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Authors
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Maynard, Jonathan J
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Fate of nitrate in seepage from a restored wetland receiving agricultural tailwater

Neil Brauer [a], Jonathan J. Maynard [b], Randy A. Dahlgren [a], Anthony T. O'Geen [a,*]

[a] Department of Land, Air and Water Resources, University of California, One Shields Avenue, Davis, CA 95616, USA
[b] USDA ARS, Jornada Experimental Range, New Mexico State University, P.O. Box 30003, MSC 3JER, Las Cruces, NM 88003-8003, USA

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ABSTRACT

Constructed and restored wetlands are a common practice to filter agricultural runoff, which often contains high levels of pollutants, including nitrate. Seepage waters from wetlands have potential to contaminate groundwater. This study used soil and water monitoring and hydrologic and nitrogen mass balances to document the fate and transport of nitrate in seepage and surface waters from a restored flow-through wetland adjacent to the San Joaquin River, California. A 39% reduction in NO_3-N concentration was observed between wetland surface water inflows (12.87 ± 6.43 mg L^{-1}; mean $\pm$ SD) and outflows (7.87 ± 4.69 mg L^{-1}). Redox potentials were consistently below the nitrate reduction threshold (~250 mV) at most sites throughout the irrigation season. In the upper 10 cm of the main flowpath, denitrification potential (DNP) for soil incubations significantly increased from 151 to 2437 mg NO_3-N m^{-2} d^{-1} when nitrate was added, but showed no response to carbon additions indicating that denitrification was primarily limited by nitrate. Approximately 72% of the water entering the wetland became deep seepage, water that percolated beyond 1-m depth. The wetland was highly effective at removing nitrate (3866 kg NO_3-N) with an estimated 75% NO_3-N removal efficiency calculated from a combined water and nitrate mass balance. The mass balance results were consistent with estimates of NO_3-N removed (5085 kg NO_3-N) via denitrification potential. Results indicate that allowing seepage from wetlands does not necessarily pose an appreciable risk for groundwater nitrate contamination and seepage can facilitate greater nitrate removal via denitrification in soil compared to surface water transport alone.

1. Introduction

There have been many efforts across the world to mitigate wetland habitat lost over the past century. This movement is echoed in California's Central Valley where stakeholders have established the goal of creating and protecting over 60,000 ha of new wetland habitat in the state (Central Valley Joint Venture, 2006). Many of these wetlands are, or will be, ephemeral, flow-through wetlands receiving irrigation return flows during the growing season (April–September). Most wetlands in CA are restored with the primary objective of enhancing waterfowl habitat, however, these systems also have the potential to retain and remove nutrient loads that would otherwise be exported directly into major waterways (Fisher and Acreman, 2004). Therefore, wetland treatment of agricultural return flows is being considered as a beneficial management practice to reduce algal and nutrient loads that contribute to seasonally low dissolved oxygen in the lower San Joaquin River, California (Lehman et al., 2004; Diaz et al., 2012).

Many studies have demonstrated that natural and constructed wetlands are generally effective at removing nitrogen from municipal and agricultural wastewaters (Brodie, 1989; Phipps and Crumpton, 1994; Kadlec and Knight, 1996; Woltemade, 2000; Jordon et al., 2003; Zedler, 2003; Beutel et al., 2009; Diaz et al., 2012). Removal efficiencies as high as 98% have been reported, though other studies report significantly lower N removal rates typically between 35 and 55% (Watson et al., 1989; Phipps and Crumpton, 1994; Comin et al., 1997; Kovacic et al., 2000; Mitsch et al., 2000; Tanner et al., 2002). A study of three wetlands used to treat subsurface tile drainage water in the Midwestern, USA demonstrated NO_3 removal rates of 28% (Kovacic et al., 2000). Similarly, high but variable NO_3 removal rates (35–100%) have been documented from water seeping through side berms of a constructed wetland in Illinois (Larson et al., 2000). Variation in

* Corresponding author. Tel.: +1 530 752 2155.
E-mail address: atogeen@ucdavis.edu (A.T. O'Geen).

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nitrate removal is a result of many factors such as hydraulic residence time, soil properties, vegetation characteristics, variability in input loads, N loading, temperature, dissolved oxygen concentration, climate and nitrogen form (nitrate, ammonium or organic) in input waters (Phipps and Crumpton, 1994; Beutel et al., 2009; O'Geen et al., 2010).

Using wetlands as a beneficial management practice to reduce non-point source pollution from agricultural drainage waters may introduce a problem as these wetlands could leach contaminants such as nitrate directly into the groundwater. This could compound an existing problem in California where groundwater NO_3-N loading rates of 200 Gg per year have been reported in areas of intensive agriculture such as the Salinas Valley and Tulare Lake Basin (Viers et al., 2012). Several studies of dairy lagoons summarized in Harter et al. (2002) document high seepage rates (up to 1 cm d^{-1}), and elevated groundwater N concentrations beneath lagoons. Similarly, Huffman (2004) found NO_3-N concentrations exceeding the EPA drinking water standard (10 mg NO_3-N L^{-1}) beneath two thirds of 34 swine lagoons in North Carolina. More studies of nitrogen fate and transport in wetlands receiving tailwater from cropland are needed because the existing literature base for this topic encompasses a wide range of environmental characteristics that govern nitrogen transformations (e.g., differences in nitrogen form, N concentration, hydrology, soil characteristics and climate).

The primary objectives of this study were to determine the fate of nitrogen in seepage waters of a restored surface-flow through wetland and to determine the importance of hydrologic- as well as soil- and biogeochemical-factors that regulate nitrate removal. We addressed these objectives by: (i) monitoring nitrogen concentration in nested piezometers (10, 50, and 100 cm) throughout the wetland and comparing them to surface water; (ii) measuring spatial patterns in selected soil and hydrological characteristics; and, (iii) developing wetland hydrologic and nitrogen mass balances to evaluate the fate of nitrate. The results from this study provide information relevant to the optimization, design, and management of restored wetlands for nitrate removal. Moreover, these findings expand upon the limited number of published studies that document nitrate removal by constructed wetlands receiving nitrate runoff from irrigated agriculture (Beutel et al., 2009).

2. Materials and methods

2.1. Site description

The restored flow-through wetland (8.7 ha) is located in the Central Valley of California adjacent to the San Joaquin River (Fig. 1). The two-year-old wetland intercepts irrigation return flows from about 420 ha of farmland before discharging into the river. Tailwaters originate from both furrow and flood irrigated crops primarily of tomatoes, melons, stone fruits, nuts, and alfalfa.

