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Efficacy of a denitrification wall to treat continuously high nitrate loads

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ABSTRACT

Denitrification walls have been proven as an effective, long-term method for remediating nitrogen in groundwater underneath agricultural lands. Utilizing walls to provide large N load reductions requires targeting a significant portion of agricultural effluent. One approach for more efficient application of walls is to locate them adjacent to zones with high groundwater flow, although treatment efficacy in these conditions is uncertain. In this study, a large wall (168 m^3) receiving high N loads was assessed using a well transect array for hydraulic and water quality evaluations and media were collected from within the wall to evaluate enzyme activity with flow distance. Porewater velocity through the wall was rapid (1.7 m day^{-1}) with short detention times (1.7–1.9 days), yet the wall treated 100 ± 28 m^3 of groundwater per day, effectively removing 228 ± 155 kg of total N per year. Maximum nitrate-N removal rates per media volume (4.9–5.5 g-N m^{-3} d^{-1}) were at the upper end of published values. Rapid reduction of potential denitrification rates in media samples from 4.89 g N m^{-3} d^{-1} to undetectable within a quarter of the wall length suggests that nitrate-N depletion drove a rapid reduction in denitrifying enzymes. Based on a carbon mass balance, dissolved organic C leaching was initially the largest C export process and the longevity of total bioavailable C was estimated as 23 ± 5.9 years. These results indicate the ability of walls to reduce high N-loads over long timespans.

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1. Introduction

Agriculture is the most extensive source of nitrate-N to groundwater and increases in nitrate-N within shallow groundwater due to modern agricultural practices have been well documented (Andersen and Kristiansen, 1984; Hallberg, 1989; Hill, 1983; Hudak, 2000; Nolan and Stoner, 2000; Nolan, 2001; Puckett et al., 1999; Refsgaard et al., 1999; Breemen et al., 2002). The efficiency of N applied and ultimately consumed by humans or livestock is generally low, with approximately 33% of all N added to agroecosystems consumed by humans or livestock, while 65% is lost to the atmosphere or aquatic ecosystems (Galloway et al., 2003; Smil, 2001, 2002). Additionally it has been estimated that in the US, farmers typically over fertilize with N by 24 to 38% (Babcock and Blackmer, 1992; Trachtenberg and Ogg, 1994). As a result of this low efficiency, nitrate-N concentrations in shallow groundwater underneath agricultural lands exceed the maximum contaminant level (MCL = 10 mg L^{-1}) in 19% of samples nationwide (Nolan and Stoner, 2000). In addition to fertilizer efficiency improvements, edge of field remediation processes can help achieve water quality standards for N.

Denitrification is a microbial process that mitigates nitrate-N pollution by reducing nitrate-N to N_2 or N_2O in hypoxic conditions utilizing an electron donor such as organic carbon. Several techniques have been utilized to increase the denitrification rate in agricultural effluent by adding a C amendment such as woodchips or sawdust. These include 'denitrification beds' and 'denitrification walls', both of which are termed denitrification bioreactors (Schipper et al., 2010). Denitrification beds are often containerized treatment systems consisting of wood chips-alone, treating concentrated discharges from natural or tile-drainage systems. Denitrification walls are traditional permeable reactive barriers (PRBs) inserted vertically into the ground to intercept groundwater flow. Denitrification is stimulated in these PRBs by adding an organic carbon amendment such as sawdust or woodchips to stimulate the denitrification process and reduce effluent nitrate-N concentrations.

Scaling-up denitrification walls for widespread application to reduce groundwater nitrate-N require efficiently maximizing treatment area and volume. When denitrification walls are installed in aquifers with low porewater velocities, volumetric treatment rates are low and N-limiting conditions are more likely

Abbreviations: DEA, denitrification enzyme activity; MBC, microbial biomass carbon; TKN, total Kjeldahl nitrogen; TMDL, total maximum daily load; PRB, permeable reactive barrier; DOC, dissolved organic carbon.

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to occur in a fraction of the groundwater flow-length within the wall. One technique to increase treatment efficiency is to deploy denitrification walls to target zones of high porewater velocities, such as adjacent to a ditch or in riparian areas where groundwater discharges to surface water. This will reduce the occurrence of N-limiting conditions and allow for high volumetric treatment rates and greater reductions in nitrate-N loading rates. In this study, this concept was evaluated by the construction of a relatively large denitrification wall (168 m^3) immediately adjacent to a stream where high porewater velocities and nitrate concentrations directed a high nitrate load through the wall.

