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INVESTIGATION OF DENITRIFICATION RATES IN AN AMMONIA-DOMINATED CONSTRUCTED WASTEWATER-TREATMENT WETLAND

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Abstract: Denitrification measurements were made under simulated field conditions using sediment cores and water collected from the Heinet/San Jacinto Multipurpose Demonstration Wetland (Riverside County, California, USA). The 9.9 ha constructed wetland is used to both polish ammonia-dominated secondary municipal effluent and provide migratory bird habitat. The wetland was originally constructed as a marsh-pond-marsh system in 1994. Over the period from January through March 1998, measured denitrification rates averaged 20.9 ± 20.9 μmol N m^{-2} h^{-1} within the emergent marsh portions of the wetland and 646 ± 353 μmol N m^{-2} h^{-1} in open water areas. The mean areal denitrification removal rate for this period was 0.70 kg N ha^{-1} d^{-1}, which accounted for about 8% of the total N removed by the wetland. Internal retention was the main N-removal mechanism. Synoptic water quality surveys indicated that denitrification was limited by a lack of nitrification within the wetland. Between April 1998 and January 1999, the wetland was reconfigured as a hemi-marsh system, having equal areas of interspersed emergent marsh and deep open water. In May 1999, measured denitrification rates averaged 1414 ± 298 μmol N m^{-2} h^{-1} within the emergent marsh zones and 682 ± 218 μmol N m^{-2} h^{-1} in the open water areas. The mean areal denitrification removal rate was 3.58 kg N ha^{-1} d^{-1}, which accounted for 40% of the total N removed by the wetland. A synoptic water quality survey indicated that nitrification within the wetland had been enhanced by the reduction of emergent macrophyte biomass and the increase in the area of interspersed deep open water. The modifications to the wetland shifted the nitrogen balance from a large internal storage component and a small denitrification component in 1998 to a more denitrifying system in 1999.

Key Words: constructed wetlands, nitrogen removal, denitrification

INTRODUCTION

Freshwater wetlands are considered a sink for natural and anthropogenic nitrogen (N). They have been shown to trap, store, transform, and release nutrients, thus significantly modifying the chemistry of the water flowing through them and affecting the water quality downstream (Hemond and Benoit 1988, Whigham et al. 1988). Many studies have shown that treatment wetlands reduce the quantity of N flowing through them (Grant and Patrick 1970, Fetter et al. 1978, Dierberg and Brezonik 1984, Knight and Winchester 1985, DeBusk and Reddy 1987, Knight et al. 1987, Knight and Ferda 1989, Gearheart 1992, Kadlec 1993, 1994, Patruno and Russell 1994). The general perception that wetlands improve water quality through the reduction of N has led to their use for the disposal and polishing of wastewater in many parts of the world (Mitsch and Gosselink 1993). However, the pathways by which wetlands process and retain N inputs are not well-understood (Nixon and Lee 1986, Johnston 1991, Cole 1998).

Typically, a "black box" approach has been used to demonstrate the efficacy of wetlands in decreasing N concentrations in wastewater effluent (i.e., Dierberg and Brezonik 1984, Bowden 1986, Gersberg et al. 1986, Cole 1998, Frankenbach and Meyer 1999). These studies treat the whole wetland as a storage unit, and the fluxes of N-species into and out of the wetland are measured to derive the net effect on water quality. Denitrification has often been invoked as the major pathway of N removal from wetlands (Reed et al. 1988, USEPA 1988), including wetlands that are dom-

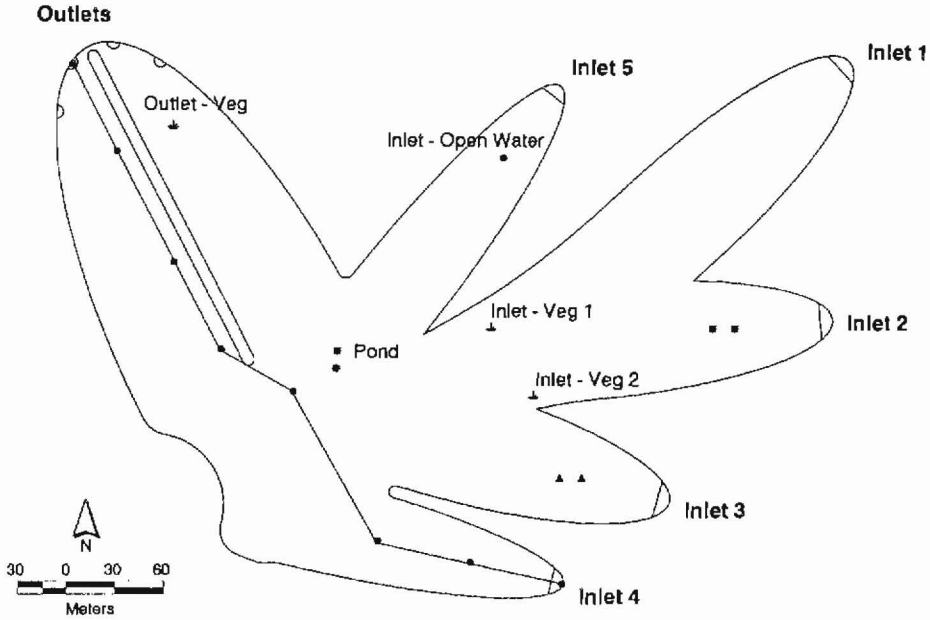

Figure 1. Location of the sampling sites within the Hemet/San Jacinto Demonstration Wetland. In 1998, the sites were: Pond, Inlet—Open Water, Inlet—Veg 1 (*Schoenoplectus acutus*).and Inlet—Veg 2 (*Schoenoplectus californicus*). In 1999, we included a fifth sampling site in the outlet (Outlet—Veg, *Schoenoplectus californicus*). The asterisks indicate where the Hydrolabs were deployed in 1998, and the triangles indicate where they were deployed in 1999. The transect line indicates where the data were collected to generate Figure 3.

inated by ammonia, where a close coupling of the processes of nitrification and denitrification has been assumed, based on studies such as those by Reddy et al. (1989) and Yoh et al. (1990). However, quantification of denitrification rates within constructed wetlands has been largely neglected (Nixon and Lee 1986, Bowden 1988, Cole 1998, Xue et al. 1999). More importantly, few measurements of denitrification under field conditions have been made in either natural or constructed wetlands (Seitzinger 1994), and we know of none from ammonia-dominated treatment wetlands.

The goal of this study was to determine if denitrification is the primary removal mechanism of N within a constructed wetland used to polish ammonia-dominated secondary effluent. The quantity of N removed by the wetland was determined through inflow/outflow mass balances, and the rates of denitrification were quantified in the laboratory under simulated field conditions. These rates were compared to the total quantity of nitrogen removed during transit through the wetland to determine the relative importance of denitrification within the treatment wetland.

