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APPLIED ISSUES

Assessing the sources and magnitude of diurnal nitrate variability in the San Joaquin River (California) with an *in situ* optical nitrate sensor and dual nitrate isotopes

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SUMMARY

1. We investigated diurnal nitrate (NO_3^-) concentration variability in the San Joaquin River using an *in situ* optical NO_3^- sensor and discrete sampling during a 5-day summer period characterized by high algal productivity. Dual NO_3^- isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) and dissolved oxygen isotopes ($\delta^{18}O_{DO}$) were measured over 2 days to assess NO_3^- sources and biogeochemical controls over diurnal time-scales.
2. Concerted temporal patterns of dissolved oxygen (DO) concentrations and $\delta^{18}O_{DO}$ were consistent with photosynthesis, respiration and atmospheric O_2 exchange, providing evidence of diurnal biological processes independent of river discharge.
3. Surface water NO_3^- concentrations varied by up to 22% over a single diurnal cycle and up to 31% over the 5-day study, but did not reveal concerted diurnal patterns at a frequency comparable to DO concentrations. The decoupling of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ isotopes suggests that algal assimilation and denitrification are not major processes controlling diurnal NO_3^- variability in the San Joaquin River during the study. The lack of a clear explanation for NO_3^- variability likely reflects a combination of riverine biological processes and time-varying physical transport of NO_3^- from upstream agricultural drains to the mainstem San Joaquin River.
4. The application of an *in situ* optical NO_3^- sensor along with discrete samples provides a view into the fine temporal structure of hydrochemical data and may allow for greater accuracy in pollution assessment.

Keywords: agriculture, diurnal, isotopes, nitrate, sensors

Introduction

Human activity has dramatically increased nitrogen (N) loading to rivers and coastal waters via atmospheric deposition and agricultural and urban land use inputs (Howarth *et al.*, 1996; Green *et al.*, 2004). While the capacity for terrestrial and aquatic systems to retain N is often high (Howarth *et al.*, 1996; Seitzinger *et al.*, 2002; Green *et al.*, 2004), increased N loading remains a significant concern for surface water eutrophication, drinking water quality and ecosystem health. For example, elevated NO_3^- concentrations from non-point sources contribute to chronically low dissolved oxygen (DO) conditions in lower reaches of the San Joaquin River (California, U.S.A.) by stimulating algal production and

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subsequent oxygen depletion during organic matter decomposition. Low DO concentrations in the San Joaquin River are a particular concern due to the inhibition of upstream migration and spawning of freshwater fish such as Chinook salmon (*Oncorhynchus tshawytscha* Walbaum) (Jassby, 2005; Volkmar & Dahlgren, 2006).

Identifying and managing linkages between human activity and surface water N dynamics requires sampling at intervals that capture the hydrologic, physical and biological variability in water quality (Kirchner *et al.*, 2004). While water quality data have traditionally been collected at weekly to monthly intervals, recent studies have shown that concentrations of inorganic N (Harrison, Matson & Fendorf, 2005; Kent, Belitz & Burton, 2005; Scholefield *et al.*, 2005; Mulholland *et al.*, 2006), DO (Mulholland, Houser & Maloney, 2005; Parker *et al.*, 2005) and trace metals (Brick & Moore, 1996; Nimick, Cleasby & McClesky, 2005), as well as the quality of dissolved organic matter (Spencer *et al.*, 2007), may vary considerably over daily time cycles in rivers and streams. Undersampling may therefore have important consequences for the accuracy of pollution assessment and the development of best management practices in urban and agricultural catchments (Stelzer & Likens, 2006).

Advances in *in situ* water quality sensors allow for the determination of nitrate (NO_3^-) concentrations at temporal scales that historically have not been feasible (Johnson & Colletti, 2002; Chapin *et al.*, 2004; Johnson *et al.*, 2007). While several recent studies have used wet chemical sensors, optical NO_3^- sensors which utilize the light attenuating properties of NO_3^- in the UV range can provide long-term and high frequency data without the need for *in situ* wet chemistry (Johnson & Colletti, 2002). For example, Johnson, Coletti & Chavez (2006) recently used an *in situ* ultraviolet spectrophotometer for the optical determination of NO_3^- concentrations hourly for 2 years off the California coast.

The goal of our study was to elucidate high-frequency biogeochemical and hydrologic processes affecting diurnal NO_3^- concentrations in the San Joaquin River during a summer period characterized by high algal productivity. In particular, we tested the hypothesis that biological processes (including algal uptake, denitrification and nitrification) exert a dominant control on NO_3^- concentrations diurnally as observed in other systems. A continuous 5-day record was collected using an *in situ* optical NO_3^- sensor along with ancillary measurements [including DO and chlorophyll-*a* (Chl-*a*) fluorescence] to offer a view of the fine temporal structure of hydrochemical data. Dual NO_3^- isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) and DO isotopes ($\delta^{18}O_{DO}$) were also measured over 2 days to help deconvolve the sources and processes controlling NO_3^- concentrations over diurnal time scales. Taken together, these measurements allow for a better understanding of the magnitude of NO_3^- variability and controls (physical and/or biological) on short-term N dynamics in aquatic and terrestrial ecosystems.

Methods

Site description

The San Joaquin River is one of two major rivers draining into the San Francisco Estuary in California, and is the focus of management due to chronically low DO concentrations during late summer and autumn in downstream reaches (Jassby, 2005). Our study was conducted at Crows Landing (37°25′42″N, 121°00′12″W), upstream of the tidal portion of the San Joaquin River (Fig. 1). The drainage area of the perennial San Joaquin River is approximately 9425 km^2 and the dominant land uses are largely agricultural (row crop agriculture, vineyard and orchard) in the valley and native vegetation and forest in the Sierra Nevada (Kratzer *et al.*, 2004). The catchment has an arid to semi-arid climate, and average daily air temperatures during our study ranged from 25.1 to 27.3 °C. Total daily flux of solar radiation was 6613–7007 W m^{-2} with 14 h of solar radiation daily as recorded by the CIMIS station near Patterson, approximately 14.5 km from the sampling site (37°26′24″N, 121°08′20″W). Peak solar radiation occurred daily at noon and ranged from 810 to 845 W m^{-2}.