2. Materials and methods

2.1. Site location and construction

The study area was located in a 65 ha container nursery in Alachua, Florida that sells plants for the landscape market, which are all grown in containers partially buried in the soil. The nursery is located in a watershed (Fig. 1a), which has total maximum daily load (TMDL) restrictions for nitrate-N (Hallas and Magley, 2008). Soils in the groundwatershed of the denitrification wall are excessively to moderately well drained and consist of >93% sand sized particles to ~2 m depth overlying a clay aquitard 2–2.4 m below the surface (USDA, 1985). Excessive N leaching from the bottom of the plant container drains through the surface soils in to a shallow aquifer resulting from a subsurface clay aquitard. This shallow groundwater is transported laterally towards the edge of the property where declining surface elevations exposes the aquitard and forces groundwater to the surface in numerous seepage slopes. The denitrification wall was installed adjacent to this break in elevation, approximately 14 m upgradient from a small stream, which begins as a significant seepage discharge (Fig. 1a). The lowest depth of the wall was installed a few inches in to the clay-rich aquitard to prevent groundwater bypass. The shallowest depth was 1.8 m above that at a height which for two years had been the highest water table measured within an adjacent well. The final dimensions of the wall are 55 m long, 1.7 m wide and 1.8 m deep (168 m^3).

The denitrification wall was constructed on September 30th, 2009. A washed and sieved quartz sand (Edgar Minerals, Inc., Edgar, Florida) was mixed with pine sawdust in a 1:1 ratio by volume. The sand and sawdust were mixed above ground, and then as soil and groundwater were excavated along the trench, the sand-sawdust media was rapidly placed in the excavated pit. The C content of the final sand-sawdust mixture was 7.4 ± 0.7%. After construction, four subsamples were collected of the final mixture within the trench.

2.2. Nitrogen and dissolved organic C groundwater measurements

Monitoring wells were installed to the bottom of the denitrification wall in three parallel transects using U.S. environmental protection agency (USEPA) guidelines (USEPA, 2008) (Fig. 1b). Wells were placed upgradient, within (center), and downgradient of the wall in three transects to monitor nitrate-N, total Kjeldahl N (TKN), and dissolved organic C loading as well as groundwater temperature, dissolved oxygen, porewater velocity, direction and elevation.

Water samples were collected within each well weekly for 20 weeks and then monthly thereafter for 660 days after construction after purging two well volumes using a submersible pump (Mini Typhoon® DTW, Proactive Environmental Products, Bradenton, FL). Samples were collected and either filtered through a 0.45 μm membrane filter (Pall Corporation, Port Washington, NY), then acidified or unfiltered and acidified directly, stored on ice and transported to the laboratory. Unfiltered samples were digested using a block digester and analyzed colorimetrically for TKN (EPA Method 351.2) on an autoanalyzer (Seal Analytical, West Sussex, UK). Filtered samples were analyzed for nitrate-nitrite colorimetrically (EPA Method 353.2) after cadmium reduction on an autoanalyzer (Seal Analytical, West Sussex, UK). Total organic C (TOC) was determined using EPA Method 415.1, after combustion as non-purgable organic C on an infrared gas analyzer (Shimadzu Corp, Kyoto, Japan). Dissolved oxygen was measured directly in the wells by slowly raising and lowering an YSI multi-probe (556 MPS, YSI Incorporated, Yellow Springs, Ohio) throughout the groundwater column.

2.3. Hydraulic measurements

Effective porosity of the wall was determined in triplicate as the fraction of saturated water volume drained at field capacity (33 kPa) (Ahuja et al., 1984; Timlin et al., 1999) in a laboratory study using recreated cores of sand and sawdust in the same ratios and same bulk density as the wall. Cores were vacuum saturated in tempe cells (Soil Moisture Equipment Co., Santa Barbara, CA), then allowed to drain to a porous surface for 72 h. This field capacity measure of effective porosity has been determined as a better predictor of the mobile groundwater volume in wall media than total porosity (Barkle et al., 2007).

The focusing of groundwater through permeable reactive barriers (PRBs) has been hampered by decreases in hydraulic conductivity due to construction, thus instigating bypass flow (Barkle et al., 2007; Schipper et al., 2004). The hydraulic conductivity (K_{sat}) was therefore determined in all nine wells using the Hvorslev slug-test method as described in the following equation (Fetter, 2001).

$$K_{sat} = \frac{r^2 \ln(L_e/R)}{2L_e t_{37}}$$

In this equation, K_{sat} is the saturated hydraulic conductivity [LT^{-1}], r is the well casing radius [L], L_e is the length of the well screen [L], R is the borehole radius, and t_{37} is the time it takes for the water level to fall to 37% of initial head change.