STUDY SITE

The Hemet/San Jacinto Multipurpose Demonstration Wetland, located 6.4 km north of Hemet, California, USA (33° 48' N, 117° 1' W), was constructed in 1994 and is owned and operated by the Eastern Municipal Water District (EMWD). The wetland covers 9.9 ha with a baseball glove-like footprint (Figure 1). The wetland was built to treat up to 18,925 $m^3 d^{-1}$ of secondary effluent from the Hemet San/Jacinto Regional Water Reclamation Facility (RWRF), as well as to provide migratory waterfowl habitat along the Pacific flyway (USBR 1993). As originally constructed, the wetland had a marsh-pond-marsh configuration, consisting of five marsh inlet arms (normal operating water depth 46 cm), a central deep water pond (normal operating water depth 2 m), and two polishing outlet marshes (normal operating water depth 46 cm) separated by a central berm to control short-circuiting of the flow (Sriles 1994). In September 1994, the wetland was planted with local hardstem bulrush [*Schoenoplectus* (formerly *Scirpus*) *acutus* (G.H.E. Muhlenberg ex J. Bigelow) (Smith 1995)] and California bulrush [*Schoenoplectus californicus* (C.A. Meyer) (Smith, personal communication)]. Within the inlet and outlet arms, the bulrush was planted in strips separated by open water areas to control short-circuiting of flows and to provide sampling access.

In January 1996, the constructed wetland began to be operated as an integral component of the RWRF treatment system. Nitrogen transformations in the wetland were investigated from January 1996 through September 1997 using inflow/outflow nitrogen mass

Table 1. Geochemical characteristics of the sediments in the Hemet/San Jacinto Demonstration Wetland. Soil classification was determined via 5 boreholes made by Inland Foundation Engineering Inc. before the wetlands were constructed (USBR 1993).

Site	% Carbon	% Nitrogen	% Phosphorus	Classification
Pond	1.64	0.18	0.086	silty sand
Inlet–open water	1.50	0.14	0.066	silty sand
Veg 1	0.61	0.03	0.065	silty sand
Veg 2	1.64	0.06	0.070	silty sand
Outlet–veg	1.95	0.14	0.069	silty sand

balances supplemented with biannual, 45-station synoptic surveys to determine the internal distribution patterns of the various nitrogen species and total organic carbon (Sartoris et al. 2000). These investigations indicated that nitrogen dynamics in the wetland were influenced not only by variations in loading from the treatment plant, but also by internal loading from decaying bulrush, which increased as the emergent vegetation became more dense. By September 1997, the mean total ammonia nitrogen removal efficiency of the wetland had decreased to 4% from a mean of 76% in May 1996 (Sartoris et al. 2000). The first two denitrification measurements of the present study were made in January and March 1998. Results of these measurements are discussed in this paper as the "pre-reconfiguration" condition.

During the winter of 1997–1998, it was decided that reconfiguring the wetland into a hemi-marsh system (Weller and Spatcher 1965, Sartoris and Thullen 1998), with equal areas of interspersed emergent marsh and deep open water, might help alleviate the problems described above. On 1 April 1998, the inflow was turned off, and the wetland was allowed to drain. In September 1998, the wetland was burned to eliminate the accumulated aboveground emergent vegetation biomass and to reduce the internal N loading and oxygen demand noted by Sartoris et al. (2000). Fifty percent of the wetland area was then deepened to 1.8 m to produce hemi-marsh conditions for the enhancement of DO within the wetland.

The upper 5 m of the soil profile under the wetland consists of silty sand that is underlain by silty clay or clayey silt, which precludes seepage loss from the wetland (USBR 1993). Ground-water depths range from 4–7 m, consequently ground-water inflow is also unlikely (USBR 1993). Sediment carbon, nitrogen, and phosphorus content is fairly uniform across the wetland (Table 1), and the sediments of the five locations sampled average 1.47%, 0.11%, and 0.071% for these elements, respectively.

The RWRF is an activated sludge process plant producing an average of 28,388 m^3 d^{-1} of secondary effluent. Wetland inflow and outflow volumes vary according to daily operational requirements of the RWRF, seasonal variations in reclaimed water demand, and requirements for occasional flushing flows to preclude avian botulism outbreaks. Hydraulic retention times varied from 9 to 14 days between January 1996 and April 1998. After the modifications to the wetland and unrelated treatment plant upgrades, hydraulic retention times varied from 8 to 12 days during 1999.

METHODS

The study periods were 8 January–31 March 1998 and 25 March–9 May 1999. During these periods, the quantity of water flowing into and out of the wetland was measured daily by totaling flow meters located in the supply and exit pipelines. Inflow quantity was measured by means of an in-pipe magnetic meter, while outflow quantity was measured by an in-pipe propeller meter. Both meters were fitted with flow totalizers, which were read and reset on a daily basis by the RWRF operations staff. Inflow and outflow water samples were collected from the supply and exit pipelines on a weekly basis and analyzed within 24 hours of collection for total ammonia, total Kjeldahl-N, nitrite-N, and nitrate-N according to EPA protocols (USEPA 1984) by EMWD personnel at the District laboratory in Perris, California. Total ammonia was determined by the titrimetric distillation procedure (EPA Method 350.2). Total Kjeldahl-N was determined by the semi-automated block digester, autoanalyzer method (EPA Method 351.2). Nitrite-N data were obtained by spectrophotometry (EPA Method 354.1), and nitrate-N data were obtained by ion chromatography (EPA Method 300.0). The nitrogen mass imported and exported by the wetland was estimated by combining the laboratory-obtained concentration data with the total inflow and outflow volumes measured on the same day that the particular water sample was collected. The imported and exported loads were calculated in this manner for each N species. Nitrogen loading rates reported for periods previous to this study were taken from Sartoris et al. (2000).

The Hemet/San Jacinto Demonstration Wetland has become an important stopover site for migrating and nesting waterfowl along the Pacific Flyway. Inputs of N from waterfowl are potentially an important component of the N budget of the wetland, and estimates of avian N loading were made. Bird censuses were conducted twice each week in 1998, and the average of those counts was used as an estimate of species-specific abundances for each day of the week in which counts took place. For waterfowl and wading marsh birds, N inputs for a particular day were generated as

the product of the number of individual birds multiplied by the N output per bird. The latter value was obtained as the product of N output for the Canada Goose (1.57 g d^{-1}, Manny et al. 1994) and the fraction of a Canada goose's (*Branta canadensis* L.) biomass (3033 g) represented by each species' biomass. For red-winged blackbirds (*Agelaius phoeniceus* L.), N input was estimated as the number of birds roosting overnight multiplied by the N content (9.6%) of an individual bird's nightly production of fecal material (0.727 g dry mass, Hayes and Caslick 1984). Total N input by birds is the sum of the N inputs for each species.