The San Joaquin River within the study area is a low gradient river (mean *c.* 0.015%) with a dominant bed material of sand. During most years, the lower portion of the river is disconnected from its headwaters in the Sierra Nevada mountain range due to water diversion for agricultural and urban use, with summer river flows re-established about 40 km upstream from our study site due to agricultural return flows. During the study period (28 July–1 August, 2005), discharge fluctuated from 32.2 to 36.3 m^3 s^{-1} (Fig. 2a) in relation to agricultural withdrawal and reservoir release. Approximately 50% of the flow at the Crows Landing

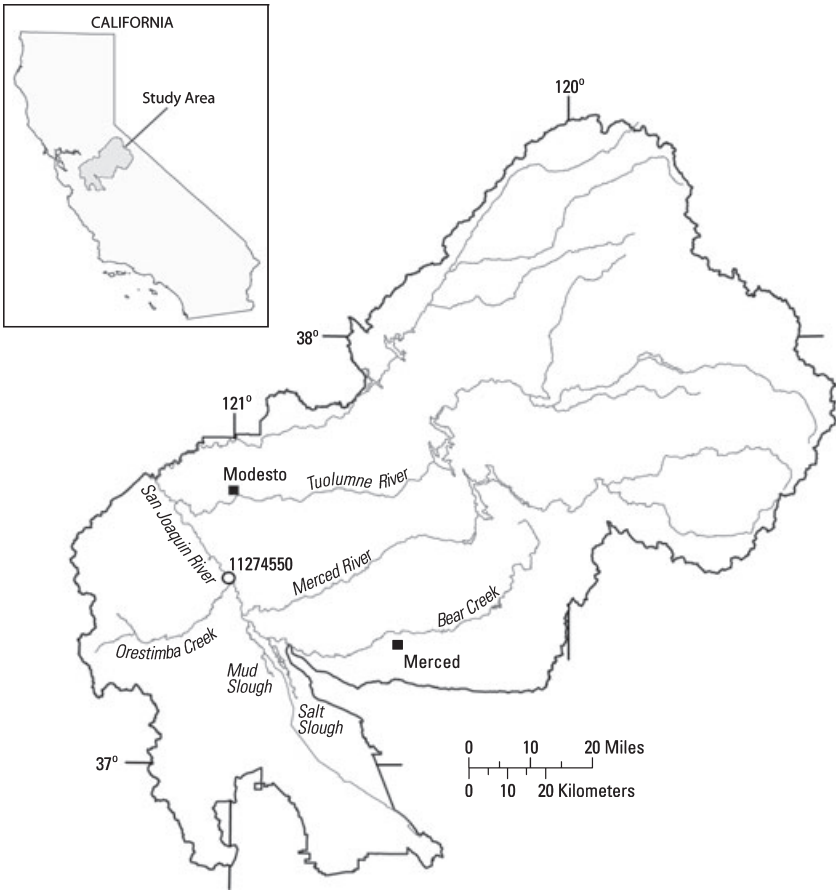

Fig. 1 Location of the study site at Crows Landing (near USGS gage 11274550) on the San Joaquin River, California.

study site originated from the Merced River (a mixture of reservoir waters from the Sierra Nevada and agricultural runoff). The remaining flow was agricultural drainage from Salt Slough (15%), Mud Slough (5%), Bear Creek (2%), Orestimba Creek (2%) and *c.* 25% from unknown sources such as ungaged agricultural drains and groundwater discharge (http://cdec.water.ca.gov; http://waterdata.usgs.gov/nwis/sw). The diurnal study was conducted during a rain-free period when peak Chl-*a* and pheophytin-*a* concentrations are common in the San Joaquin River (Kratzer *et al.*, 2004; Jassby, 2005). The phytoplankton community in the San Joaquin River is dominated by centric diatoms (Thalassiosirales), with growth rates limited by light and flow regime rather than nutrients (Leland, Brown & Mueller, 2001; Jassby, 2005; Ohte *et al.*, 2007).

In situ measurements

An *in situ* optical instrumentation package was deployed in the centre of the channel (*c.* 60 m wide) for the 5-day period from noon on 28 July to noon on 1 August, 2005. Samples were collected every 30 min from a depth of approximately 2 m (roughly mid-depth of the water column). Dissolved measurements were made on samples drawn through a filtered flow path using a SHURflo model 1100 pump (SHURflo, Cypress, CA, U.S.A.), acid-rinsed Tygon tubing and a 10 μm pre-filter/0.2 μm membrane filter (Osmonics Memtrex GE, Minnetonka, MN, U.S.A.; 25.4 cm). Filtered water was pumped through an ISUS optical nitrate sensor (Satlantic Inc., Halifax, NS, Canada), which calculated NO_3 concentrations using the absorption spectrum from 217 to 240 nm. In addition, a linear baseline correction of the raw ISUS data was required to correct for a constant offset relative to lab NO_3^- concentrations ($y = 1.168 \times ISUS_{NO3} - 0.022$). Manufacturer reported instrument precision is ±0.5 μM and accuracy is reported at ±2 μM.