Porewater velocity and direction were measured periodically in wells at 0.4, 0.8 and 1.2 m from the bottom of the denitrification wall using a heat-pulse flowmeter (GeoFlo Model 40, Kerfoot Technologies, Mashpee, MA). The direction and velocity readings of the flowmeter are calibrated by pumping a known velocity and direction in a tank containing the well screen surrounded by the same standard sand filter pack used in the field well installation. This procedure yielded an r^2 for velocity of 0.999 and a standard deviation for direction of ± 2 degrees around the true value. Heat-pulse groundwater flowmeters have been field-verified as accurate representations of porewater velocity and direction as compared to piezometer gradients with average velocity uncertainties of only 0.02–0.04 m d^{-1} and direction uncertainties of 4.9–7.4 degrees (Alden and Munster, 1997).

Water level elevations and temperature were measured hourly over 462 days by pressure transducers placed in the wells (Global Water. Gold River, CA). To provide a confirmation on the flowmeter results and a more continuous measurement of groundwater mobility, porewater velocities were determined using Darcy's law based on measured head gradients from transducers, K_{sat}, and effective porosity.

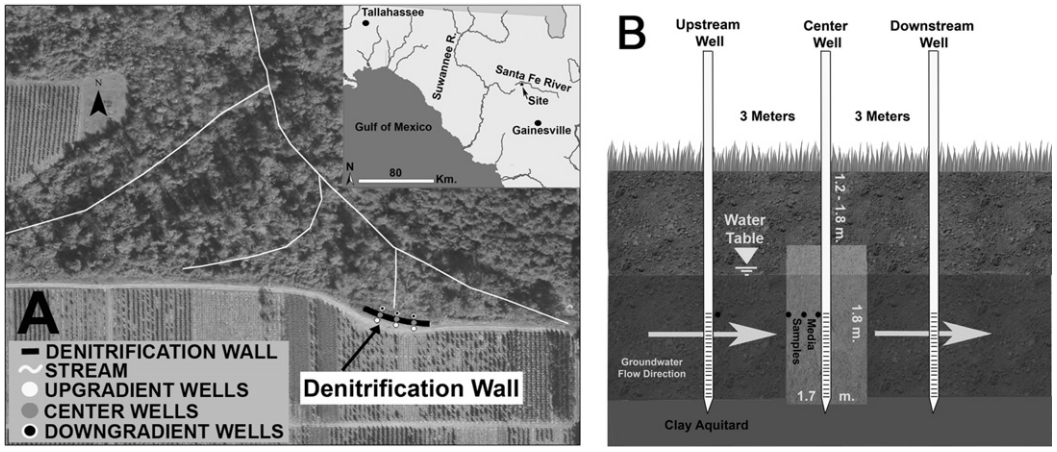

Fig. 1. (A) Geographic location of the watershed and an aerial image delineating the land-use, denitrification wall location and streams at the study site. (B) A cross-section diagram of the denitrification wall delineating the well transects and the media sampling transect. Map created by author using publicly available aerial imagery from ACPA (2006).

2.4. Nitrate-N removal rate estimates

Nitrate-N removal rates within the wells were determined as daily mass nitrate-N loss per volume of reactor media using the following equation (Schipper and Vojvodic-Vukovic, 2000).

$$Nr = \frac{vA\Delta_n}{V_s}$$

In this equation, Nr is the nitrate-N mass removal rate per volume of wall [g-N m^{-3} d^{-1}], v is the porewater velocity [LT^{-1}], A is the cross-sectional area conducting ground water [L^2], calculated as $A = L^2\phi$, where ϕ is effective porosity [L^3 L^{-3}], Δ_n is the decrease in nitrate-N N concentration [ML^{-3}] and V_s is the media volume of wall the nitrate-N travels through [L^3] (L^2 × the travel distance within the wall). Porewater velocity (v) and media volume (V_s) were determined from velocity and directional readings measured with the groundwater flowmeter.

2.5. Media sampling

Media sampling for microbial biomass C, bulk density, particle density, total porosity, potential denitrification rate and denitrification enzyme activity (DEA) was conducted on March 28th 2011, 540 days after the denitrification wall installation. Within the denitrification wall, three media sample transects were done by collecting samples 37 cm apart horizontally at the leading edge (0 cm), midpoint (37 cm), and adjacent to all three center wells (74 cm) (Fig. 1b) with an auger. Three additional samples were collected at the same depths in native soils. All samples were collected below the water table, ~45 cm below the top of the wall.