Measurements of denitrification were made in January and March 1998 and in May 1999. Triplicate sediment cores were collected from four sites in 1998 (Figure 1), which were chosen to represent the four different regions of the wetland: deep water pond (Pond); open water band within inlet arm 5 (Inlet–Open Water); emergent marsh containing *Schoenoplectus acutus* in inlet arm 1 (Inlet–Veg 1); and emergent marsh containing *Schoenoplectus californicus* in inlet arm 2 (Inlet–Veg 2). In 1999, a fifth site was added to measure denitrification in a vegetated band containing *Schoenoplectus californicus* of the outlet arm (Outlet–Veg). The cores consisted of schedule-40 PVC pipe (ID, 7.62 cm; length, 33 cm), which were inserted into a one-way valve that was connected to a smaller diameter pipe used to drive the cores into the sediments. Once collected, the cores were sealed on the top and bottom with black rubber caps that were secured with hose clamps. At the time of core collection, about 30 L of water were collected in carboys to serve as feed water for the core incubation experiments. The sealed cores and carboys were shipped overnight express to the University of Colorado at Boulder, where they were placed in an incubator maintained at a temperature similar to the water temperature at the time of sampling (incubation temperature: 15°–18° C). Each set of sediment cores, along with a blank core consisting of water from the respective field site, was maintained within a flow-through system. The cores were continually supplied with the water collected from the field sites. Flow through the cores was provided by a Rainin peristaltic pump set at a flow rate of ca. 0.3 L hr^{-1}; turnover time of the water overlying the sediments was about 3 hours. The overlying water was gently stirred by a magnetic stir bar at a rate of 22 RPM.

Denitrification measurements were made using membrane inlet mass spectrometry (MIMS), an innovative method developed by Kana et al. (1994, 1998). Briefly, triplicate water samples were collected from the inlet and outlet line of each sediment core by overflowing 5-ml glass test tubes. The test tubes were sealed by the bottom of another glass test tube fitted with a Tygon collar. The samples were analyzed within 4 hours of collection for dissolved N_2 and Ar using the MIMS system. The quadrupole mass spectrometer, which attains the greatest precision with ratio data, was tuned to masses 28 and 40, and the ratio of N_2: Ar was continuously displayed by the MIMS software. Instrument standardization was conducted every six samples using two air-equilibrated DI standards maintained at 15.0°C and 21.5°C. The equations of Colt (1984) were used to calculate the dissolved N_2 and Ar concentrations of the standards.

The flux of N_2 was determined by the following equation:

$$F = [(C_o - C_i)\ V_l\ /\ A] - F_b \qquad (1)$$

where, F is the N_2 flux (μmol N m^{-2} h^{-1}), C_o and C_i are the concentration of N_2 into and out of the cores (μmol N L^{-1}), V_l is the volumetric flow rate (L h^{-1}), A is the sediment surface area within the cores (m^2), and F_b is the N_2 flux in the blank core (μmol N m^{-2} h^{-1}). The flux of the blank core was calculated from the above equation excluding the term F_b.

At the time of the core collections, a synoptic survey was conducted to monitor N concentrations throughout the demonstration wetland. Surveys had been conducted on a biannual basis since May 1996 and are described in Sartoris et al. (2000). Pertinent to this study, 1-L water samples were collected at the sampling sites, and they were analyzed for N species by EMWD personnel as described previously. Hydrolab Corporation Recorder multi-probe water quality data loggers were deployed over a 3-day sampling period to determine ambient water temperature, dissolved oxygen concentration (DO), and pH on an hourly basis. In January 1998, Hydrolabs were deployed just below the water surface and just above the sediment-water interface of the deep water pond and also mid-water column within a vegetated strip in inlet arm 2 (Figure 1). In March 1998, an additional monitoring site was established within an open water strip of inlet arm 2. In May 1999, the Hydrolabs were deployed as in March 1998; however, the inlet arm 2 monitoring site was moved to inlet arm 3.

We estimated rates of volatilization using the following data: pH and temperature data recorded by the Hydrolabs on an hourly basis; hourly windspeed data obtained by M. Bishop (U.C. Riverside); and the total ammonia concentrations determined during the synoptic surveys. The percent unionized ammonia present at the sampling sites were calculated as in Denmead et al. (1982):

$$A = C\ /\ [1 + 10^{(0.09018\ +\ (2729.92/T) - pH)}] \qquad (2)$$

where, A and C are aqueous NH_3 and ammoniacal ni-

trogen ([NH_3 + NH_4^+]) concentrations in water (g N m^{-3}) and T is temperature (K). The equilibrium NH_3 vapor pressure was calculated from the following (Denmead et al. 1982):

$$\rho_o = 0.00488\ AT/10^{(1477.8/T-1.6937)} \quad (3)$$

where ρ_o is in mbar. Finally, the rate of volatilization was determined as in Freney et al. (1985):

$$F = k\ u_z\ (\rho_o - \rho_z) \quad (4)$$

where, F is the flux of ammonia from the wetland (μg N m^{-2} s^{-1}), k is the exchange coefficient (0.885 x 10^{-3}, Freney et al. 1985), u_z is the mean windspeed (m s^{-1}), and ρ_z is the ammonia concentration at reference height z (we assumed ρ_z = 0 to calculate a worst-case volatilization rate).

Aerial photographs were taken in April 1998 and May 1999 to record the areal coverage of emergent vegetation within the wetland. Photos were taken with color infrared and natural color film from a height of 427–488 m above the ground (nominal scale 1:2500). The spatial extent of vegetation was derived by scanning polygons hand-drawn around interpreted vegetated areas on clear Mylar sheets or by color scanning the photographs when the vegetation was very sparse. Photo interpretation was validated by ground inspection. The scanned data were rectified using landmarks visible on both the photographs and the wetland diagram (see Sartoris et al. 2000). Coordinate transformation, area computation, mapping, and plotting were performed using ARC/INFO Geographic Information System software.

RESULTS

The mean loading rates of total N into and out of the wetland over the entire period that it was in operation through May 1999 are presented in Figure 2. The quantity of N loading into the wetland increased three-fold over the 2.5-year period of operation in its original configuration, and ammonia accounted for >95% of the total N load. During the 1998 study period (January to March), the total N load into the wetland was 17.26 kg N ha^{-1} d^{-1}, and about half this amount exited the wetland through the outlets. Once the wetland had been modified and had gone back online in 1999, nitrogen loading increased considerably. The mean daily load of total N for the 1999 study period (25 March to 4 May) was 24.80 kg N ha^{-1} d^{-1}, and 15.93 kg N ha^{-1} d^{-1} exited the outlets. The influent inorganic nitrogen load consisted of 72% total ammonia-N and 17% nitrite plus nitrate-N, which indicates that the modifications on the treatment plant improved the quality of water entering the demonstration wetland.