Unfiltered water was pumped using a SeaBird 5T pump with a Teflon screen and passed through a WET Labs (Philomath, OR, U.S.A.) single-band fluorometer to measure Chl-*a* fluorescence (excitation

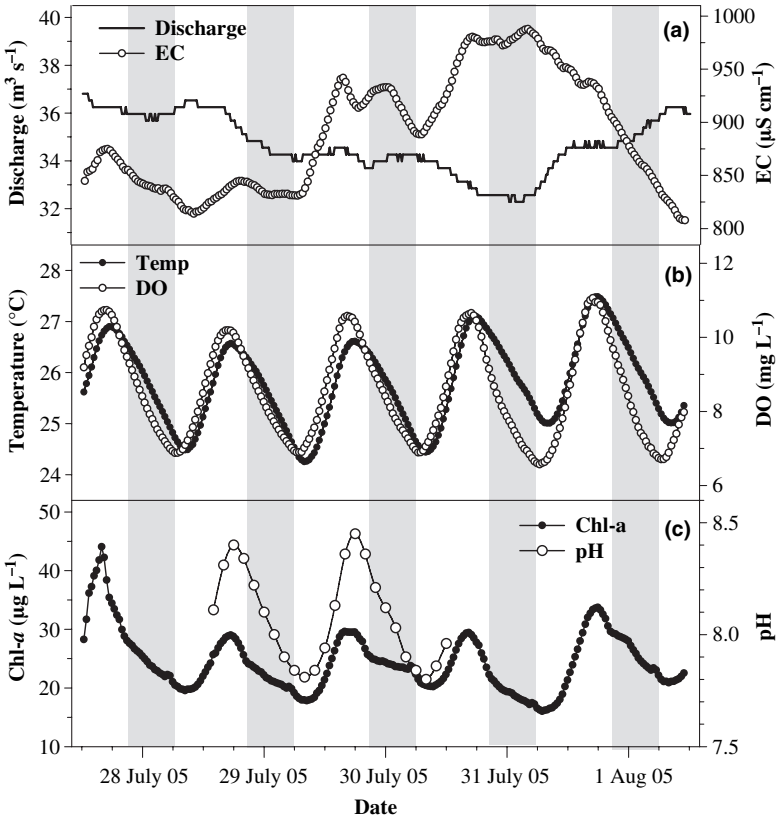

Fig. 2 Ancillary parameters measured during the diurnal study period in the San Joaquin River at Crows Landing: (a) discharge (m^3 s^{-1}) and electrical conductivity (µS cm^{-1}); (b) temperature (°C) and dissolved oxygen (mg L^{-1}); (c) chlorophyll-*a* fluorescence (ex. 460 nm, em. 695 nm; µg L^{-1}) and pH. Grey shaded blocks indicate night-time.

460 nm, emission 695 nm). A Seabird CTD (SeaBird, Bellevue, WA, U.S.A.) was used in conjunction with the optical instrumentation for water temperature and electrical conductivity, while DO concentrations were measured with an Aanderraa model 4175 oxygen optode (Aanderaa Instruments, Bergen, Norway). Chromophoric dissolved organic matter fluorescence was also measured as described in Spencer *et al.* (2007). All *in situ* optical instrumentation was controlled and logged using a WET Labs DH-4 datalogger.

Discrete sample collection and analyses

Discrete samples were collected every 2 h from 28 July until 30 July, 2005 (12:00–12:00 hours, $n = 25$) from 1 m below the water surface and pumped through a 10 µm pre-filter and 0.2 µm membrane filter (Osmonics Memtrex, 25.4 cm) in the field. Samples were stored on ice in pre-combusted amber glass bottles in the dark until returned to the lab, where inorganic N samples were frozen and the rest of the samples were kept chilled until analysis. NO_3^- concentrations were measured using a benchtop spectrophotometric method (Doane & Horwath, 2003) with a detection limit of 0.05 mg N L^{-1}. Ammonium (NH_4^+) was also measured spectrophotometrically using the Berthelot reaction with a detection limit of 0.02 mg N L^{-1}. Total dissolved nitrogen (TDN) was measured as NO_3^- following persulphate oxidation.

Nitrate isotopes ($\delta^{15}N$ and $\delta^{18}O$) were analysed at the USGS Menlo Park Stable Isotope Laboratory using the denitrifier method (Sigman *et al.*, 2001; Casciotti *et al.*, 2002). Thawed samples were injected into sealed vials containing denitrifying bacteria (*Pseudomonas aureofaciens* Kluyver) and soy broth media. The resulting N_2O gas was analysed via a Micromass IsoPrime continuous flow mass spectrometer for both $\delta^{15}N$ and $\delta^{18}O$, and reported in ‰ relative to the Air and VSMOW standards respectively. Analyses of $\delta^{15}N$–NO_3 were corrected using an internal lab nitrate standard, calibrated against international nitrate isotopic standards IAEA-N3 ($\delta^{15}N$ +4.7‰) and USGS-34 ($\delta^{15}N$ −1.8‰). Analyses of $\delta^{18}O$–NO_3 were corrected for exchange, fractionation and blank against international nitrate isotopic standards USGS-34 ($\delta^{18}O$ −27.9‰) and USGS-35 ($\delta^{18}O$ +57.5‰)

(Böhlke, Mroczkowski & Coplen, 2003). All samples were analysed in duplicate, with average differences of 0.3‰ for $\delta^{15}N$ and 0.5‰ for $\delta^{18}O$.