2.6. Media characterization

Bulk density was calculated as the oven dry weight (105 °C for 48 h) divided by the volume of sample collected with a peat auger. Moist media samples were analyzed within 4 days for microbial biomass C by the 24 h. chloroform fumigation–extraction method (Vance et al., 1987) as the difference in TOC (Section 2.2) between untreated and chloroform-fumigated media with an extraction efficiency (k_{EC}) factor of 0.37 applied (Sparling et al., 1990). To determine changes in sawdust properties with time, the total C and fiber content of media were compared between the four subsamples collected in the trench at day 0 and samples from the transect collected 540 days after installation. Oven-dried samples were homogenized and ground with a plant grinder (Thomas Scientific, Swedesboro, NJ) for fiber analysis and a ball-mill for total C and total N. Percentages of neutral detergent fiber (NDF), hemicellulose, cellulose and lignin were determined as mass loss after a sequential neutral detergent-acid digestion technique within a fiber analyzer (ANKOM, Fairport, New York). Samples for total C and total N were analyzed using a thermal conductivity detector after dynamic flash combustion (FlashEA®1112, Thermo Fisher Scientific, Miami, OK).

2.7. Media denitrification rate

Potential denitrification rate was evaluated on samples which were flooded with water collected from the closest upgradient wells (NO_3 = 3.8–7.8 mg L^{-1}) and denitrification enzyme activity (DEA) was analyzed using methods outlined in Tiedje (1982) with adaptations by White and Reddy (1999). Firstly, samples were homogenized and placed in a sealed glass serum bottle. Potential denitrification rate samples were flooded with the aforementioned well water treatments and DEA samples were saturated with 2 ml of N_2-purged deionized water and 3 ml of a 56 mg KNO_3-N L^{-1}, 288 mg dextrose CL^{-1}, and 2 mg chloramphenicol L^{-1} solution, which were both purged with 99.99% O_2-free N_2 gas. Chloramphenicol is added to inhibit the synthesis of new enzymes thus the DEA metric provides a snapshot of existing denitrifying enzymes only in the absence of N or C limitation. In all samples, headspace air was evacuated and replaced with N_2 gas, then approximately 15% of the headspace N_2 was replaced with acetylene gas (C_2H_2) (Balderston et al., 1976; Yoshinari et al., 1977). Bottles were shaken on a longitudinal shaker for 1 h and incubated at 25 °C for the DEA analysis and 22 °C for the potential denitrification rate analysis, which was the maximum temperature observed in the wall.

Headspace gas was sampled incrementally with time. All headspace samples were analyzed for nitrous oxide production with a gas chromatograph that was equipped with a 3.7 × 108 (10mCi) ^{63}NI electron capture detector (300 C) (Shimadzu GC-14A, Kyoto, Japan). A stainless steel column (1.8 m long by 2 mm i.d.) packed with Poropak^{TM}Q (0.177–0.149 mm; 80–100 mesh) was used (Supelco, Bellefonte, PA). Operating temperatures were 120, 30 and 230 °C for the injector, column and detector respectively. Measured values were adjusted to account for N_2O dissolved in the aqueous phase employing Bunsen absorption coefficients (Tiedje, 1982). All denitrification rates were determined by fitting a least-squares regression line to the cumulative N_2O production over

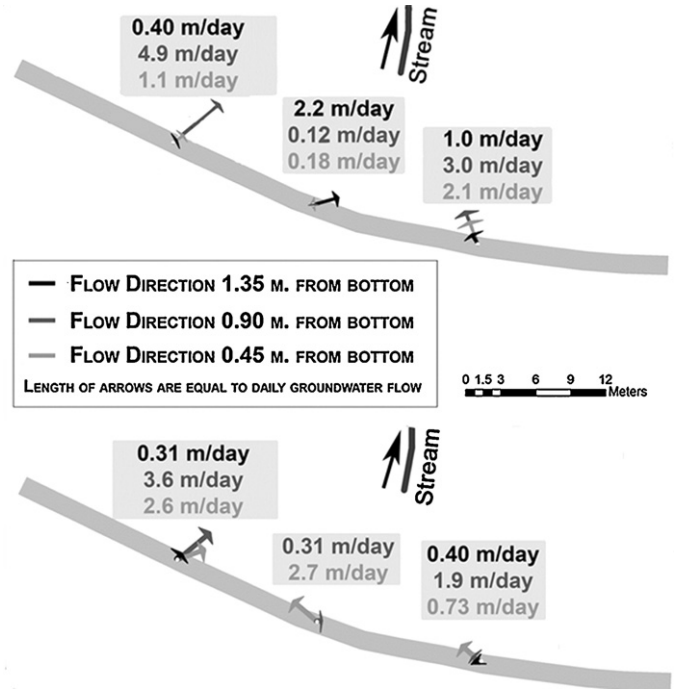

Fig. 2. Porewater velocities and directions measured using a heat-pulse flowmeter; (A) May 13, 2010 and (B) July 13, 2010.

time. Denitrification rates were quantified on a volumetric basis as determined from bulk density measurements.