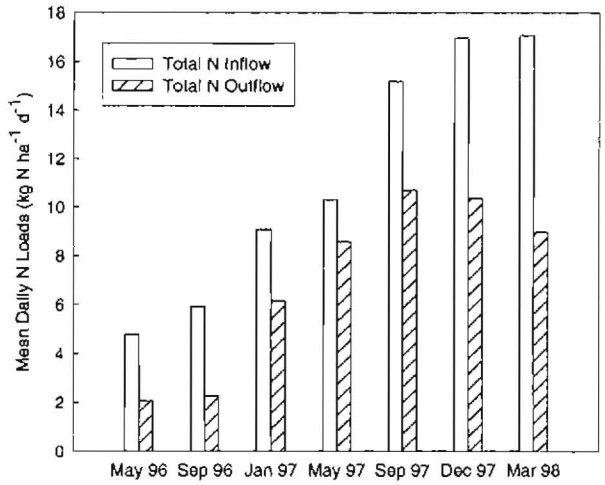

Figure 2. Total nitrogen loading into and out of the Hemet/San Jacinto Demonstration Wetland over the period of its operation (Jan 1996–Mar 1998 and Mar - May 1999). Mean loads were determined for the periods when synoptic surveys were conducted.

A transect of nitrogen concentrations across the wetland (see Figure 1) illustrates within-wetland nitrogen transformations as the influent traversed the wetland (Figure 3). In 1998, total ammonia-N levels decreased along the transect, and concentrations were approximately two-thirds their original concentration upon exiting the outlet. Nitrite-N and nitrate-N remained low throughout the wetland (<1 mg/L, except one location), and organic N constituted between 5 and 14% of the total N within the wetland. After modifications to the wetland were completed, total ammonia-N levels did not change as dramatically as was observed in 1998, but nitrate-N became more prevalent across the transect (12% of the total N in the effluent). Nearly half of the effluent consisted of non-ammonia species, which is in contrast to the effluent's constituency in 1998.

A second potential source of nitrogen to the demonstration wetland is waterfowl. A histogram of total N inputs into the wetland from waterfowl for January to April 1998, is shown in Figure 4. From January to March, N inputs were generally between 100 and 200 g d^{-1}. In early March, when a large influx of red-winged blackbirds occurred, inputs jumped 10-fold to nearly 1000 g d^{-1}. When the cumulative inputs were converted to an areal basis, the loading rates of N by waterfowl were insignificant compared to the loading from the treatment plant. We estimate that waterfowl inputs were equivalent to 0.02 kg ha^{-1} d^{-1}, which is roughly 0.1% of the average loading rate from the RWRF for the period of January–March, 1998.

Figure 5 illustrates typical DO concentrations in the

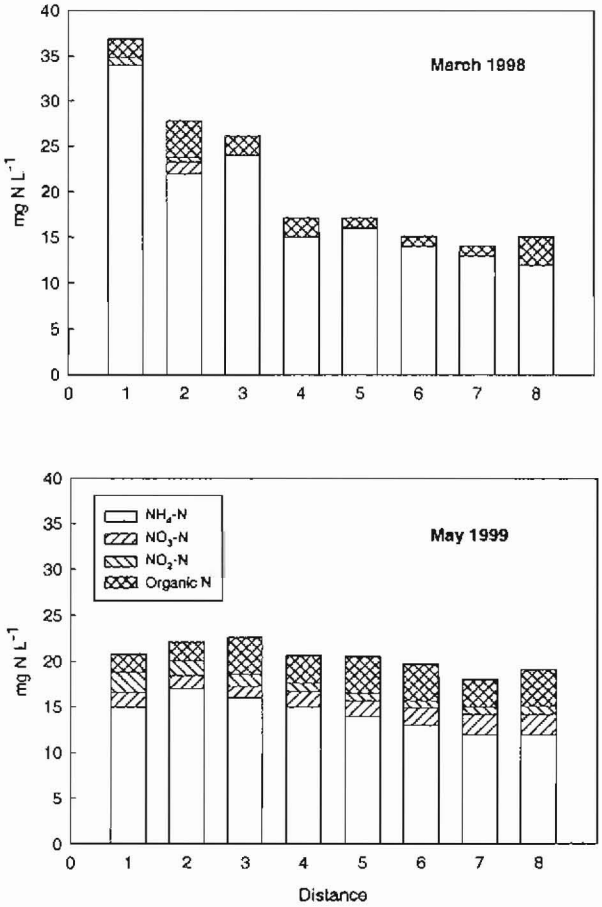

Figure 3. Transect of nitrogenous species across the Hemet/San Jacinto Demonstration Wetland. Data are from the synoptic survey conducted on 31 March 1998 and on 4 May 1999.

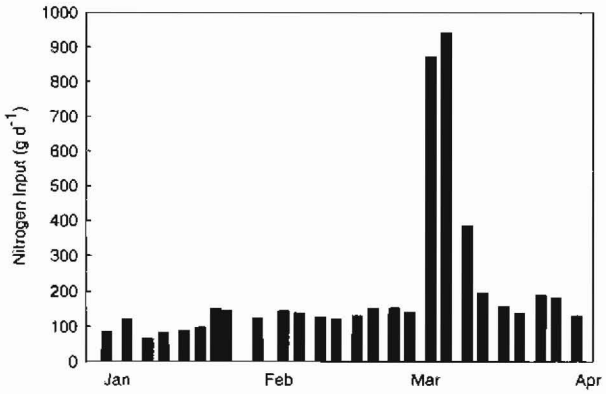

Figure 4. Total nitrogen contributed to the Hemet/San Jacinto Demonstration Wetland by waterfowl during the period that the wetland was operational in 1998.

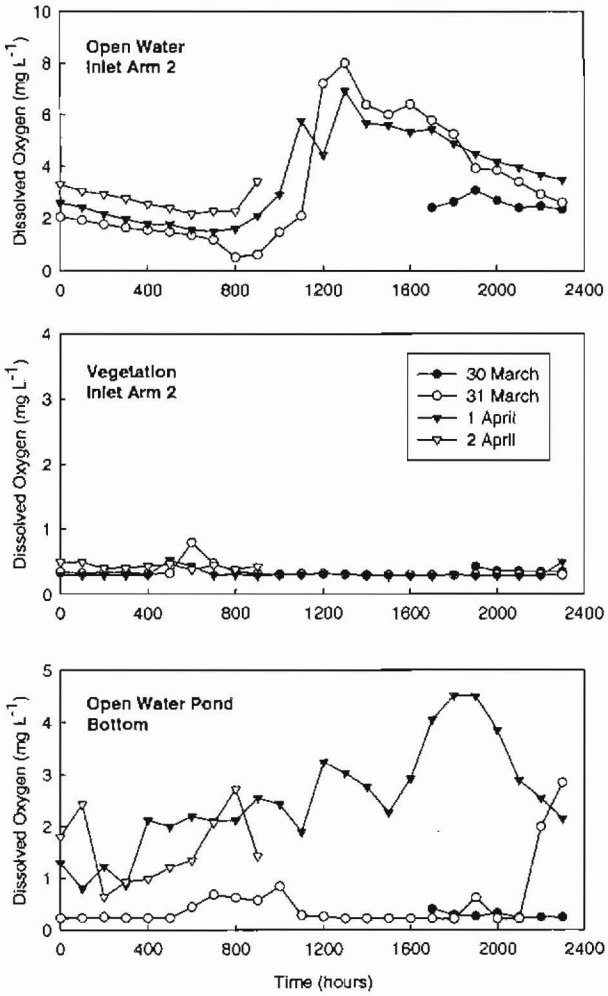

Figure 5. Diel patterns of dissolved oxygen for the 4-day deployment period of the Hydrolabs in March 1998. The top two panels represent data collected from the open water and vegetated strips that were adjacent to each other; water depth was about 0.5 m. Note change in scale between graphs.