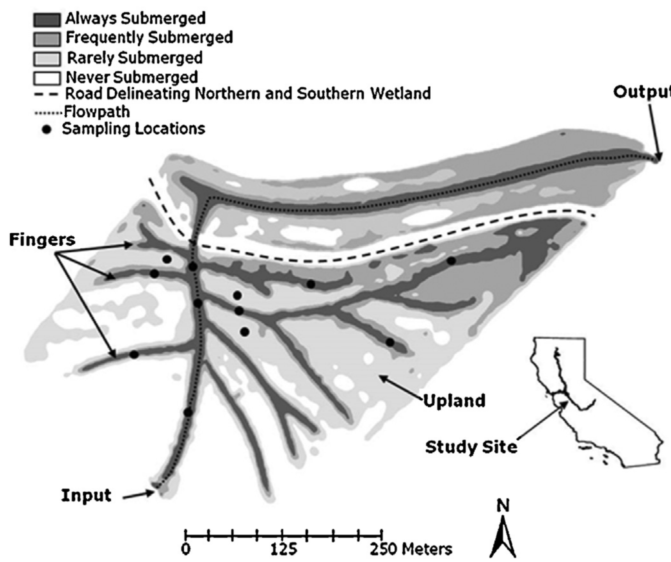

Fig. 1. Schematic showing site location, wetland morphology, sampling locations and areas of submersion. Dashed line represents a road. Dotted line is the main flowpath hydrologic zone. The upland hydrologic zone represents landscape positions that are rarely submerged.

The climate is Mediterranean, having hot and dry growing seasons and cool, wet winters. No precipitation occurred during the irrigation season.

The wetland has a dendritic form with three distinct hydrologic zones (Fig. 1): (i) the main flowpath, characterized by deep water (~0.75 m), measurable flow velocity, high sedimentation rates (~10–35 kg m^{-2} yr^{-1}) and minimal vegetation; (ii) the fingers, shallow (~0.1–0.5 m) areas with no measurable flow velocity, low sedimentation rates (~0.5–5 kg m^{-2} yr^{-1}), and partially vegetated with *Polygonum lapathifolium* (smartweed); and (iii) upland zones that experienced intermittent flooding, but had saturated conditions that extend within 25 cm of the soil surface and densely vegetated with smartweed, grasses and riparian trees such as willow and cottonwood (Table 1 and Fig. 1).

2.2. Hydrologic characterization

The wetland received agricultural return flows during the irrigation season from April to September, with no rainfall occurring during this time. Surface water inflow and outflow volumes were measured at 30-min intervals using v-notch weirs and barometric pressure compensated water level loggers (Solonist, Georgetown, ON). A digital elevation model (DEM) was created using a Trimble RTK GPS (Trimble, Sunnyvale, CA) with ±3 cm accuracy. The DEM was used to relate water depth measured at two locations (30-min intervals) with water depth throughout

Table 1
Wetland physical and hydrologic characteristics.

Wetland attributes	
Total area (ha)	8.7
Flowpath	1.8
Fingers	2.2
Upland	4.7
Vegetation	*Typha latifolia*, *Polygonum lapathifolium*
Depth range (m)	0–1.3
Average temperature flowpath (°C)	22.3
Average temperature fingers (°C)	21.5
Hydraulic residence time-modeled (days)	0.9

the wetland, as well as to determine changes in the wetted surface area (calculated at each 30-min interval) throughout the irrigation season. Vertical hydraulic gradients were calculated at 12 piezometric monitoring locations in the southern section of the wetland, using biweekly water height measurements at 10- and 100-cm depths (Table 2). Surface water residence time was calculated using a plug-flow model (Gujer, 2008). Temperature was measured at 15-min intervals near the output.

Wetland evapotranspiration was estimated using meteorological data obtained from the California Irrigation Management Information System (CIMIS, 2007) Patterson station, approximately 15 km from the study site. ET rates for vegetated upland areas were presumed to approximate the CIMIS values calculated for grass cover. Evaporation for the sparsely vegetated wetland area was assumed to be 1.28 times that of the grass ET value (Snyder et al., 2005). ET volumes were calculated at 30-min intervals to account for fluctuations in the wetted surface area. A season-long seepage volume was calculated by subtracting total outflow volume from total inflow volume, accounting for water loss due to ET.

An independent measurement of the seepage rate for the northern and southern sections of the wetland (Fig. 1) was determined on 6/4/2007 through 6/9/2007 by preventing all inflow and outflow, and measuring the rate of water level drop over a 120-h period. Seepage volumes were then calculated for each 30-min interval by multiplying the seepage rate by the wetland wetted surface area (Table 2). Assuming similar seepage rates across the different hydrologic zones, we calculated the percentage of the water surface area covering each hydrologic zone at 30-min increments based on the high-resolution DEM and water height at the output location. The seepage volume was summed for each 30-min increment to obtain a total seepage volume for each hydrologic zone.

2.3. Water collection and analysis

Pore water was collected from piezometers at 12 locations (Fig. 1) on a biweekly basis at depths of 10, 50 and 100 cm below the soil surface. Screened sections of the piezometers were surrounded in a layer of pure silica sand and sealed above and below with bentonite clay to prevent water intrusion from adjacent horizons (Young, 2002). Prior to sampling, piezometers were purged and allowed to recharge for 1–2 h. Water samples were maintained at 3 °C between the time of collection and analysis (<24 h). Aliquots of samples were filtered through a pre-rinsed 0.4 μm polycarbonate membrane filter (Millipore) for quantification of NO_3-N (LOD ~ 0.01 mg L^{-1}), NH_4-N (LOD ~ 0.01 mg L^{-1}), and DOC (LOD ~ 0.1 mg L^{-1}). Determination of NO_3 was made using the vanadium chloride method (Doane and Horwath, 2003) and NH_4 using the Berthelot reaction with a salicylate analog of indophenol blue (Forster, 1995). DOC was measured using a Dohrmann UV enhanced-persulfate TOC analyzer (Phoenix 8000; Teledyne Tekmar, Mason, OH). A non-filtered sample was used to determine total N (TN) following oxidation with 1% persulfate using the method described above for NO_3-N. Surface water samples were collected adjacent to the piezometers and at input and output locations on a weekly basis and were analyzed as described above.

Depth splines were used to model nitrate distribution over the 100-cm depth of the piezometer monitoring nests. The segmentation procedure involved fitting an equal-area or mass-preserving quadratic spline across the discrete set of pore water NO_3-N sampling depths (10, 50 and 100 cm), producing a continuous depth function segmented at 1-cm intervals (Bishop et al., 1999; Malone et al., 2009). Mean values at each 1-cm depth increment were calculated across all sampling dates and sampling locations within each hydrologic zone. The segmenting algorithm was implemented using the 'GSIF' and 'aqp' packages for R (Beaudette et al., 2013).

Table 2
Summary of hydrologic parameters and water budget for the 2007 irrigation season.

Hydrologic characteristics	Value
Average inflow ($m^3\,d^{-1}$)	5232
Average outflow ($m^3\,d^{-1}$)	1394
Measured seepage rate northern section (cm d^{-1})	10.4 ± 2.2
Measured seepage rate southern section (cm d^{-1})	9.4 ± 5.0
Vertical hydraulic gradient	
Flowpath (m m^{-1})	0.27 ± 0.18
Fingers (m m^{-1})	0.33 ± 0.27
Upland (m m^{-1})	0.39 ± 0.21
Measured seepage estimate[a]	
Near input (m^3)	257,200 ± 55,700
Near output (m^3)	178,150 ± 95,000
Total seepage (m^3)	435,350 ± 150,700
Mass balance seepage calculation[b]	
Surface water input (m^3)	423,822
Evapotranspiration (m^3)	37,075
Total seepage (m^3)	302,840
Flowpath seepage (m^3)	139,108
Fingers seepage (m^3)	121,351
Upland seepage (m^3)	42,381
Surface water output (m^3)	83,907

Standard deviations follow ±symbols.
[a] Seepage volumes estimated using measured seepage rates.
[b] Seepage volumes estimated by subtracting total outflow volume from total inflow volume, accounting for water loss due to ET and wetted surface area of wetland zones.