Samples for $\delta^{18}O$ of DO were collected in pre-evacuated 50 mL glass vials with 2–4 mg of copper sulphate preservative. The sample bottle was held under the sample water and a 2.5 cm × 21G needle was inserted through the septum, letting the vacuum draw in water to fill the sample bottle. Samples were analysed for $\delta^{18}O$ using a modification of the method in Wassenaar & Koehler (1999). Briefly, 10 mL of water is replaced with ultrapure He and the bottles are shaken for 30 min on an orbital shaker. Gas samples from the bottles are then injected through an injection port on a Carlo Erba 1500 elemental analyser interfaced with a Micromass Optima mass spectrometer. The raw data were corrected using the values of air samples injected and analysed using the same apparatus, with $\delta^{18}O$ values of O_2 reported in ‰ relative to SMOW. Analytical precision is typically ±0.2‰.

Filtered samples (0.7 μm GFF) for $\delta^{18}O$ of water were stored in glass vials with polyseal caps until analysis. Aliquots of 2 mL of water were equilibrated with CO_2 gas under controlled temperature conditions using the method of Epstein & Mayeda (1953), and the resulting CO_2 was analysed for isotopic composition using a Finnigan MAT 251 mass spectrometer (Thermo, San Jose, CA, U.S.A.). Raw data were corrected for instrument drift and temperature-dependent isotope fractionation before reporting final data. The $\delta^{18}O$ values are reported in ‰ relative to the VSMOW standard. Analytic precision is ±0.1‰.

Data analysis

Time-series analysis was performed in the MATLAB computing environment (MathWorks, Inc., Natick, MA, U.S.A.) to determine the power spectrum of ISUS *in situ* NO_3^- concentrations and other continuous parameters. Data were detrended and padded from 238 to 256 data points for calculation via a fast Fourier transform (FFT) algorithm. Although detrending largely removed the impact of endpoints, endpoints of [$f(0)$ and $f(N$ samples/2)] were ignored and for clarity are not presented. The Fourier components were squared and summed to give signal power presented as a function of frequency (cycles per day) in a periodogram. Additional regression analyses to evaluate relationships between parameters was performed using S-PLUS statistical software (Insightful Corp., Seattle, WA, U.S.A.).

Results

Discharge fluctuated over a relatively small range during the study period (32.2–36.3 $m^3\ s^{-1}$, Fig. 2a) in relation to agricultural diversions, discharges and reservoir releases, but did not show diurnal variability. Electrical conductivity (Fig. 2a) also did not vary diurnally, but was negatively correlated with discharge ($r^2 = 0.52$, $P < 0.01$). Temperature, DO, pH and Chl-*a* fluorescence showed diurnal patterns independent of discharge with daily maxima in late afternoon and minima in the early morning (Fig. 2b,c). Diurnal changes in water column pH and DO, along with a strong negative correlation between DO concentrations and $\delta^{18}O$–DO ($r^2 = 0.90$, $P < 0.01$), are consistent with aquatic photosynthesis and respiration (Mulholland *et al.*, 2005; Parker *et al.*, 2005).

The concentrations of NO_3^- measured *in situ* and in the lab were strongly correlated ($r^2 = 0.97$, $P < 0.01$, $n = 25$) and showed that NO_3^- ranged from 1.72 to 2.47 mg N L^{-1} over the 5-day study period and varied by up to 0.62 mg N L^{-1} over a single diurnal cycle (Fig. 3). NO_3^- was the dominant form of N in the San Joaquin River during our study and was responsible for most of the observed variability in TDN concentrations (Table 1). Dissolved organic nitrogen (DON) accounted for 24–34% of TDN during the study, but did not show diurnal variability. NH_4^+ data suggest a weak diurnal pattern with highest concentrations during mid-morning, but NH_4^+ accounted for only 1–4% of the TDN concentrations (Table 1).

The power spectrum of DO (Fig. 4a) and temperature (not shown) revealed that 91% and 87%, respectively, of the total power over the study period was explained by the diurnal maxima occurring at 1 cycle day^{-1}. In contrast, the power spectrum for NO_3^- concentration showed maximum periodicities at frequencies ranging from 0.40, 0.81, 1.4 and 2.0 cycles day^{-1} (Fig. 4b), with the two maximum frequency peaks (0.81 and 1.4 cycles day^{-1}) accounting for 32% of the total power. This periodicity appears to reflect a local daytime peak that was observed in early afternoon, with daytime minima in early morning and/or late evening. Additional peaks

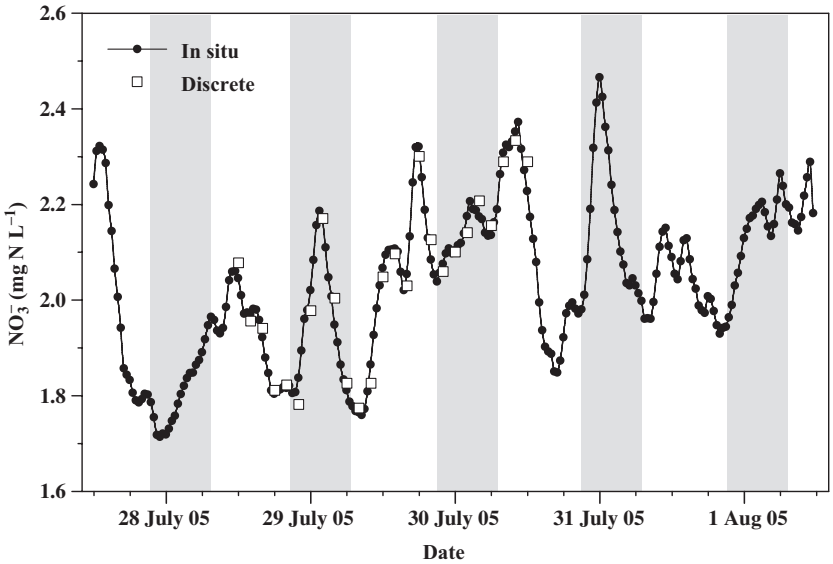

Fig. 3 Temporal trends in NO_3^- concentrations from the *in situ* NO_3^- sensor (solid circles with line) and lab NO_3^- concentrations (open squares) during the diurnal study period in the San Joaquin River at Crows Landing. The r^2 between *in situ* and discrete lab NO_3^- concentrations was 0.97 ($P < 0.01$, $n = 25$, slope = 1).