three regions sampled in 1998. In the shallow open water strip of inlet arm 2, DO levels showed a typical diel pattern where DO concentrations peaked around noon, decreased in the late afternoon, and remained low throughout the evening. Within the corresponding vegetated site (see Figure 1), DO concentrations remained low throughout the deployment period; often, values were <0.3 mg L^{-1}. DO levels were the most variable in the bottom waters of the deep water pond site, and they seemed to be related to the combination of wind speed and direction. A weather station maintained at the treatment wetland by the University of California at Riverside recorded wind speed and direction during the study period. Unfortunately, the anemometer was not calibrated for absolute wind direc-

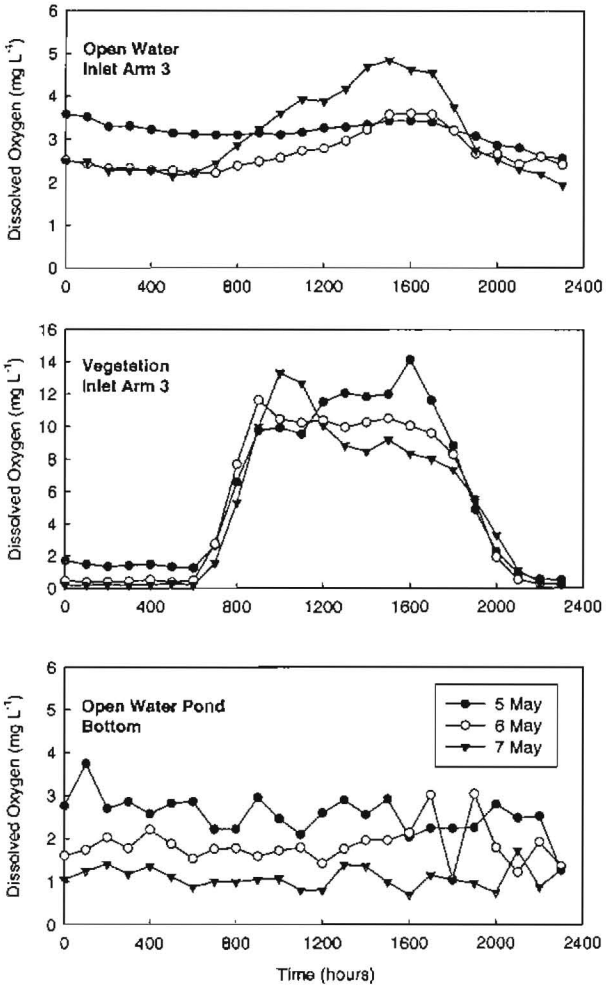

Figure 6. Diel patterns of dissolved oxygen for the 3-day deployment period of the Hydrolabs in May 1999. The top two panels represent data collected from the open water and vegetated strips that were adjacent to each other; water depth was about 2 meters and 0.5 m, respectively. Note change in scale between graphs.

tion, but we observed on 30–31 March 1998, when mean wind direction was 260° and DO levels were <1.0 mg L^{-1}, that the correlation between wind speed and DO levels was insignificant based on Sigma Stat 5.0 linear regression analysis ($r^2 = 0.01$, $n = 31$, $p > 0.05$). On 1 and 2 April 1998, the mean wind direction shifted by 55°, and there was a small but significant correlation between wind speed and DO concentration ($r^2 = 0.32$, $n = 34$, $p<0.001$). We speculate that the wind came from the southwest at this time, crossed the maximum fetch of the deep water pond, and mixed DO into the bottom waters, as suggested by the similar top and bottom water DO readings obtained on 1–2 April with the Hydrolabs.

Table 2. Denitrification rates of cores collected from the Hemet/San Jacinto Demonstration Wetland. The inlet open water sites were shallow in 1998 and were deep in 1999. The sample size is 3 for each site, except for Inlet–Veg 2, March 1998, when only 1 core was collected. The error represents the standard error. See Figure 1 for site locations.

Site	DO (mg L^{-1})	NO3 (mg N L^{-1})	Denitrification (μmol N m^{-2} h^{-1})
January 1998–incubator temperature 15° C			
Pond	1.00	<0.2	1799 ± 947*
Inlet–open water	0.25	<0.2	177 ± 44
Inlet–veg 1	0.65	<0.2	0 ± 0
Inlet–veg 2	0.30	<0.2	63 ± 62
March 1998–incubator temperature 18° C			
Pond	0.22	0.05	66 ± 7
Inlet–open water	2.9	0.7	198 ± 198
Inlet–veg 1	0.30	0.05	0 ± 0
Inlet–veg 2	0.30	0.05	0
May 1999–incubator temperature 18° C			
Pond	11.6	1.6	389 ± 110
Inlet–open water	3.0	1.5	975 ± 310
Inlet–veg 1	10.4	1.7	685 ± 172
Inlet–veg 2	10.4	1.3	1456 ± 411
Outlet–veg	6.0	2.1	2444 ± 175

* An algal bloom occurred within the feed water during the experiment. See text for explanation.

Typical DO concentrations measured during 1999 are contrasted in Figure 6. We observed that DO levels in the open water strips showed diel variations, but they were dampened compared to that of 1998. During modification, the open water strips of the inlets and outlets were deepened, and hence, bottom water DO levels were lower. DO levels within the vegetated strips, which had been hypoxic during 1998, ranged from 10–14 mg L^{-1} at mid-day to < 2 mg L^{-1} at night. It should be noted, however, that at this time, the emergent vegetation in this strip was only beginning to re-establish itself after being burned the previous fall, so it was sparse compared with the 1998 vegetation. We observed no consistent pattern of DO in the deep water pond, and DO levels decreased with the passage of each day as the thermocline strengthened.