2.4. Wetland N budget

Inflow and outflow seasonal loads for total nitrogen, nitrate, and ammonium were calculated using the period-weighted approach from weekly constituent concentration and weekly water flux (Moldan and Cerny, 1994). Nitrate seepage loads for each hydrologic zone were also calculated with the period-weighted approach using average biweekly nitrate concentration at the 100-cm depth and weekly seepage flux.

2.5. Denitrification potential (DNP)

Soil samples were collected on June 24, July 13, and August 16 in 2007, and analyzed for DNP. Samples were taken with an auger adjacent to the piezometer sites (n = 12), at depths of 10, 50 and 100 cm, placed on ice upon collection and maintained at 3 °C until analysis (<3 days). DNP was measured using the acetylene block technique (Tiedje, 1982; Hunt et al., 2006). Duplicate field moist subsamples (25 g) were placed in 125 mL Erlenmeyer flasks. A 25 mL volume of amendment solution was added to each sample. The amendments were ambient (distilled water), glucose (2 g L^{-1}), NO_3-N (200 mg L^{-1}), and glucose (2 g L^{-1}) plus NO_3-N (200 mg L^{-1}). All amendment solutions contained chloramphenicol (1 g L^{-1}) to inhibit microbial growth during the incubation period. The bottles were capped with septa stoppers and flushed with nitrogen gas at a flow rate of 1.5 L min^{-1} for two minutes. Then each bottle was injected with 15 mL of pure acetylene (generated from calcium carbide). Samples were incubated at room temperature (22 ± 2 °C; approximately mean field water temperature; Table 1) on an orbital shaker at 150 rpm. Headspace samples were taken at 30, 60 and 90 min for 50- and 100-cm depth samples, and at 10, 20 and 40 min for 10-cm depth samples. Gas samples were placed in Exetainer Borosilicate glass vials (Labco Limited, Buckinghamshire, UK), and analyzed for N_2O on a gas chromatograph with an electron capture detector (Hewlett Packard, Palo Alto, CA).

Denitrification rates were calculated from the linear portion of the curve produced when cumulative N_2O concentration was plotted against time (White and Reddy, 2003).

An estimate of nitrate removal attributable to denitrification was calculated to compare DNP values attained in the laboratory incubations with the loss of nitrate during seepage determined from piezometer samples. Mean depth-weighted denitrification potentials ($mg\ NO_3$-$N\ m^{-2}\ d^{-1}$) for each depth increment in each hydrologic zone were multiplied by the residence time (d) of the seepage water in each hydrologic zone and daily average wetted surface area (m^2) to yield daily nitrogen removal ($kg\ d^{-1}$) loads. These values were then summed to yield a seasonal nitrate removal load attributable to denitrification.

2.6. Soil analysis

Sub-samples of soil collected for the DNP experiment were analyzed for total organic carbon and total nitrogen by combustion using a C/N analyzer (Costech Analytical Technologies, Inc., Valencia, CA). Soil NO_3 and NH_4 concentrations were determined by 1 M KCl extraction (Mulvaney, 1996). Particle size distribution was measured using the hydrometer method (Gee and Or, 2002). Redox potential was measured in situ at one-minute intervals over a 6-month period during the irrigation season and compiled as hourly averages using a data logger (Campbell, Logan, UT) for the 12 monitoring sites. Platinum electrodes were placed in triplicate at each depth (10, 50 and 100 cm) and the average potential difference between the platinum electrodes and a calomel reference electrode was measured (Mitsch and Gosselink, 2000); results are reported on a standard H^+ reference electrode basis.

2.7. Statistical analysis

Linear mixed effects models were used to analyze data from water analysis and DNP incubations using S-Plus (Insightful Corp., 2001). As samples were taken at the same location several times throughout the season, location was treated as a random effect in the model to account for autocorrelation between measurements at the same site. The NH_4-N, NO_3-N, DNP and DOC values were log transformed prior to statistical analysis to better approximate a normal distribution. For each analysis, the initial model accounted for main effects, as well as all possible two-way interactions between main effects. Interactions that were not significant were removed from subsequent models to gain sensitivity. Mean separation was determined using a conditional *t*-test. Raw (non-transformed means) are reported in Tables 4 and 5 to reflect measured field conditions. Constituent values are reported as mean ± standard deviation unless otherwise indicated.

3. Results

3.1. Wetland hydrology

A plug flow model for the wetland estimated a hydraulic residence time (HRT) of 0.9 days for surface waters at the average inflow rate of $5232\ m^3\ d^{-1}$ (Table 1). Inflow and outflow rates were highly variable throughout the irrigation season with inflow rates decreasing to zero in August (Fig. 2a). Irrigation season water flux was substantially higher at the input ($423{,}822\ m^3$) compared to the output ($83{,}907\ m^3$) (Table 2). Evapotranspiration accounted for approximately 9% of the water budget ($37{,}075\ m^3$). Using mass balance calculations, seepage accounted for $302{,}840\ m^3$ or roughly 72% of the water balance (Table 2). The total wetland seepage budget estimated from measured seepage rates was $435{,}350 \pm 150{,}700\ m^3$, which agrees reasonably well (±1SD) with our mass balance calculation ($302{,}840\ m^3$). Seepage rates were similar across the wetland ranging from $10.4 \pm 2.2\ cm\ d^{-1}$ in the southern section to $9.4 \pm 5.0\ cm\ d^{-1}$ in the northern section (Table 2). Moreover, similar vertical hydraulic gradients were found within each of the three hydrologic zones (Table 2). Given the high variability associated with the measured seepage values and similar vertical hydraulic gradients, we chose to carry out all further estimates of the N- and hydrologic-budgets using the mass balance approach.

The wetted surface area of the wetland varied based on water height and ranged from 1.8 to 8.7 ha, with a mean surface area of 4.0 ha during the irrigation season (Table 1 and Fig. 1). Highly variable inflow water fluxes resulted in fluctuating water levels throughout the study period. Upland zones were dry 47% of the time during the irrigation season. The finger zone experienced dry conditions 10% of time, while the flowpath zone remained permanently flooded (Fig. 2b). As a result, seasonal seepage volumes differed among the hydrologic zones due to differences in hydroperiod. As a percentage of total seepage, the flowpath zone accounted for 46% ($139{,}108\ m^3$), the finger zones 40% ($121{,}351\ m^3$), and the upland zones 14% ($42{,}381\ m^3$) (Table 2).