Table 1 Nitrogen concentrations (mg N L^{-1}) in discrete samples collected at Crows Landing, San Joaquin River, California

Date	Time (h)	NO_3^-	NH_4^+	TDN	DON
28 July	12:00	2.08	0.05	2.87	0.74
28 July	14:00	1.96	0.05	2.89	0.89
28 July	16:00	1.94	0.05	2.81	0.82
28 July	18:00	1.81	0.03	2.77	0.93
28 July	20:00	1.82	0.03	2.67	0.82
28 July	22:00	1.78	0.05	2.70	0.88
28 July	00:00	1.98	0.07	2.88	0.83
29 July	02:00	2.17	0.05	3.05	0.83
29 July	04:00	2.00	0.06	2.92	0.86
29 July	06:00	1.83	0.07	2.88	0.99
29 July	08:00	1.77	0.07	2.56	0.71
29 July	10:00	1.83	0.10	2.72	0.79
29 July	12:00	2.05	0.03	2.83	0.76
29 July	14:00	2.10	0.02	3.00	0.88
29 July	16:00	2.03	0.03	2.74	0.68
29 July	18:00	2.30	0.03	3.21	0.88
29 July	20:00	2.13	0.02	3.00	0.86
29 July	22:00	2.06	0.02	2.89	0.82
30 July	00:00	2.10	0.02	3.00	0.89
30 July	02:00	2.14	0.02	2.97	0.81
30 July	04:00	2.21	0.02	3.05	0.82
30 July	06:00	2.16	0.02	2.91	0.73
30 July	08:00	2.29	0.06	3.10	0.75
30 July	10:00	2.33	0.07	3.27	0.87
30 July	12:00	2.29	0.02	3.26	0.95

Samples were collected between 28 and 30 July, 2005.
NO_3^-, nitrate; NH_4^+, ammonium; TDN, total dissolved nitrogen; DON, dissolved organic nitrogen.

in NO_3^- concentrations occurred on one or more dates throughout the 5-day study, including large peaks near midnight on 29 and 31 July (Fig. 3).

Diurnal patterns of $\delta^{18}O_{DO}$ (Fig. 5) occurred independent of discharge in the San Joaquin River and ranged from 12.5‰ to 22.8‰, with highest values in early morning and lowest values in late evening. Nitrate ($\delta^{15}N_{NO3}$) isotope values varied from +10.6 to +12.5‰ in our study, while $\delta^{18}O_{NO3}$ values ranged from +4.1 to +10.8‰ (Fig. 6). There was significant isotopic variability between samples, but no concerted diurnal $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ trend was apparent. For example, $\delta^{18}O_{NO3}$ increased from +5.7 to +10.8‰ over a 2-h period on 29 and 30 July and then remained elevated through the remaining discrete sampling period (Fig. 6b). NO_3^- concentration was not correlated with $\delta^{15}N_{NO3}$ ($r^2 = 0.06$, $P = 0.25$) and weakly positively correlated with $\delta^{18}O_{NO3}$ ($r^2 = 0.19$, $P = 0.03$), while $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ showed a weak negative correlation ($r^2 = 0.20$, $P = 0.03$; Fig. 7).

Discussion

The use of discrete sampling and an *in situ* NO_3^- sensor revealed variability in NO_3^- concentrations of up to 22% over a single diurnal cycle and 31% over the 5-day study in the San Joaquin River. However, lack of a concerted diurnal NO_3^- pattern suggests the importance of anthropogenic activities (e.g. water inputs and diversions upstream) that alter NO_3^- concentrations in the San Joaquin River at variable frequencies. The lack of a clear diurnal signal does not necessarily imply limited riverine N cycling in the San

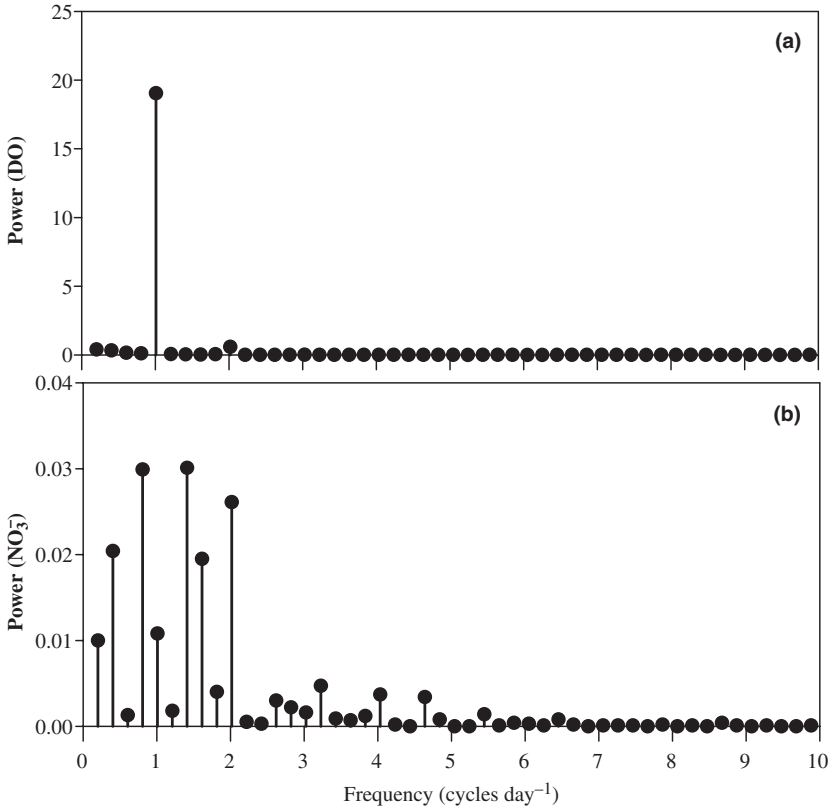

Fig. 4 Power spectrum for (a) DO concentration and (b) NO_3^- concentration during the study period in the San Joaquin River at Crows Landing. Frequencies greater than 10 cycles day^{-1} hold no power in the calculation and are not shown.