The mean denitrification rates measured at the four wetland sites in 1998, along with the DO and nitrate data collected at the time of sampling the wetland, are shown in Table 2. In general, when denitrification was detected, the rates were highly variable for a given site. Coefficients of variation ranged from 15% (Pond, March) to 173% (Inlet–Veg 2, January). Denitrification rates were highest within the two open water sites, and except in one core (Inlet–Veg 2, January), we detected no denitrification within the vegetated areas. Overall, denitrification rates within open water regions averaged 646 ± 353 μmol N m^{-2} h^{-1}, which is nearly

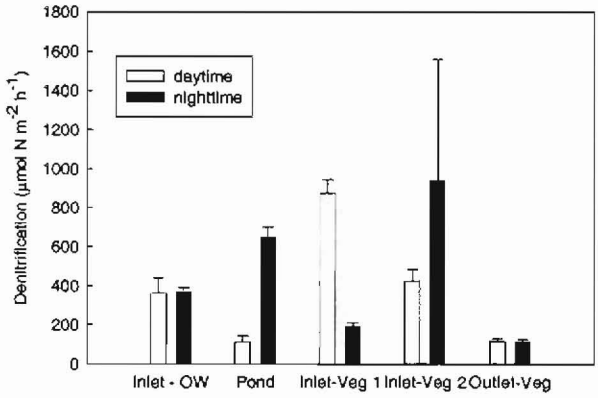

Figure 7. Comparison of light and dark treatments on denitrification rates. Error bars represent the standard error.

twenty times greater than the average rates measured within vegetated areas (20.9 ± 20.9 µmol N m^{-2} h^{-1}).

At the time of core collection, both DO and nitrate concentrations at the sites were generally low (<1.0 mg L^{-1}). Only at the two open water sites (Pond, January and Inlet–Open Water, March) did DO concentrations rise to or above 1.0 mg L^{-1}. During the first denitrification measurements (Pond, January), the incubator was maintained on a day-night cycle that was similar to that of the wetland in California. Over the course of the incubation, an algal bloom occurred within the feed water, and nitrate levels rose to 2.5 mg L^{-1}. The presence of high levels of nitrate stimulated denitrification, and thus, the cores registered very high rates that probably were not representative of field conditions at this site.

In 1999, we tested the effect of light and dark conditions on denitrification rates by incubating a series of cores under the two regimes. We analyzed the results using one-way ANOVA (Sigma Stat 5.0). The results were somewhat inconclusive, as we found that denitrification rates were significantly higher ($F = 79.6$, $n = 6$, $p < 0.001$) in the dark for the cores collected from the deep water pond, and the opposite was true ($F = 91.7$, $n = 6$, $p < 0.001$) for the cores collected from Inlet–Veg 1 (Figure 7). At the other three sites, we found no significant effect ($F = 0.68$, 0.001, 0.002; $p > 0.05$) from light exposure or absence. Consequently, we maintained the incubator in total darkness for the remainder of the measurements, and we assume the rates can be extrapolated to a 24-hour period.

After modifying the wetland, DO and nitrate levels were higher than observed in 1998, and we found higher denitrification rates at least within the reestablishing vegetated zones. Variability in the rates was generally lower than in 1998, and the CVs ranged from a low of 10% at the Outlet–Veg site to a high of 78% at the Inlet–Veg 1 site. Overall denitrification rates averaged 682 ± 218 µmol N m^{-2} h^{-1} within open water areas and 1414 ± 298 µmol N m^{-2} h^{-1} within the emergent marsh zones.

For a nitrogen balance, the point measurements of denitrification must be scaled up to a wetland-wide rate. This was accomplished by computing the product of the areal coverage of the four wetland regions (deep water pond, open water strip, and the two types of vegetation) delineated using the photographic images and the average denitrification rates for each region. The wetland-wide denitrification rate for the 1998 study period is 0.70 kg ha^{-1} d^{-1}, and for the 1999 study period is 3.58 kg ha^{-1} d^{-1}.

The range in water temperature and pH recorded within the three regions of the wetland during the 3-day Hydrolab deployment periods is shown in Table 3. When water temperatures were cooler (1998), the pH of the surface waters in the three regions ranged from a low of 6.90 to a high of 7.64. In May 1999, water temperature averaged ca. 22 °C and pH ranged from 7.37 to 8.39. In January, the %NH_3 present in the wetland never exceeded 0.50%. In March, when pH rose above 7.5 in three cases, the highest percentage of NH_3 present was ca. 1.1%. In terms of NH_3 concentration, the values ranged from 0.03 to 0.23 mg N L^{-1} in 1998. In May 1999, the pH on occasion drift-

Table 3. The range in water temperature (C) and pH in a vegetated band of an inlet, an open band of an inlet, and the deep open water pond. The mean is shown in parentheses below each range. The values reflect the range and mean in data collected on an hourly basis over a three-day period. A Hydrolab was not deployed in an open water band in January 1998.

	January 1998		March 1998		May 1999	
Site	Temp	pH	Temp	pH	Temp	pH
Vegetation	15.6–16.7 (16.2)	7.02–7.19 (7.07)	11.8–13.6 (12.5)	6.90–7.23 (7.02)	17.3–27.9 (22.5)	7.37–8.39 (7.66)
Open water	—	—	11.7–15.2 (13.1)	7.13–7.64 (7.26)	18.3–24.9 (22.3)	7.50–7.66 (7.55)
Pond	11.8–14.3 (12.4)	6.97–7.09 (7.01)	10.4–12.2 (11.5)	6.92–7.25 (7.08)	15.6–26.6 (21.1)	7.85–8.11 (7.98)

ed above 8, and as a consequence, the NH_3 levels reached between 6 and 14% or 1 to 2 mg N L^{-1}.

To obtain wetland-wide rates, ammonia volatilization was estimated on an hourly basis. An average daily rate was determined for each station in which a Hydrolab was deployed, and the rates were extrapolated to a wetland-wide rate as in the denitrification rates. In May 1999, when water temperatures and pH were the highest, volatilization averaged 0.0002 kg N ha^{-1} d^{-1}. Rates were even lower in 1998, when temperatures and pH were lower.

DISCUSSION

This study examined the role of denitrification in the removal of nitrogen from a constructed wetland for wastewater treatment (CWWT) that receives ammonia-dominated effluent. Furthermore, this study was designed to compare the relative importance of denitrification during two phases of the wetland's establishment. First, denitrification rates were measured at a mature stage in the wetland's development, when internal loading of ammonia-N and anoxia caused by extensive biomass accumulation had reduced treatment efficiency (Sartoris et al. 2000). Second, denitrification rates were measured after the wetland had been reconfigured, and a new "set-point" representing an early stage in wetland development had been established. The balance between N inputs, outputs, and internal storage at these two developmental stages is illustrated in Figure 8.