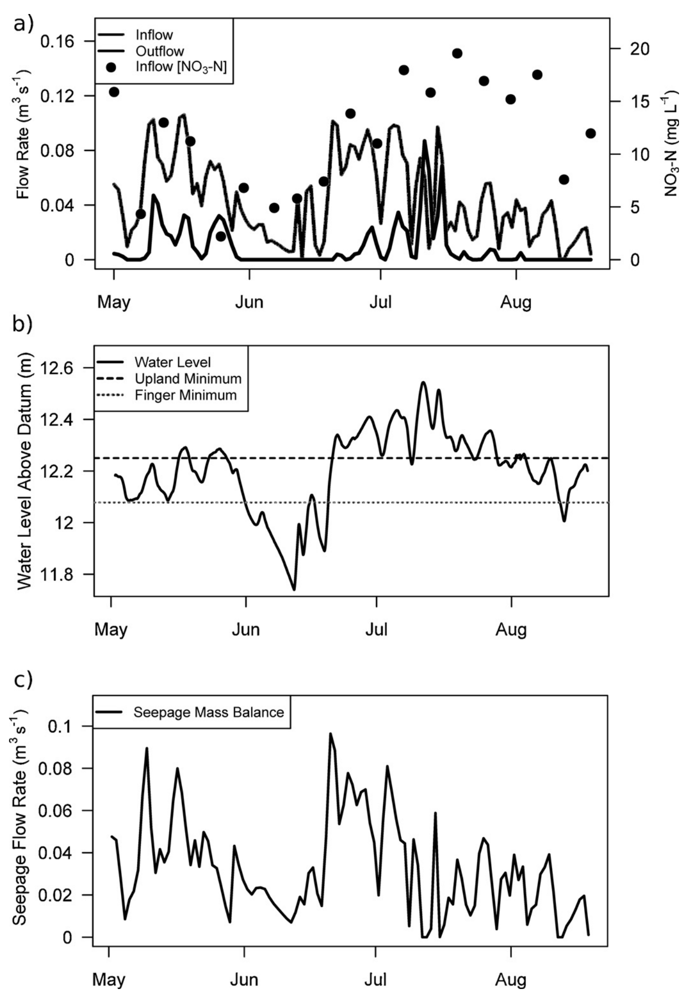

Fig. 2. (a) Temporal trends in flow rate (input and output) and nitrate input concentration over the 2007 irrigation season; (b) temporal fluctuations in wetland water depth as measured above reference datum. Black horizontal dashed line shows the elevation at which the surface of the upland zone is completely dry (upland minimum) and the grey horizontal dashed line shows the elevation at which the surface of the finger zone is completely dry (finger minimum); and (c) temporal trends in seepage estimated from the mass balance.

3.2. Surface water quality

Input concentrations of NO_3-N were highly variable throughout the irrigation season (Fig. 2). Nitrate was the dominant form of nitrogen in both inflow (~80%) and outflow (~81%) waters (Table 3). Nitrogen concentrations in the water column varied among hydrologic zones (Table 4). Mean NO_3-N concentrations were twice as high in the finger and flowpath zones compared to uplands. Mean DOC concentration did not vary significantly among hydrologic zones or between inflow and outflow (Tables 3 and 4).

3.3. Seepage water quality

The water sampled from piezometers (10-, 50- and 100-cm) was termed seepage water. Nitrate concentration was markedly lower in seepage water than in surface waters (Table 4). Concentrations of NO_3-N were significantly ($p < 0.05$) lower at the 50-cm depth than the 10-cm depth, but there was not a significant difference in NO_3-N concentrations between the 50- and 100-cm depths among the three hydrologic zones (Fig. 4). Modeled nitrate removal rates from Fig. 4 in the top 10-cm soil depth relative to the water column were 932, 631 and 143 mg NO_3-N $m^{-2}\,d^{-1}$ in the flowpath, finger and upland zones, respectively.

In the wettest hydrologic zones (fingers and flowpath) there was a significant increase in NH_4-N concentrations from the surface water to the 10-cm depth (Table 4). NH_4-N concentrations decreased at the 50- and 100-cm depths and were not significantly different from those in the surface waters (Table 4).

DOC concentration in seepage water ranged from 3.2 to 6.0 mg L^{-1} (Table 4). There were no significant differences in DOC between the surface water, 10-, and 50-cm depths; however, DOC concentration decreased significantly at the 100-cm depth of the upland sites. Among the hydrologic zones, DOC in seepage water was significantly higher in the uplands (Table 4).

3.4. Soil physical and chemical characteristics

Soil texture was generally similar among hydrologic zones and no abrupt changes in texture were observed with depth (Table 5). Sedimentation was highest in the flowpath zone totaling over 35 kg $m^{-2}\,yr^{-1}$ compared to sedimentation rates <5 kg $m^{-2}\,yr^{-1}$ in the fingers and uplands. Saturated hydraulic conductivities estimated for these textural classes were similar to measured seepage rates (Table 2; USDA-NRCS, 2014).

Average soil organic carbon concentration was relatively low in all hydrologic zones (Table 5). Organic carbon decreased with depth in all hydrologic zones. Average total nitrogen was similar across all hydrologic zones and decreased with depth. The C:N ratio ranged from 8.9 to 11.7 and was relatively consistent with depth in all hydrologic zones (Table 5). Average KCl-extractable NO_3 and NH_4 were highest in the flowpath zone (Table 5) and generally decreased with depth.

3.5. Redox potential

Redox potential was predominantly below nitrate reduction levels (~250 mV) in the fingers and flowpath (Fig. 3), which were submerged with water 90% and 100% of the time during the irrigation season (Table 5 and Fig. 2b). In upland zones, redox potentials periodically exceeded nitrate reduction levels, and were below 250 mV 17% of the time at the 50-cm depth, and 39% of the time at the 100-cm depth. Upland zones were submerged 53% of irrigation season from late June through late July (Fig. 2b), corresponding to low but variable Eh values (Fig. 3). Large swings in Eh occurred over 24–48 h in response to fluctuations in water level (Figs. 3 and 2b), indicating that nitrate reduction levels were reached shortly after inundation.

3.6. Denitrification potential (DNP)

DNP was highest in the flowpath zone at the 10-cm depth and increased when nitrate, or a combination of glucose and nitrate was added (Table 6). In the flowpath zone at the 10-cm depth, mean DNP increased from 151 to 2437 mg NO_3-N $m^{-2}\,d^{-1}$, when nitrate was added over ambient conditions. In contrast, there was very little response when nitrate was added at the lower depths.

Table 3
Water quality summary of surface water input and output locations ($n = 25$) during the 2007 irrigation season (mean ± standard deviation).

Water quality parameter	Input	Output
EC ds m^{-1}	1.54 ± 0.36	1.44 ± 0.27
pH	8.6 ± 0.4	8.4 ± 0.6
DO mg L^{-1}	9.64 ± 1.66	6.44 ± 2.23*
TN mg L^{-1}	16.17 ± 7.38	9.76 ± 5.24*
NO_3-N mg L^{-1}	12.87 ± 6.43	7.87 ± 4.69*
NH_4-N mg L^{-1}	0.87 ± 1.40	0.35 ± 0.46
DOC mg L^{-1}	4.3 ± 1.3	4.3 ± 1.2

EC: electrical conductivity; DO: dissolved oxygen; TN: total nitrogen; DOC: dissolved organic carbon.
* Asterisk adjacent to output values indicates significant differences ($p < 0.05$) with respect to the input.