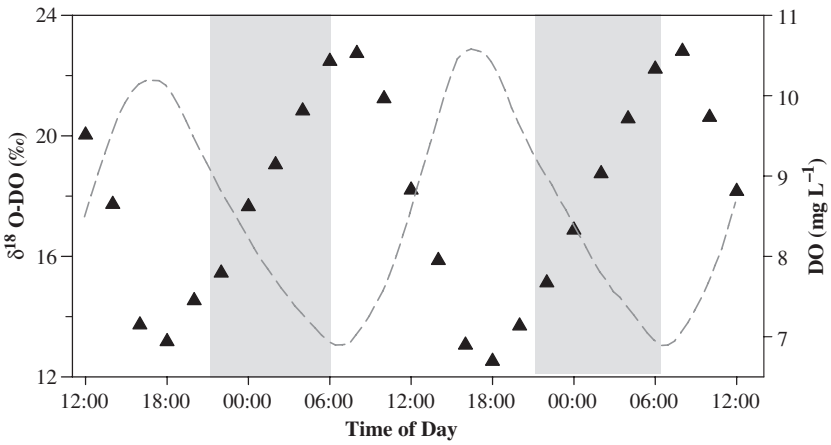

Fig. 5 Diurnal $\delta^{18}O_{DO}$ variability (‰) and in situ DO concentrations (mg L^{-1}) at Crows Landing, San Joaquin River during the discrete sampling period (28–30 July, 2005). DO concentrations are represented by dashed lines.

Joaquin River, but instead may reflect a masking effect from anthropogenic N loads over the study period. These results have several important implications for water quality management. First, they highlight the uncertainty inherent in traditional weekly to monthly sampling intervals, particularly in river systems strongly influenced by anthropogenic N loading. Our results also suggest that important physical and biogeochemical processes occur in catchments which alter the concentration and isotopic composition of NO_3^- on timescales of hours to days.

Factors potentially influencing NO_3^- concentrations in the river system may be biological (assimilation, nitrification and denitrification) or physical processes related to the timing and sources of runoff from the agricultural landscape. Evidence for the relative importance of biological and physical processes is described below and represents a critical challenge for

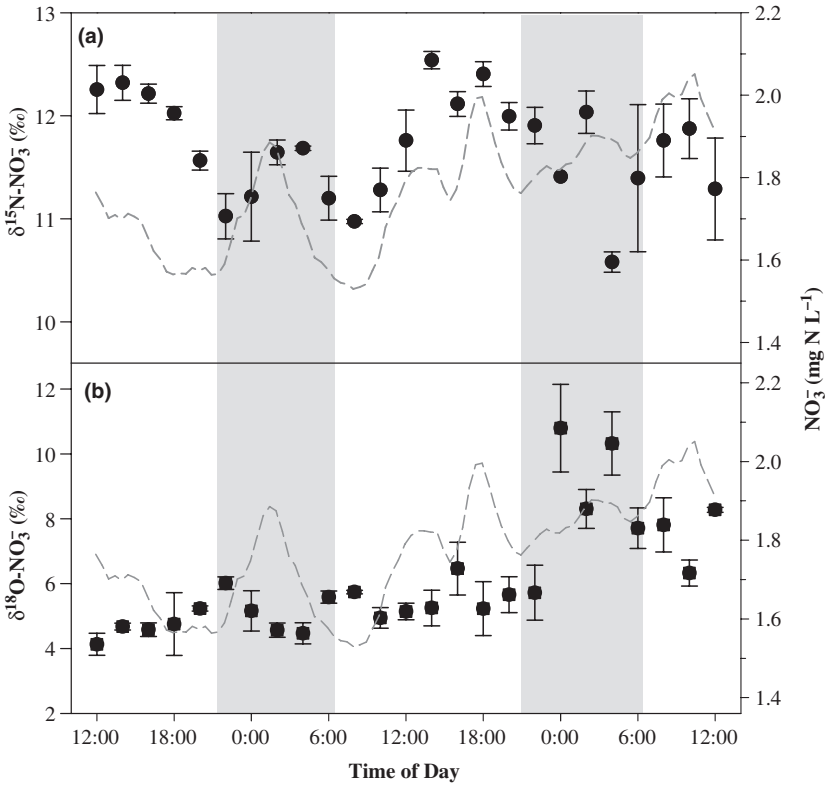

Fig. 6 Diurnal (a) $\delta^{15}N_{NO3}$ and (b) $\delta^{18}O_{NO3}$ variability (‰) and in situ NO_3^- concentrations (mg N L^{-1}) at Crows Landing, San Joaquin River during the discrete sampling period (28–30 July, 2005). NO_3^- concentrations are represented by dashed lines.

