction or decreased iron oxidation in the surface layer or both. These changes were more apparent at the South Marsh site, which is closer to the coast than more inland sites like Azevedo Pond.

Solid phase Fe^{3+} may reduce the inhibitory effects of sulfide on nitrification by binding sulfide and keeping it from building up in pore water (Dollhopf et al. 2005). Dollhopf et al. (2005) found that an Fe^{3+} inventory of ~2000 mmol m^{-2}, which is ~3× higher than the highest Fe^{3+} inventory in Elkhorn Slough, protected nitrifiers from inhibition by sulfide. Despite relatively low Fe^{3+} concentrations and the appearance of sulfide in surficial sediments, nitrification was not completely inhibited in Elkhorn Slough. While the potential nitrification rates we measured were lower than rates in sediments that do not become hypoxic or anoxic (Caffrey et al. 2003), the Elkhorn Slough nitrifying community appears to be capable of adjusting to changing environmental conditions, specifically of tolerating low levels of sulfide. Laboratory experiments have shown that the activity of nitrifiers in sediments is inhibited at sulfide concentrations between 0.1 (Joye & Hollibaugh 1995) and 1 mmol l^{-1} (Dollhopf et al. 2005) when Fe^{3+} concentrations are low.

One possibility is that different members of the nitrifying community may be responsible for nitrification under different sets of environmental conditions. The AOA sequences that were isolated in this study were similar to other observations in Elkhorn Slough reported by Francis et al. (2005). In contrast to previous studies in Elkhorn Slough, the AOB sequences that were recovered during this study were more distantly related to the previously found *Nitrosomonas* or *Nitrosospira* sequences (Caffrey et al. 2003), but were similar to sequences found in other coastal environments. The South Marsh clones were most similar to

Nitrosospira, while North Marsh clones were most similar to *Nitrosomonas* sp. Nm 143.

While we do not know which of these groups was responsible for nitrification at the different sites, quantitative PCR from these sites showed that AOA *amoA* genes were 13× more abundant than AOB *amoA* genes at North Marsh (Caffrey et al. 2007a), which is the site with the highest potential nitrification rates. The abundance of the 2 groups was comparable at South Marsh (1.2 AOB:1 AOA), while AOB *amoA* were 5× more abundant than AOA *amoA* at Azevedo Pond (Caffrey et al. 2007a), which had the lowest potential nitrification rates of the 3 sites. This suggests that different groups might have been responsible for nitrification as environmental conditions changed during this upwelling event. High abundances of AOB have been associated with high nitrification rates in several estuaries (Cebron et al. 2003, Bernhard et al. 2007), while nitrification rates are correlated with AOA *amoA* abundance in oceanic water columns (Wuchter et al. 2006, Lam et al. 2007) and in some estuarine sediments (Caffrey et al. 2007a). While high abundances of AOA *amoA* have been measured at low DO levels (Coolen et al. 2007, Lam et al. 2007), the question remains open whether AOA can nitrify at DO concentrations as low as those at which AOB can (Sliekers et al. 2005).

Benthic fluxes that were measured in this study are in the range of previous measurements at similar Elkhorn Slough sites (Table 3; Caffrey 2002, Caffrey et al. 2002) and similar to fluxes measured in other estuarine systems (Cowan et al. 1996, Feuillet-Girard et al. 1997, Dalsgaard 2003). Benthic fluxes in shallow photic estuaries like Elkhorn Slough are controlled by redox and light conditions. $NO_3^-+NO_2^-$ fluxes were into sediments at both of our study sites, indicating that $NO_3^-+NO_2^-$ production by nitrification was low relative to NO_3^- removal by processes such as denitrification (Table 3). The direction of NO_3^- fluxes changes from out of sediments during aerobic conditions to into sediments during hypoxia (Cowan et al. 1996), presumably due to reduced nitrification, although increased demand for an alternative terminal electron acceptor

to support benthic respiration may also be a factor under hypoxic conditions. Inhibition of nitrification may also reduce denitrification rates (Kemp et al. 1990) and anammox in systems with low concentrations of NO_3^- in the overlying water. Similarly, hypoxic conditions can lead to the release of DIP from sediments as P bound to Fe oxides is released when Fe^{3+} is reduced (Koop et al. 1990, Hietanen & Lukkari 2007). NH_4^+ fluxes that were measured in intact cores and those calculated from extractable NH_4^+ concentrations were similar and showed little change over time. Given that NH_4^+ can be released during aerobic decomposition, fermentation or sulfate reduction (Canfield et al. 2005), NH_4^+ fluxes may be less sensitive to changing redox conditions than other fluxes.

This study shows how changing light conditions during an upwelling event affect microbial processes and sediment biogeochemistry in Elkhorn Slough. In shallow photic estuaries, the activity of microphytobenthos, specifically the oxidation of surface sediments, has a major impact on microbial processes and potentially on the microbial community. Small reductions in irradiance, whether from fog caused by upwelling, or from increases in turbidity due to sediment resuspension, lead to lower water column DO concentrations and reduced sediment redox potential, ultimately inhibiting aerobic microbial processes such as nitrification. As a result, eutrophic shallow estuaries like Elkhorn Slough are delicately poised such that their sediment biogeochemistry responds quickly to relatively small changes in the light environment.

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