Table 4
Sample mean (±standard deviation) and statistical groupings for NH_4-N, NO_3-N and DOC for pore water samples for specific depths and hydrologic zones in the wetland. Water column samples were collected at each piezometer location and do not include values from input and output locations.

Depth	Wetland hydrologic zones		
	Flowpath	Finger	Upland
mg NH_4-N L^{-1}			
Water column	0.57_{Ax} (0.74)	1.18_{Ax} (1.90)	0.39_{Ax} (0.39)
10 cm	4.07_{Ay} (1.99)	1.61_{By} (1.58)	0.18_{Cy} (0.09)
50 cm	1.57_{Axy} (1.88)	0.55_{Bxy} (0.54)	0.88_{By} (1.55)
100 cm	1.35_{Axy} (1.47)	0.45_{Axy} (0.41)	0.23_{Axy} (0.24)
mg NO_3-N L^{-1}			
Water column	13.00_{Ax} (3.86)	15.13_{Bx} (7.50)	6.45_{Ax} (5.61)
10 cm	3.35_{Ay} (2.78)	8.21_{By} (7.54)	3.22_{Ay} (4.30)
50 cm	0.67_{Az} (1.42)	4.72_{Bz} (5.24)	1.55_{Az} (2.73)
100 cm	0.73_{Az} (1.46)	3.06_{Bz} (2.68)	1.34_{Az} (1.29)
mg DOC L^{-1}			
Water column	3.68_{Ax} (1.17)	3.61_{Ax} (0.75)	4.78_{Ax} (1.94)
10 cm	3.79_{Ax} (1.64)	3.87_{Ax} (1.52)	5.11_{Bxy} (1.97)
50 cm	3.93_{Ax} (1.04)	3.27_{Ax} (0.69)	6.00_{By} (1.83)
100 cm	3.45_{Ax} (0.84)	3.15_{Ax} (0.75)	3.69_{Bx} (1.18)

Uppercase letters (A, B, C) denote statistical groupings among environments for a given depth $p < 0.05$. Lowercase letters (x, y, z) denote statistical groupings among depths for a given environment $p < 0.05$. In the water column, $n = 15$, 30, and 3 for flowpath, finger, and upland zones, respectively. At 10 cm, $n = 20$, 36, and 7 for flowpath, finger, and upland zones, respectively. At 50 cm, $n = 19$, 31, and 10 for flowpath, finger, and upland zones, respectively. At 100 cm, $n = 20$, 38, and 18 for flowpath, finger, and upland zones, respectively.

Table 5
Mean (±standard deviation) of soil chemical and physical parameters at the 10, 50, and 100 cm depths in each of the three hydrologic zones in the wetland ($n = 12$).

Hydrologic zone	Depth	Total C g kg^{-1} soil	Total N g kg^{-1} soil	C:N	KCl-NO_3-N mg kg^{-1} soil	KCl-NH_4-N mg kg^{-1} soil	pH	% time below 250 mV	Sand%	Silt%	Clay%
Flowpath	10 cm	10.1 (1.2)	1.13 (0.1)	8.94 (0.2)	7.22 (1.1)	27.92 (8.2)	7.04 (0.1)	97 (2.6)	8 (<0.1)	50 (0.2)	42 (0.2)
	50 cm	6.69 (1.2)	0.67 (0.1)	9.94 (0.4)	2.97 (0.6)	1.63 (0.8)	7.17 (<0.1)	100 (0.1)	10 (5.4)	54 (7.3)	39 (2.0)
	100 cm	5.14 (0.4)	0.53 (<0.1)	9.61 (0.5)	1.67 (0.4)	0.38 (0.1)	7.16 (0.1)	100 (0.1)	10 (3.1)	55 (1.3)	35 (4.1)
Finger	10 cm	9.53 (0.8)	0.97 (<0.1)	9.87 (0.2)	4.87 (1.3)	4.11 (1.7)	7.10 (<0.1)	100 (0.1)	22 (1.5)	44 (1.4)	34 (1.7)
	50 cm	7.18 (0.5)	0.67 (<0.1)	10.78 (0.4)	2.77 (0.5)	0.42 (0.1)	7.12 (<0.1)	100 (0.1)	14 (1.5)	54 (0.9)	33 (1.4)
	100 cm	5.57 (0.4)	0.52 (<0.1)	10.75 (0.2)	1.43 (0.4)	0.42 (0.1)	7.20 (0.1)	100 (0.1)	10 (1.7)	53 (2.4)	37 (2.0)
Upland	10 cm	14.30 (1.0)	1.23 (<0.1)	11.66 (0.5)	1.61 (0.1)	0.99 (0.3)	6.83 (<0.1)	27 (13.4)	29 (3.2)	39 (1.6)	32 (2.8)
	50 cm	9.15 (1.4)	0.84 (0.1)	10.91 (0.4)	1.36 (0.3)	0.12 (<0.1)	6.90 (0.1)	17.2 (6.7)	16 (2.8)	48 (2.8)	36 (2.8)
	100 cm	6.02 (0.4)	0.57 (<0.1)	10.54 (0.8)	0.36 (0.3)	0.03 (<0.1)	7.02 (<0.1)	39 (4.9)	12 (2.9)	51 (4.3)	37 (1.6)

C:N calculated on a mass basis.

There was no significant difference in DNP for any wetland zone between ambient conditions and glucose C-source amendment. However, adding glucose and nitrate significantly increased DNP in all three hydrologic zones (Table 6). The largest increase was seen in the flowpath zone soils at 10 cm (Table 6). In the upper 10 cm, it is also notable that the maximum measured DNP under non N-limiting conditions was much higher in the flowpath zone.

DNP was relatively low and similar for the 50- and 100-cm depths for all hydrologic zones and amendments. Amending the 50- and 100-cm depth soils with glucose or nitrate produced no significant response in DNP. When glucose and nitrate were added there was a slight increase in DNP in a few instances (Table 6).

Several factors and combinations of factors appeared to influence DNP in this wetland. The first statistical model tested was the most complex and hypothesized that $\log_{10}$ DNP was the result of wetland zone, sample depth, amendment, and soil organic carbon content. The two-way interactions of depth and hydrologic zone, depth and amendment, and hydrologic zone and amendment were also included in this preliminary model. Soil organic carbon content had no significant effect on DNP. There were also no significant interaction effects between depth and hydrologic zone, or zone and amendment.

Depth had a highly significant effect on DNP ($p < 0.001$). DNP was an order of magnitude higher at 10 cm than at the 50- or 100-cm depths for all treatment/wetland zone combinations (Table 6). There was no significant difference in DNP between the 50- or 100-cm depths. Because of the huge disparity in DNP among depths, the 10-cm depth was separated from the 50- and 100-cm depths for further analysis.