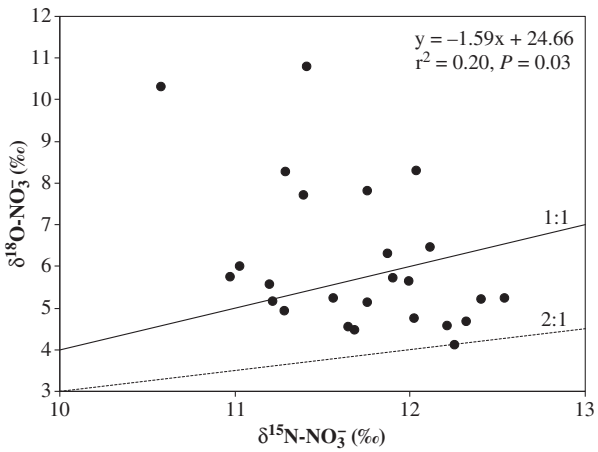

Fig. 7 Correlation between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ values during the discrete sampling period (28–30 July, 2005) at Crows Landing, San Joaquin River. The slope of the 1 : 1 (indicative of assimilation) and 2 : 1 trend lines (indicative of denitrification) shown for comparison.

the development of best management practices that limit NO_3^- impairment in surface waters. This is a particular concern in agricultural catchments like the San Joaquin Valley, where a better understanding of N sources and cycling is needed to address chronically low DO conditions observed in lower reaches (Jassby, 2005; Volkmar & Dahlgren, 2006).

Biological controls on NO_3^- variability

Diurnal patterns of DO concentrations and $\delta^{18}O_{DO}$ occurred independent of discharge in the San Joaquin River and were consistent with biological processes (e.g. photosynthesis and respiration) and atmospheric O_2 exchange (Mulholland *et al.*, 2005; Parker *et al.*, 2005). Declining δ^{18}O–DO to below atmospheric equilibrium (+24.2‰) during the day is likely due to photosynthesis using substrate water with lower δ^{18}O (−11.0‰ ± 0.3‰) than atmospheric O_2. In contrast, increasing δ^{18}O–DO at night is presumably due to preferential consumption of O_2 with low δ^{18}O by respiring organisms coupled with the inward diffusion of atmospheric O_2 with a higher δ^{18}O (Parker *et al.*, 2005).

While the periodicity in NO_3^- concentrations does not support a strong diurnal signal (e.g. one cycle - day^{-1}), a late day minima in NO_3^- concentration occurs in conjunction with peak Chl-*a* fluorescence on several days, inferring algal uptake as a possible factor influencing NO_3^- variability. Ohte *et al.* (2007)

reported that only 32% of the Chl-*a* load at Crows Landing was delivered from upstream tributaries from June to October 2001, indicating significant algal production (and presumably N uptake) in the mainstem San Joauqin River. Other diurnal studies have observed a drawdown of NO_3^- concentrations attributed to algal assimilation in both fresh waters (Kent *et al.*, 2005; Mulholland *et al.*, 2006) and coastal waters (Johnson *et al.*, 2006). However, additional evidence to support algal assimilation as a dominant driver of NO_3^- trends is lacking in our data set. For example, the apparent decoupling of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ contrasts with the *c.* 1 : 1 enrichment in the residual NO_3^- pool expected if phytoplankton uptake were significantly influencing NO_3^- concentrations (Casciotti *et al.*, 2002; Granger *et al.*, 2004). In addition, Ohte *et al.* (2007) reported that algal N demand in the San Joaquin River only accounted for a small fraction (e.g. 6.5%) of the total N load along a 119 km length.

The $\delta^{15}N_{NO3}$ values in our study (+10.6 to +12.5‰) are within a range often attributed to NO_3^- consuming processes such as denitrification and/or NO_3^- contributions from wastewater sources (Kendall, Elliott & Wankel, 2007). Previous studies using dual isotopes have shown that denitrification results in a coupling of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ (*c.* 2 : 1 enrichment) in the residual NO_3^- pool in fresh waters as N and O atoms originate from the same NO_3^- molecule (Kendall *et al.*, 2007). The negative slope and weak correlation between $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in our data set also does not provide evidence for denitrification as a dominant driver of NO_3^- variability during our study period. In addition, surface water DO concentrations at our site varied diurnally from 6.5 to 11.1 mg L^{-1} (*c.* 77–140% DO saturation) and did not become completely reduced or anoxic as observed by Harrison *et al.* (2005) and Laursen & Seitzinger (2004) in NO_3^--rich agricultural streams. Similarly, Kratzer *et al.* (2004) also reported a lack of direct evidence for significant denitrification using dual isotopes collected biweekly in the San Joaquin River during a 2001 study.

The decoupling of $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ suggests the influence of mixing from multiple sources and/or additional biogeochemical processes such as nitrification occurring during our study. The formation of NO_3^- via nitrification incorporates oxygen atoms from both water and dissolved O_2 (Andersson & Hooper, 1983; Kumar, Nicholas & Williams, 1983; Casciotti *et al.*, 2002), decoupling $\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$ in the residual NO_3^- pool. Changes in surface water pH and temperature have been shown to affect nitrification rates (Warwick, 1986) and could theoretically contribute to NO_3^- variability in our study. Laursen & Seitzinger (2004) suggested that elevated NO_3^- concentrations in an agricultural reach were related to higher rates of whole system nitrification during the day, and we similarly observed daytime peaks in NO_3^- concentrations on several days. In addition, low NH_4^+ concentrations relative to NO_3^- concentrations suggest that diurnal variability in nitrification rates would account for only a small fraction of the observed NO_3^- variability.

While generally consistent with the apparent isotope decoupling in our study, $\delta^{18}O_{NO3}$ values were higher than expected from *in situ* nitrification using two oxygen atoms from water ($\delta^{18}O = -11.0 \pm 0.3$‰) and one from dissolved O_2 ($\delta^{18}O = +13.1$ to $+22.8$‰). The low $\delta^{18}O_{NO3}$ values from 29 July 2005 suggest soil microbial nitrification; however, the higher $\delta^{18}O_{NO3}$ values the following day could represent a different source of NO_3^- or some additional biogeochemical process causing isotope fractionation. Other possible explanations for the discrepancy in aquatic systems are described by Kendall *et al.* (2007) and include nitrification in soil water with higher than expected $\delta^{18}O$ (due to evaporation) and nitrification using O_2 with a high $\delta^{18}O$ (possibly due to respiration).