Nitrogen Balance—The Mature Wetland

Nitrogen Inputs. Total nitrogen inputs from the RWRF dominate N loading into the wetland. Over the 8 January–31 March 1998 study period, the wetland received 17.26 kg N ha^{-1} d^{-1} of TN, and ammonia was the dominant nitrogenous species flowing into the wetland (Figure 3). Even though waterfowl counts were high at the wetland, estimated N input from birds was only 0.02 kg N ha^{-1} d^{-1}, a negligible component of the N balance. Two other possible inputs of N were ground-water seepage and precipitation. We assume that ground water attributed nothing to the N budget, as the underlying soils are clayey silt and ground-water depths range from 4 to 7 m (USBR 1993), which precludes ground-water seepage into the wetland. We also assume that precipitation did not contribute significantly to N inputs, as Hemet is located in an arid climate. Typically rainfall is ca. 10 cm per annum (http://www4.ncdc.noaa.gov/cgi-win/wwcgi.dll?wwDI~StnSrch~StnID~20001573). However, during this study period, Hemet received ca. 36 cm of rainfall. Assuming an even spread of rainfall over the surface of the wetland, precipitation contributed roughly

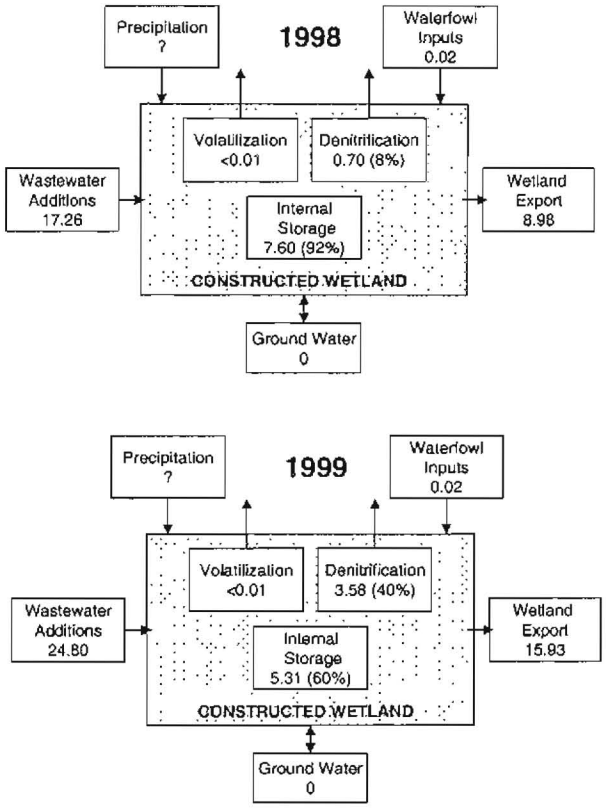

Figure 8. Inputs, outputs, and internal storage of total nitrogen for the 1998 and 1999 study periods; all units are in kg N ha^{-1} d^{-1}. In both 1998 and 1999, 8 kg N ha^{-1} d^{-1} was removed through internal processing by the wetland. The parentheses within the shadowed boxes show the percentage of TN removed permanently via denitrification and temporarily through internal storage.

14% of the average daily inflow to the treatment wetland. Since we did not analyze the precipitation for nitrogenous species, the quantity of N attributable to precipitation during this period is unknown.

Nitrogen Outputs. There are three pathways of permanent nitrogen loss from the Hemet San/Jacinto Demonstration Wetland: export of dissolved nitrogenous species through the outlet weirs, gaseous escape of NH_3 through volatilization, and loss of N_2 and N_2O via denitrification. TN export during the study period averaged 8.98 kg N ha^{-1} d^{-1}, or about half of the wastewater additions. Loss of N through volatilization was negligible, as <0.01 kg N ha^{-1} d^{-1} was estimated to be lost from the wetland through this process.

Denitrification played a minor role in the removal of N from the wetland during this period. The wetland-wide denitrification rate was 0.70 kg N ha^{-1} d^{-1}, which represents less than10% of the TN removed by the wetland (Figure 8). Denitrification relies on the pres-

ence of nitrate as a precursor and organic carbon compounds to provide electrons for denitrifying heterotrophs to form N_2 and N_2O (Knowles 1982). Most likely, DOC was not limiting to denitrification in this study because concentrations ranged from 9.25 to 11.7 mg L^{-1} (L. Barber, unpublished data). Nitrogen inputs were dominated by ammonia, and nitrate levels were probably too low to support much direct denitrification (Table 2; Figure 3). Instead, the coupled process of nitrification-denitrification was a more likely pathway for denitrification within this ammonia-dominated wetland. This process has been found to be particularly important within the sediment-rhizome matrix where macrophytic release of DO within anoxic sediments facilitates these transformations (Reddy et al. 1989, Caffrey and Kemp 1992). However, this did not occur readily in the wetland during 1998, as denitrification rates were zero within the vegetated areas in nearly all cases. Instead, nitrification of ammonia within the water-column of the open water areas and subsequent denitrification within the sediments must have occurred, since denitrification was only consistently detected within the open water sites. An example of the importance of DO in the ammonia-rich water-column is found during the March sampling. At this time, cores were collected from the deep water site (Pond) on 31 March, when DO levels were <0.5 mg L^{-1} (Figure 5). Nitrification was probably limited by the relative lack of DO; hence, denitrification rates were lower than the shallow open water site (Inlet—Open Water), which had higher DO concentrations (Table 2).

Internal Storage of N. The difference between the measured inputs and outputs of nitrogen represent the internal storage component of the wetland. Loss of N to the sediments via burial or assimilation of N by macrophytes, periphyton, and algae are the two routes for internal storage. Both of these processes represent a temporary loss of N from the wetland because dissolved inorganic N is released back to the water column when organic material within the sediments or within the senescent biomass is mineralized. We estimate that internal storage during the 1998 study period was 7.60 kg N ha^{-1} d^{-1}. Thus, of the 8.98 kg N ha^{-1} d^{-1} removed by the wetland, more than 90% was stored internally (Figure 8).

Nitrogen Balance—The Immature Wetland

Nitrogen Inputs. Again, total nitrogen inputs from the RWRF dominate N loading into the wetland during the 1999 study period. Loading rates of TN were about 50% greater than in 1998, with an average rate of 24.80 kg N ha^{-1} d^{-1}. Nitrite-N + nitrate-N was a more important component of the TN loads, and together, they averaged 4.16 kg N ha^{-1} d^{-1}. No waterfowl counts were made in 1999, but we assume that the loading rate from birds was equivalent to the previous year. Precipitation was most likely an insignificant component of the input budget of N, as rainfall was only 6 cm during this study period and represented <1% of the average daily inflow.