At the 10-cm depth, both wetland zone and amendment had a significant effect on DNP, but there was no significant interaction between amendment and wetland zone. With all amendments, DNP showed the following pattern among hydrologic zones: flowpath > fingers > uplands (Table 6). This same pattern was observed with the nitrate removal rates that were calculated from piezometer/pore water depth profiles. The calculated removal rates (top 10 cm) were similar in magnitude to DNP removal rates.

3.7. Nitrate budget

The wetland was highly effective at removing nitrate with an estimated 75% total NO_3-N removal efficiency for surface and subsurface flowpaths combined (Table 7). The wetland received 5127 kg of NO_3-N from input water originating from agricultural return flows and exported 714 kg of NO_3-N (~14% of input load) in output water during the 6-month irrigation season (Table 7). Approximately 4122 kg NO_3-N (80% of input load) infiltrated into the wetland soil as seepage, and of this amount, 547 kg NO_3-N (13% of total seepage input load) was lost as seepage below 100 cm. Thus, 3866 kg of the inflowing NO_3-N load was either immobilized biologically via plant and microbial uptake or lost from the system via biotic and abiotic transformations (e.g., denitrification) (Table 7).

Patterns of N loading (TN, NO_3, and NH_4) from inflows were similar among zones; increasing in the middle of the season

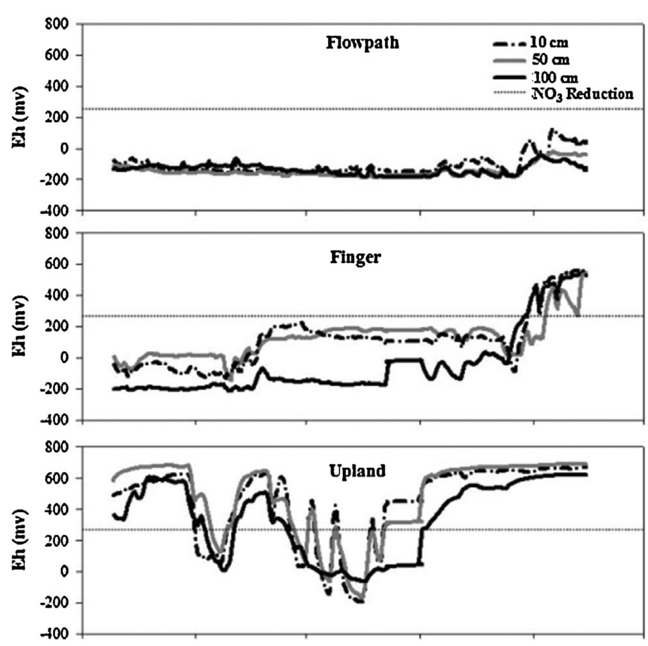

Fig. 3. Redox status (hourly means) for each hydrologic zone at 10-, 50- and 100-cm depths. Nitrate reduction threshold level (~250 mV) is shown by the dotted line.

Table 6
Sample mean, statistical groupings and maximum measured denitrification potential for specific depths and hydrologic zones in the wetland ($n = 12$).

Depth	Amendment[a]	Wetland hydrologic zone								
		Flowpath			Finger			Upland		
		$mg\ NO_3\text{-}N\ m^{-2}\ soil\ d^{-1}$								
		Mean	SD[b]	Max	Mean	SD	Max	Mean	SD	Max
10 cm	Ambient	151_{Ax}	212	702	98_{Bx}	194	1026	42_{Cx}	87	355
	C	249_{Ax}	388	1365	115_{Bx}	316	1877	32_{Cxy}	53	236
	N	2437_{Ay}	4046	15145	104_{Bx}	255	1505	20_{Cx}	22	75
	C + N	3168_{Az}	4323	13089	274_{Bz}	625	2946	57_{Cy}	58	259
50 cm	Ambient	11_{Aw}	9	26	14_{Aw}	16	89	12_{Aw}	10	33
	C	12_{Aw}	12	45	14_{Aw}	18	94	13_{Aw}	23	100
	N	19_{Av}	13	49	12_{Aw}	13	75	12_{Aw}	11	46
	C + N	16_{Awv}	17	64	25_{Av}	64	374	22_{Av}	37	259
100 cm	Ambient	11_{Aw}	6	26	56_{Awv}	187	1018	11_{Aw}	11	38
	C	11_{Aw}	7	27	24_{Aw}	76	459	9_{Aw}	6	93
	N	6_{Aw}	5	18	10_{Aw}	9	37	18_{Aw}	24	93
	C + N	19_{Awv}	21	99	12_{Aw}	8	40	13_{Aw}	9	37

Uppercase letters (A, B, C) denote statistical groupings among environments $p < 0.05$. Lowercase letters (v, w, x, y) denote statistical groupings among depths $p < 0.05$.
[a] C: glucose; N: nitrate.
[b] SD: standard deviation.

(Fig. 5a–c). Outflow N loads were consistently low throughout the study period (Fig. 5). NO_3 loads lost via deep seepage were low during the beginning of the season (May–June) and remained low in flowpath and upland zones. In the finger zone, however, a dramatic increase in NO_3 seepage loads occurred from late June through September (Fig. 5d). Seasonal retention efficiencies for NO_3-N loads in seepage water were 95, 81, and 70% for the flowpath, finger, and upland zones, respectively (Table 7). A moderate decrease in surface water NO_3-N concentration between inflow (12.87 mg L^{-1}) and outflow (7.87 mg L^{-1}) locations indicates some NO_3-N removal via surface processes (e.g., algal uptake, diffusion into soil), however, high measured rates for DNP in surface soil (10-cm depth) and significantly lower pore water NO_3-N concentrations at 50- and 100-cm depths indicate that subsurface denitrification was a dominant nitrogen removal mechanism (Tables 4 and 6).

Notably, the NO_3-N removal rate estimated via non-nitrate limited DNP values considering all wetland zones was similar to that calculated from the mass balance. Considering all hydrologic zones, NO_3-N removal estimated from DNP was 5085 kg NO_3-N. This estimate was slightly higher than the estimate of NO_3-N removed via the mass balance (3866 kg NO_3-N) (Table 7).

4. Discussion

4.1. Nitrate mass balance

Despite the large amount of water lost as vertical seepage (~72%), overall NO_3-N removal was high (~75%) in this restored wetland and comparable to that of other regions with temperate climates. Other studies of wetlands receiving agricultural runoff report NO_3-N removal efficiencies ranging from 0 to as high as 99% (summarized by O'Geen et al., 2010). Comparisons of wetland-N treatment capability, however, is challenging in agricultural settings, because climate, flow characteristics (e.g., flow pulses), N species and N load vary across a wide range of temporal and spatial scales. Wetland characteristics (shape, size, depth, age, soil characteristics and vegetation) also vary widely (O'Geen et al., 2010).