Physical controls on NO_3^- variability

The high variability in NO_3^- concentrations and a general lack of isotopic evidence for a single biological control suggests the importance of physical transport of NO_3^- from tributaries and drains to the San Joaquin River. For example, Ohte *et al.* (2007) reported that minor drainages along the middle to downstream reaches of the San Joaquin River accounted for only 7% of the total river discharge, but 20% of the NO_3^- load. Runoff from agricultural drains with flow gauges accounted for *c.* 25% of the discharge at Crows Landing during our study period, with another *c.* 25% from unknown sources such as smaller ungauged drains and groundwater discharge (http://cdec.water.ca.gov; http://waterdata.usgs.gov/nwis/sw).

Samples collected from agricultural drains in the San Joaquin River catchment on the first day of our study had NO_3^- concentrations as high as

8.9 mg N L^{-1} (mean = 5.6 mg N L^{-1}; R. Dahlgren, unpubl. data), significantly higher than measured at our San Joaquin River mainstem sampling site (1.7–2.1 mg N L^{-1}). Ohte *et al.* (2007) reported that gauged tributaries accounted for 70% of the NO_3^- load at the same study site from June until October, 2001, suggesting that time-varying contributions of irrigation return flows could result in high NO_3^- variability as observed in our study. Similarly, Burow, Shelton & Dubrovsky (2008) reported median groundwater NO_3^- concentrations (median = 6.4 mg N L^{-1}, n = 102 wells) in the east side of the San Joaquin River catchment that were higher than river concentrations and attributed this to anthropogenic N loading and oxic groundwater conditions. Changes in the relative importance of ground water may also affect NO_3^- concentrations, particularly given the relatively high groundwater discharge rates (4.17 cfs km^{-1}) reported by Phillips, Beard & Gilliom (1991) for the San Joaquin River mainstem reach that includes our study site.

Values of $\delta^{15}N_{NO3}$ for the San Joaquin River mainstem at Crows Landing (+11.9‰) and major upstream drainages were similar on the first day of diurnal sampling (+8.3 to +12.5‰; C. Kendall, unpubl. data), suggesting that the source isotopic signature of NO_3^- was similar throughout the catchment. In contrast, $\delta^{18}O_{NO3}$ values differed between sites (+4.3‰ in the Merced River, +7.4 to +8.5‰ in upstream agricultural drainages; C. Kendall, unpubl. data). The decoupling of $\delta^{18}O$ and $\delta^{15}N$ values as previously discussed suggests that the mixing of waters from various sources (including animal wastewater) may explain the observed lack of correlation between the dual NO_3^- isotopes. For example, the rapid increase in $\delta^{18}O_{NO3}$ on the second day of discrete sampling may be indicative of an increased NO_3^- contribution from agricultural sources with a distinct $\delta^{18}O$ value that reflects the variability of water $\delta^{18}O$ values (−0.5 to +21‰; C. Kendall, unpubl. data) in the tributaries and catchments or fields where the NO_3^- was formed.

Management implications

Data from our San Joaquin River study supports a growing body of evidence for significant variability in NO_3^- concentrations in rivers and streams over short time scales (Harrison *et al.*, 2005; Kent *et al.*, 2005; Scholefield *et al.*, 2005). While the relative importance of biological and physical controls is unclear, rapid changes in the concentration and isotopic composition of NO_3^- remains a critical challenge for the accuracy of load assessments, and in development of best management practices that limit NO_3^- impairment in surface waters. This is a particular concern in agricultural catchments like the San Joaquin Valley, where a better understanding of catchment N sources and cycling is needed to address chronically low DO conditions observed in lower reaches (Jassby, 2005; Volkmar & Dahlgren, 2006).

Diurnal patterns in Chl-*a*, DO concentrations and $\delta^{18}O_{DO}$ independent of discharge provide clear evidence for *in situ* biological production. Concerted trends in diurnal NO_3^- concentrations were not evident in our data set, despite high variability over short time scales (e.g. up to 22% over a single 24-h period). Dual NO_3^- isotopes ($\delta^{15}N_{NO3}$ and $\delta^{18}O_{NO3}$) did not indicate a single dominant biological or physical mechanism driving NO_3^- variability, likely reflecting the complex mixture of sources and processes expected in biogeochemically and/or hydrologically active catchments (Kendall *et al.*, 2007). For example, the multiple potential sources of NO_3^- rarely have constant isotopic values and the initial composition may be altered by various fractionation processes before, during and after mixing. We hypothesize that algal assimilation and agricultural return flows contributed to the observed variability in NO_3^- concentrations based on existing data, but that nitrification and denitrification also deserve further attention in light of evidence for these processes in other agricultural river systems (Laursen & Seitzinger, 2004; Harrison *et al.*, 2005).

While the mechanisms driving NO_3^- variability are not yet clear, the observation that NO_3^- concentrations varied by up to 22% over a single diurnal cycle and up to 31% over the 5-day study suggests that sampling at weekly to monthly intervals may represent an important source of uncertainty in pollution assessment. The recent development of *in situ* NO_3^- sensors allows for data collection at intervals that capture the hydrologic, physical and biological variability in water quality (Kirchner *et al.*, 2004). In addition, the development of optical NO_3^- sensors eliminates the need for wet chemistry and allows for the high frequency determination of NO_3^- concentrations in aquatic systems over periods of days to weeks.

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