Nitrogen Outputs. TN export during this study period averaged 15.93 kg N ha^{-1} d^{-1} or about 60% of the loading from the RWRF. Loss of N through volatilization was negligible, but denitrification played a more important role in the removal of nitrogen. The wetland denitrified 3.58 kg N ha^{-1} d^{-1}, a rate five times greater than that measured in 1998. In addition to receiving a more nitrified effluent, DO concentrations within the wetland were high enough to support the conversion of ammonia to nitrate, which increased wetland nitrate concentrations. During the sediment core incubations, we found a significant positive relationship ($r^2 = 0.70$, $n = 15$, $p < 0.05$) between the rate of nitrate decrease within the cores (delta NO_3) and denitrification rates (Figure 9a). The slope of the line for the vegetated sites is 20% steeper than the slope of the line for the non-vegetated sites. We also found that as the number of culms increased within the sediment cores, the rate of denitrification increased (Figure 9b). These observations suggest that the vegetation aided denitrification through the coupled process of nitrification-denitrification, and as a consequence, for a given amount of water-column nitrate, the vegetated areas yielded a higher denitrification rate.

Internal Storage of N. We estimate that internal storage during the 1999 study period was 5.31 kg N ha^{-1} d^{-1} or roughly 60% of the TN removed by the wetland. The modifications to the wetland, along with the treatment plant upgrades, shifted the nitrogen balance from a large internal storage component and a small denitrification component in 1998 to a more denitrifying system in 1999.

Comparison to Other Studies

It is difficult to compare our denitrification data to those of other studies because very few quantitative data exist, and methodologies vary and are not directly comparable (Seitzinger et al. 1993). Two recently published studies, which report a nitrogen budget (Frankenbach and Meyer 1999) and *in situ* denitrification rates (Xue et al. 1999) for CWWTs, merit comparison with our study. In a small (0.74 ha) constructed treatment wetland located in Arcata, California, Frankenbach and Meyer (1999) measured the total N content of the influent and effluent, the plant biomass, and the sediments, and they estimated denitrification by mass

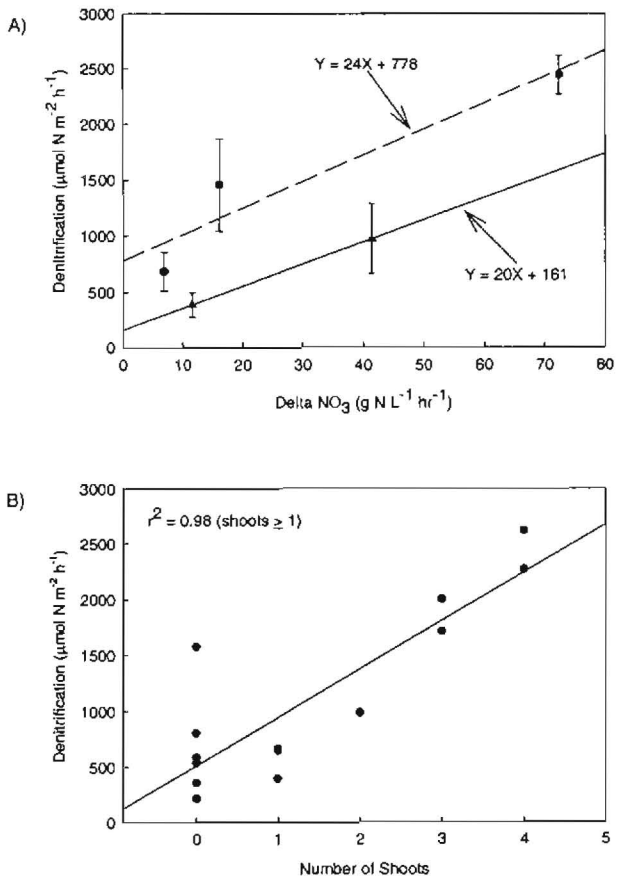

Figure 9. (A) The relationship between the change in nitrate concentration of the feed water and the denitrification rates for the May 1999 sediment core measurements. The solid line illustrates the slope of the data for the open water sites (triangles), and the dashed line illustrates the slope of the data for the vegetated sites (circles). (B) The relationship between the number of shoots within the sediment cores and denitrification rates. The data points for zero shoots are from the open water sites, and they were not included in the correlation analysis.

balance. The composition of the influent differed from that of the Hemet wetland in that the main nitrogen component was organic N (ca. 50%), and the HRT was considerably shorter (20 hours). This wetland was inefficient in the removal of TN, as only 17% was removed on an annual basis. The estimated wetland-wide denitrification rate was 9.88 kg N ha^{-1} d^{-1} (in comparison, the loading rate was 61.34 kg N ha^{-1} d^{-1}), and it accounted for about 90% of the TN removed by the wetland. We question whether plant biomass might have accounted for a larger percentage of the internal storage component of the Arcata wetland. TN content of the rhizomes, phytoplankton, and periphyton was not measured, and the litter component was assumed to be zero. Furthermore, precipitation inputs were not included in the model used to derive flow rates out of the wetland (flow rates were not measured), which could affect the TN balance, given that Arcata is located in a moderately wet climate. As a consequence, the mass balance-derived denitrification rates may be less than Frankenbach and Meyer (1999) estimated, and the internal storage component may be larger than reported.

During two time periods, January–February and May–June, Xue et al. (1999) measured denitrification rates by the acetylene block technique in small constructed wetlands (0.6–0.8 ha) located in Illinois. These wetlands received agricultural tile drainage that ranged from 0 to 10.5 mg L^{-1} of NO_3-N and had an HRT of 7 days. The denitrification rates ranged from 143 to 843 µmol N m^{-2} h^{-1}. Based on the mean TN load during their measurement periods and the measured denitrification rates, Xue et al. (1999) estimate that denitrification could, on average, account for 33% of the TN removal in the Illinois wetlands. Even though the Illinois and Hemet/San Jacinto wetlands are different in size, have different HRTs, receive effluents of different qualities, and are located in different climates, the measured denitrification rates overlap one another (Table 2).

Given the increasing use of constructed wetlands to treat a variety of wastewater types, more direct measurements of denitrification are required to determine the relative importance of denitrification and internal storage as nitrogen removal mechanisms in CWWTs. Denitrification rate estimates for CWWTs that are derived using a subtractive approach and a nitrogen budget model have the inherent problem of loading the errors of estimation or omission of compartments into the unmeasured compartment, in this case denitrification. If the denitrification rates are overestimated, then the internal storage compartment will be underestimated. Consequently, the useful treatment life of a particular CWWT may be overestimated. The Hemet/San Jacinto Demonstration Wetland reached maturity at a relatively rapid rate, but this is not unprecedented in the Southwest. A similar sized constructed wetland located in Kingman, Arizona apparently required burning after not quite five years of operation (Pinney et al. 2000). Vegetation clearing was also required at the Sweetwater Wetlands in Tucson, Arizona, after approximately three years of operation, in order to maintain flow and reduce mosquito breeding habitat (Prior 2000). We hope that the reconfiguration of the Hemet/San Jacinto Demonstration Wetland into a hemi-marsh will result in a more sustainable treatment system.

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