The fact that there was no significant difference between NO_3 concentrations at the 50- and 100-cm depths for a given wetland zone suggests that nearly all NO_3 removal in this system occurred at depths above 50 cm. Depth profiles suggest that nitrate removal is uniformly low at depth across all wetland zones (Fig. 4). Trends in DNP for N-unlimited conditions (C + N amended soils) were consistent with the nitrate losses observed in piezometer water samples.

4.2. Denitrification in wetlands

Denitrification potentials in this wetland were highly variable depending on amendment, depth and wetland environment. DNP measured in this study, ranged from non-detectable to over 15,000 mg NO_3-N m^{-2} d^{-1} (Table 6), which spans the range of DNP rates reported by several studies (Gale et al., 1993; Hunt et al., 2006; Zaman et al., 2008). Average DNP in the main flowpath zone (2437 mg NO_3-N m^{-2} d^{-1} in 10-cm soil depth of N amended treatment) was higher than rates reported from wetlands receiving agricultural runoff in other regions, however, DNP in fingers and uplands (20–104 mg NO_3-N m^{-2} d^{-1} in 10-cm soil depth of N amended treatments) was similar to that in other studies (Xue et al., 1999; Smith et al., 2000; Poe et al., 2003).

Wetland soil properties that influence spatial patterns in denitrifying bacterial communities are pH, redox potential, temperature, soil texture, labile organic carbon, and nitrogen (D'Angelo and Reddy, 1999; Hill and Cardaci, 2002; Bruland and

Table 7
Wetland hydrologic and nitrate mass balance for the 2007 irrigation season.

	NO_3-N kg	Retention%
Surface water input	5127	
Evapotranspiration	–	
Total seepage input	4122	
Flowpath seepage input	1929	
Fingers seepage input	1880	
Upland seepage input	313	
Total seepage output	547	87
Flowpath seepage output	103	95
Fingers seepage output	349	81
Upland seepage output	95	70
Surface water output	714	–
NO_3-N removed	3866	75

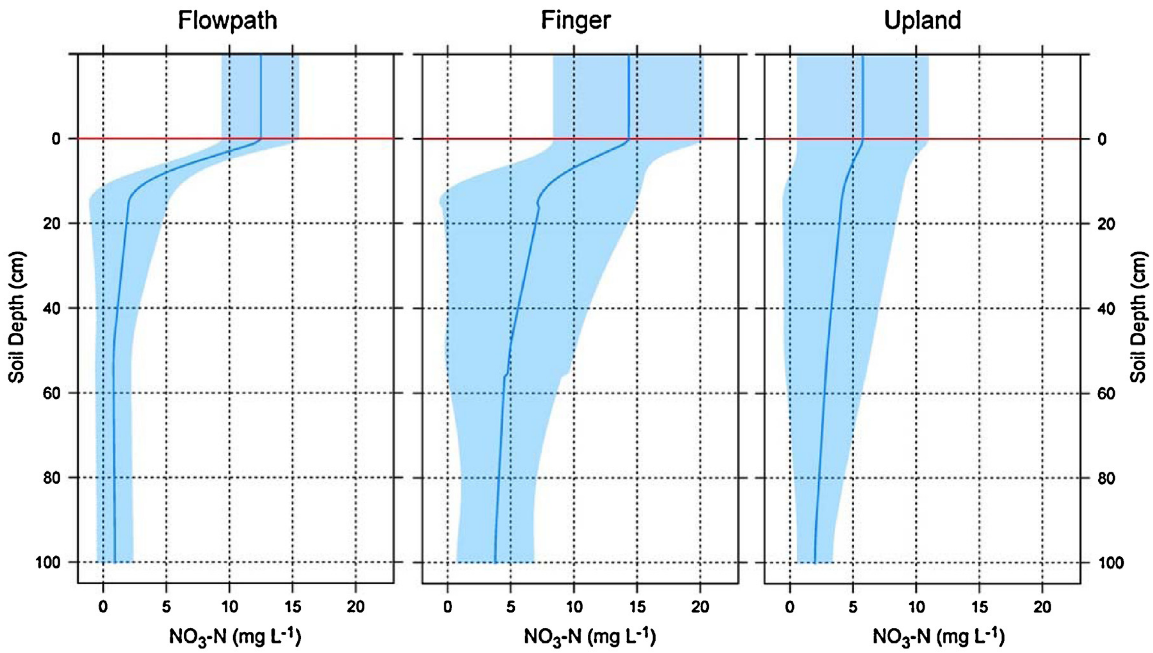

Fig. 4. Spline functions of wetland pore water NO_3-N depth distribution for each hydrologic zone. Solid blue line shows the mean NO_3-N value for each hydrologic zone. Shaded light blue area represents ±1 standard deviation of the mean. The solid horizontal red line represents the soil–water column interface, with all values above the red line showing the water column NO_3-N concentration. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Richardson, 2006; Burchell et al., 2007; Hernandez and Mitsch, 2007). With the exception of KCl-extractable N these properties were similar throughout the wetland in the upper 10 cm, so it is hard to assess the apparent differences in denitrifier activity based on these soil properties alone (Table 5). Organic carbon, KCl-extractable N, and pore water nitrate were substantially lower at the 50- and 100-cm depths, so it is possible that denitrifier activity was limited at the lower depths by lack of substrate. The observed lower denitrification potentials at depth are consistent with other studies of constructed wetlands (Zaman et al., 2008).

Some studies in constructed wetlands have found DNP to be spatially uniform (Bruland and Richardson, 2006). In contrast, we found large differences in space, with DNP being higher in the main flowpath. Differences in DNP between hydrologic zones at the 10-cm depth may be explained by spatial variability in organic carbon content, differences in redox potential, sedimentation and organic matter quality. Highly variable inflow

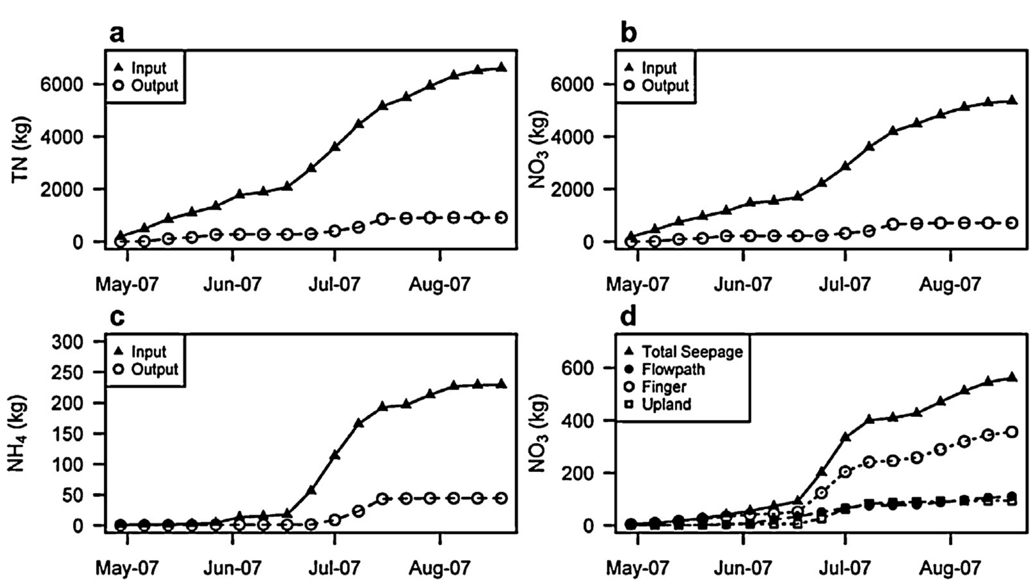

Fig. 5. Cumulative load for (a) total nitrogen, (b) nitrate, (c) ammonium, at wetland input and output locations, and (d) nitrate exported via deep seepage in the different hydrologic zones.