2.8. Statistical analyses

A two-way ANOVA was used to screen comparisons for statistical significance. Single pairwise comparisons between denitrification wall and native soils for K_{sat}, bulk density and porosity were analyzed for statistical significance with the application of a student's *t*-test. Post hoc determinations of the statistical significance for multiple pairwise comparisons for nitrate-N, TKN, DOC, denitrification rate, DEA, Microbial biomass C, total C between locations was calculated with a Tukey's HSD test. Statistical significance for all tests was determined at an alpha level of 0.05 using JMP 8.0 (SAS Inc., Cary, NC).

3. Results and discussion

3.1. Groundwater hydrology

The saturated hydraulic conductivity (K_{sat}) of the denitrification wall averaged $1.2 \times 10^{-2} \pm 3.4 \times 10^{-4}$ cm s^{-1}, which was greater than the K_{sat} of the surrounding soils which averaged $7.0 \times 10^{-3} \pm 5.4 \times 10^{-3}$ cm s^{-1}. The effective porosity of the wall media was $50.0 \pm 5.3\%$ ($n = 3$) of the total volume.

The porewater velocity and direction was measured with the heat-pulse groundwater flowmeter in May and July, 2010(Fig. 2). In general the groundwater traveled perpendicularly through the denitrification wall with a curvature towards the main surface water discharge. In both May and July the average porewater velocity was 1.7 m day^{-1} (Table 1), which is much faster than velocities for other walls (0.007–0.47 m day^{-1}) (Schipper and Vojvodic-Vukovic, 2000; Schipper et al., 2005; Robertson and Cherry, 1995). The average detention time (1.8 days), based on the projected flowpaths through the wall (not wall width), is at the lower end of the range of values reported in previous studies (1–10 days)

Table 1
Groundwater velocity, flow length and detention time within the denitrification wall wells for transects 1–3 (T1–T3).

	May		
	Velocity (m day^{-1})	Flow-length (m)	Detention time (days)
T1	2.1	1.9	0.9
T2	0.9	2.8	3.1
T3	2.1	2.1	1.0
Ave.	1.7 ± 0.7	2.3 ± 0.5	1.7 ± 1.2
	July		
	Velocity (m day^{-1})	Flow-length (m)	Detention time (days)
T1	1.3	3.0	2.3
T2	1.5	3.6	2.4
T3	2.2	2.1	1.0
Ave.	1.7 ± 0.5	2.9 ± 0.8	1.9 ± 0.8

(Schipper and Vojvodic-Vukovic, 2001; Schipper et al., 2005) and (10–13 days) (Robertson et al., 2000). Based on these flowmeter measurements and determinations of effective porosity, the wall treats approximately 84 m^3 d^{-1}. Utilizing the Darcy equation from measurements of effective porosity, K_{sat}, and head gradients yield a volumetric treatment rate of 100 ± 28 m^3 d^{-1}, which overlaps the direct measurements taken with the flowmeter.

3.2. Dissolved organic carbon export

In the first sampling event 37 days after denitrification wall installation, dissolved organic C concentration (DOC) was measured at 34 ± 5.1 and 70 ± 71 mg L^{-1} ($n = 3$) in the center and downgradient well and after one year concentrations had decreased to 2.3 ± 1.1 and 2.6 ± 1.4 mg L^{-1} ($n = 3$) respectively (Fig. 3). Over the entire sampling period, the influent water had an average DOC concentration of only 1.1 ± 0.66 mg L^{-1} ($n = 29$). Although they did not report DOC concentrations (Cameron and Schipper, 2010, 2011), reported initially high biological oxygen demand (BOD), which declined over the first few months. This leaching of DOC and BOD has the potential to impact adjacent surface water bodies, particularly when media with high bioavailability are utilized (Cameron and Schipper, 2010, 2011). Lastly, the significant loss of C during start-up will reduce the total available

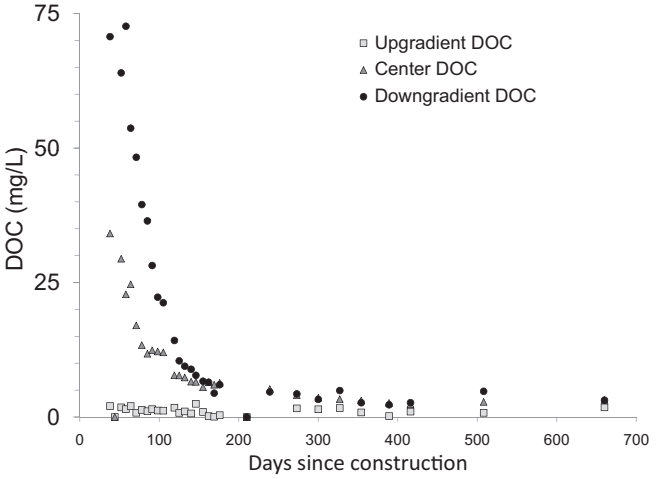

Fig. 3. Temporal trends of dissolved organic carbon in the monitoring wells.