water fluxes resulted in fluctuating water depths across the wetland, with brief dry-down periods in the finger zones and long dry periods in the upland zones (Fig. 2a and b). Higher redox potentials in the upland and finger zones may have contributed to spatial differences in DNP (Fig. 3 and Table 6). Many studies report that DNP is more strongly correlated with available carbon (e.g., microbially labile C) rather than total organic carbon (D'Angelo and Reddy, 1999; Hill and Cardaci 2002; Hernandez and Mitsch, 2007; Puckett et al., 2008). Only organic carbon was measured for this study, so it is possible that there may be substantial differences in carbon availability between the environments that may affect DNP. Also, DOC, which may serve as an important energy source for denitrifiers, was relatively constant across hydrologic zones and soil depths.

Sediment deposition in the flowpath zone was substantially higher than in the fingers or upland zones, which offers a possible explanation for the disparities in DNP despite similar soil conditions (Table 6). Areas of active sediment deposition may receive organic matter of different quality compared to that of the native soil from which the wetland was constructed (Baskerud, 2002). It is also possible that the sediment, which originated from surrounding farmland, is a seed source of denitrifying bacteria. In fact, studies have shown that frequently tilled agricultural soils in the region have more facultative anaerobes and higher denitrification rates compared to untilled soils (Calderon et al., 2001).

Since this wetland has only received tailwaters for two seasons, it is plausible that we are witnessing the initial stages of recruitment of microbial populations and the associated evolution of wetland biogeochemical processes. Thus, in older wetlands DNP may be expected to be more uniform. Other studies have shown that spatial variation in denitrification corresponds to patterns in nitrate concentration, increasing in areas of high N loading (Poe et al., 2003). Hernandez and Mitsch (2007) found higher denitrification potentials in constructed wetland soils where emergent macrophytes were present, when compared to unvegetated constructed wetland sediments. Since vegetation was sparse in both the finger and the flowpath zones, it is unlikely that the relative amount of vegetation had much effect on the observed denitrification potentials.

A disproportionately high amount of the nitrogen was removed in the flowpath zone (1826 kg, 95% retention) compared to the fingers (1531 kg, 81% retention) and uplands (218 kg, 70% retention) (Table 7). This trend was a result of higher nitrate loading rates and significantly higher DNP rates in the flowpath compared to other hydrologic zones. The higher mean N-amended DNP rates in the flowpath suggest a larger denitrifier microbial population in this zone. The finger zone, although accounting for 40% of seepage, is responsible for the majority (64%) of NO_3-N lost via deep seepage. This was the result of significantly lower DNP rates relative to the flowpath zone and significantly higher pore water NO_3-N concentrations at the 100-cm depth (Tables 4 and 6). As with any biological process, temperature strongly regulates denitrification rate (Pfenning and McMahon, 1996). Lab incubations were performed at the mean field temperature (~22 °C), which was similar to the mean temperature of the flowpath (22.3 °C) and 0.5 °C higher than that of the fingers (21.5 °C). Warm daytime temperatures (average maximum daily temperature ~29 °C) are likely to substantially increase denitrification rate over diurnal timescales.

4.3. Other nitrate removal processes

Other NO_3-N removal pathways may play an important role in this wetland. NH_4 accumulation in pore water, and elevated KCl-extractable NH_4 concentrations in the soil at 10 cm suggests that sulfur or ferrous iron-driven nitrate reduction (DRNA) may play a role in nitrogen cycling in this system (Burgin and Hamilton, 2007). Redox potential frequently reached the sulfate reduction level (−200 mV) (Fig. 3) suggesting the presence of free sulfide. Anecdotal evidence such as H_2S smell in groundwater samples, as well as visual identification of iron monosulfides (black masses and coatings) in the sediment verifies the presence of sulfide in the system (Maynard et al., 2011). At high concentrations, free sulfide is known to inhibit the final two reduction steps in the denitrification sequence, which may drive the reduction to ammonium rather than N_2O and N_2 (Burgin and Hamilton, 2007). Sorption of ammonium from seepage water to cation exchange sites in the soil may also account for accumulation of ammonium in the upper 10 cm of sediment (Austin, 2006). Equilibrium with the sediment bound ammonium would result in elevated ammonium concentrations in the associated pore water.

Despite the predominately unvegetated main flowpath, plant uptake (including algae) may play a substantial role in nitrogen cycling in this wetland. There may be diffusion of NO_3-N from surface water into the upland areas via the shallow water table (30–70 cm) in the upland zone located approximately at the same elevation as the wetland water surface. The dense vegetation in the upland areas may assimilate a significant amount of N thereby increasing N removal rates.

5. Conclusions

This study demonstrated that soils of recently restored wetlands have the capacity to remove large nitrate loads from vertically percolating water with low risk to groundwater in California's Central Valley. Biogeochemical processes in this wetland (respiratory denitrification, possibly DNRA and plant uptake) facilitated significant removal of nitrate inputs from agricultural tailwaters. The active flowpath of the wetland had the highest DNP at the 10-cm depth under all N amended conditions, and also experienced the greatest sediment deposition rates, nitrogen load and seepage volume (46% of seepage). While the flowpath had a significantly higher DNP relative to the other zones; the finger environments had a significantly higher DNP relative to the upland environments. These significant differences in DNP between zones may have resulted in the substantial differences in NO_3 removal efficiencies, with 95, 81 and 70% reduction in NO_3 seepage load in the flowpath, finger and upland zones, respectively (Table 7). Nevertheless, high NO_3 removal efficiency in the flowpath resulted in a high overall net decrease of NO_3 load (87%) from seepage water for the entire wetland.

In contrast to the notion that seepage water from wetlands may be considered as a source of groundwater nitrate contamination, this study shows that under the conditions present in our wetland, seepage through wetland soils can actually prevent some nitrate contamination of groundwater. Before recommending constructed wetlands that utilize seepage as a beneficial-management practice for treating agricultural tailwaters, further study is necessary to determine the fate and transport of other contaminants (e.g., pesticides, phosphate, salts). Studies are also needed to evaluate long-term nitrate removal efficiency over the life of these wetlands. While sealing the constructed wetland floor is considered an important aspect of treatment-wetland design, as it prevents the seepage of contaminants into groundwater bodies (Brodie, 1989; United States Environmental Protection Agency, 1995), it is not economically practical in most agricultural settings. Moreover, sealing wetlands can discourage surface water exchange with soils, which is where denitrification is most favorable.

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