Table 2
Volumetric nitrate removal rates in May and July for the three transects (T1–T3).

	May 2010	July 2010
	NO_3 removal rate ($g\,N\,m^{-3}\,d^{-1}$)	NO_3 removal rate ($g\,N\,m^{-3}\,d^{-1}$)
T1	3.25	2.00
T2	1.33	1.95
T3	5.46	4.91
Ave.	3.35	2.95

C pool of the denitrification wall and will need to figure in to a C mass balance.

3.3. Nitrate concentrations in well transects

Over the 660 days of sampling, average nitrate-N concentration significantly decreased from 6.2 ± 0.65 to $1.6 \pm 0.40\,g\,m^{-3}$ ($n = 30$) between the upgradient and downgradient well transects for a 77% average reduction. The nitrate-N reduction between the upgradient and the well within the wall (center) was much greater (Fig. 4), with reductions in transects 1, 2 of $100 \pm 1.6\%$, and $100 \pm 0.43\%$ and $75 \pm 9.7\%$ reductions in Transect 3 which had the highest influent nitrate-N concentration ($9.3 \pm 1.2\,g\,m^{-3}$) and shortest detention time (0.5 days) (Average reduction = 88%). The 88% reduction in nitrate-N concentration measured in well transects in half the wall flow-distance, indicates the strong possibility that all nitrate-N traveling the width of the wall is lost. The increase in nitrate-N concentration between wells within the wall (0.8 ± 0.26) and downgradient wells ($1.6 \pm 0.40\,g\,m^{-3}$) can likely be attributed to groundwater bypassing the edge of the wall for the following reason. The two transects at the ends of the wall had an average nitrate-N increase of ($1.2 \pm 0.8\,g\,m^{-3}$), while the center transect had no increase ($\sim 0\,g\,m^{-3}$). Therefore it is likely that higher nitrate-N concentrations in the outer two transects downgradient of the wall may be attributed to groundwater bypassing the wall.

Over the study duration, temperature (Table 3) was not correlated with nitrate-N reductions between the upgradient and center wells. Nitrate-N reductions tended to be higher the first few months after wall installation, even with low groundwater temperatures. This is possibly due to the confounding effect of elevated concentrations of bioavailable and soluble carbon (Fig. 3) during this initial start-up period causing temporarily elevated nitrate-N reduction rates.

3.4. Nitrate removal rates

The mass nitrate-N removal rates per volume of reactor media, averaged $3.4\,g\text{-}N\,m^{-3}d^{-1}$ in May 2010 and $3.0\,g\text{-}N\,m^{-3}\,d^{-1}$ in July 2010,225 and 286 days after installation when porewater velocity and direction were directly measured (Table 2). The nitrate-N removal rate of transect 3 (Ave = $5.2\,g\text{-}N\,m^{-3}\,day^{-1}$) where some nitrate-N is still present in the center well is likely more representative of actual rates without nitrate limitation. These values are at the upper end of the range of reported nitrate-N removal rates for other sand-sawdust denitrification walls ($0.014–5\,g\text{-}N\,m^{-3}\,d^{-1}$ (Robertson et al., 2008 (assuming a 50% effective porosity); Schipper et al., 2010). This high denitrification rate is possibly due to elevated groundwater temperature (Table 3; average of $19 \pm 2.7^{\circ}C$) and greater C additions (average total $C = 7.4 \pm 0.7\%$) than some other studies.

Table 3
Groundwater temperatures within the denitrification wall (2009-2010).

	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct
Temp. (°C)	20	18	16	15	15	17	19	20	21	22	22	22

3.5. Total Kjeldahl N concentration in well transects

The total Kjeldahl N (TKN) concentration increased from $0.3 \pm 0.12\,g\,m^{-3}$ upgradient of the wall to $0.9 \pm 0.25\,g\,m^{-3}$ in the center of the wall to $1.0 \pm 0.31\,g\,m^{-3}$ downgradient of the wall within all three transects (Fig. 4). Elevations in ammonium levels generally do not occur in field conditions within denitrification walls (Elgood et al., 2010; Robertson and Cherry, 1995; Schipper and Vojvodic-Vukovic, 1998), although ammonium production and leaching have been observed in mesocosms and in laboratory experiments (Cameron and Schipper, 2011; Greenan et al., 2006).

This rise in TKN is possibly in the form of organic-N associated with DOC leaching, net microbial mineralization (ammonification) or ammonium (NH_4^+) production as a result of dissimilatory nitrate reduction to ammonium (DNRA). The TKN concentration and export rates were largely constant, whereas the DOC export declined in an exponential fashion (Fig. 3) and the relationship between the two was weak ($r^2 = 0.06$). Net ammonium mineralization (ammonification) generally occurs when organic matter C:N ratios decline below 100 (Reddy and DeLaune, 2008). The C:N ratio of the wall media on day 0 was approximately 231 ± 27, which declined in the duration of the study to 140 ± 28. The high C:N ratio indicates ammonium is likely to be retained in microbial biomass to maintain a microbial C:N ratio of 10:1 and net ammonium *immobilization* would occur (Reddy and DeLaune, 2008). DNRA occurs in highly reducing environments (Eh < 0 mv) with high electron pressure, which would arise in conditions with high electron donor (sawdust) to electron acceptor (nitrate-N) ratios as is likely in the denitrification wall (Reddy and DeLaune, 2008). Therefore it is plausible that some of the total nitrate-N load reduced is not lost to the atmosphere but is instead converted to ammonium via the DNRA process. Nitrogen present as TKN is bioavailable and thus can still impact receiving water bodies. Adding TKN-nitrogen to nitrate-N, the total N concentration significantly decreased from $6.6 \pm 0.6\,g\,m^{-3}$ to $2.6 \pm 0.5\,g\,m^{-3}$ ($n = 29$) between the upgradient and downgradient wells for a 62% reduction.

3.6. Total nitrogen load reductions

Two methods were used to provide estimates of volumetric treatment rates to provide rigor to the conclusions. Based on measured concentrations and volumetric treatment rate estimates using the groundwater flowmeter ($84\,m^3\,d^{-1}$) and head gradients ($100 \pm 28\,m^3\,d^{-1}$), the wall reduces nitrate-N load by $190 \pm 20\,kg\,yr^{-1}$ ($n = 2$) and $249 \pm 161\,kg\,yr^{-1}$ ($n = 28$) and increases TKN load by $21 \pm 9\,kg\,yr^{-1}$ and $28 \pm 17\,kg\,yr^{-1}$ utilizing the two methods respectively. Because approximately 11% of nitrate-N is converted to TKN presumably due to the DNRA process, the total N load reduction in groundwater estimated from the groundwater flowmeter and head gradients is 170 ± 23 and 228 ± 155 respectively. The total N content of the wall media increased from $0.032 \pm 0.01\%$ to $0.051 \pm 0.007\%$, which represents a small total microbial N assimilation of 36 ± 13 kg in the 540 days of the study. It is difficult to include this relatively small N-mass in the load reduction rates, as this microbial N is likely not accumulative.

3.7. Media potential denitrification rate

Potential denitrification rates and denitrification enzyme activity values ($4.89 \pm 2.5\,g\text{-}N\,m^{-3}\,d^{-1}$, $n = 3$) reported in (Table 4) are not significantly different from rates determined from the well

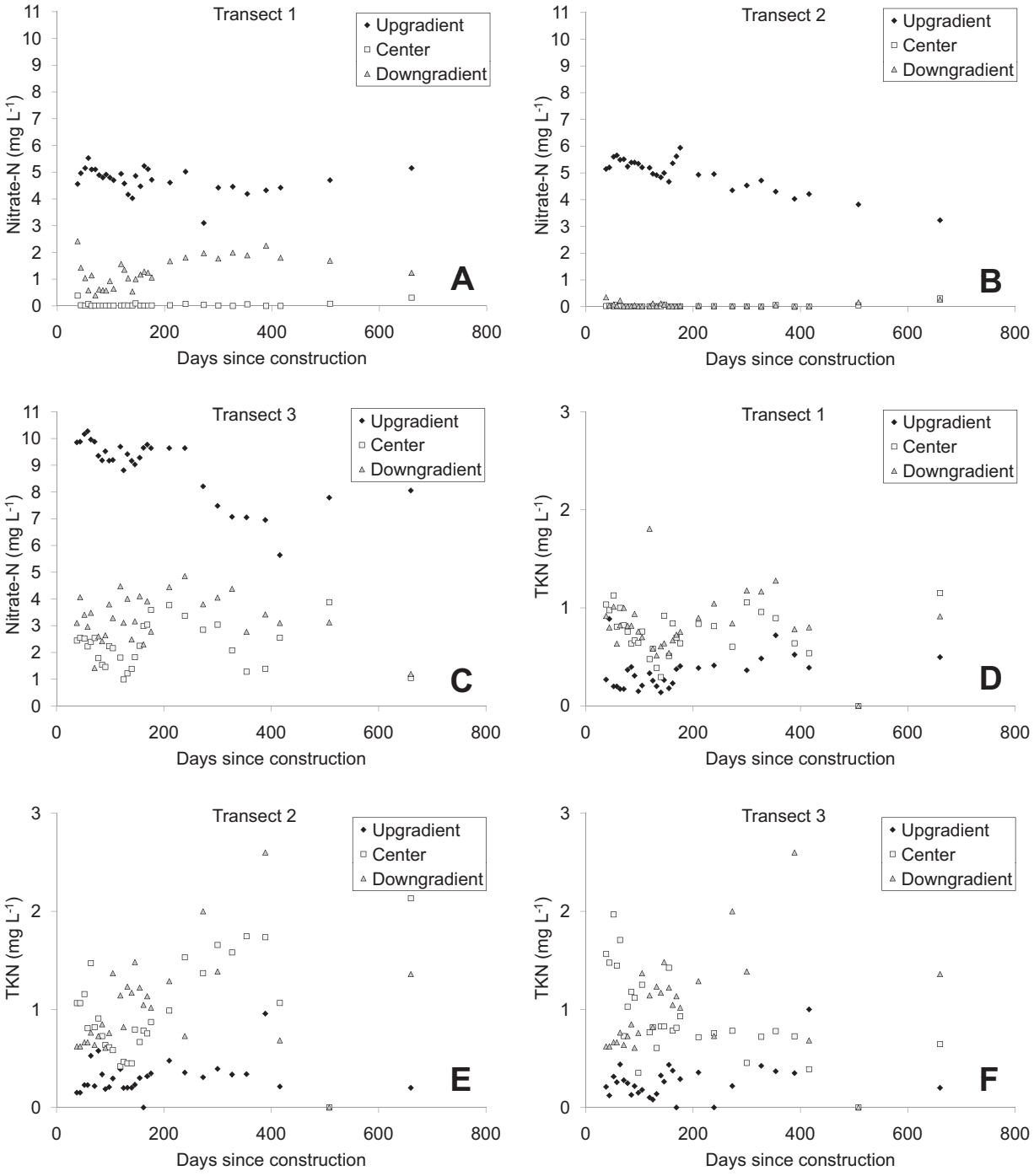

Fig. 4. Temporal trends ofNO_3-N for transects 1–3 (A–C) and TKN for transects 1–3 (D–F).

transects (3.15 ± 1.70 g-N m^{-3} d^{-1}, $n = 6$). Within the samples, N_2O emissions were detected from all media samples collected at the leading edge of the wall but not consistently detected from any samples collected 37 and 74 cm horizontally into the wall (Fig. 1b). Nitrous-oxide emissions were sporadically detected from samples collected beyond the leading edge of the wall in transect 3, where some nitrate-N was present in the center well but not consistently enough to calculate a rate. When media samples were incubated over 59 h, denitrification was detected in some samples where nitrate-N but not chloramphenicol was added indicating the ability of the microbial population to respond to nitrate-N additions. These results suggest that nitrate-N rapidly declines after groundwater enters the wall and subsequently the pool of denitrifying enzymes rapidly declines with flow distance making detection difficult.

3.8. Microbial biomass carbon and total carbon

Microbial biomass C, Total C and fiber analysis results are shown in Table 5. The average microbial biomass C of denitrification wall media (44 ± 19 mg C kg^{-1}, $n = 9$) was four times higher than native soils (10 ± 12 mg C kg^{-1} soil, $n = 3$) both collected at the same depth of approximately 2.3 m. These microbial biomass

Table 4
Potential denitrification rate, and denitrification enzyme activity (DEA) values of the denitrification wall media. Values are reported from samples collected approximately where the groundwater first contacts the wall (0 cm) and further in to the wall (37 and 74 cm) for all three transects (T1–T3) as a rate per m^3 of denitrification wall. Sampling locations are shown in Fig. 1b.

	Distance along transect (cm)	Denitrification rate (g-N m^{-3} d^{-1})	DEA (NO_3 and dextrose) (g-N m^{-3} d^{-1})
T1	0	3.00	1.49
	37	ND	ND
	74	ND	ND
T2	0	3.98	1.49
	37	ND	ND
	74	ND	ND
T3	0	7.68	3.53
	37	ND	ND
	74	ND	ND
Ave.		4.89 ± 2.5	2.17 ± 1.2

ND – N_2O emissions were not consistently detected with time.

C values are much lower than those reported in Florida wetland surface soils (920–2020 mg C kg^{-1} soil; White and Reddy, 2003), Florida fertilized surface soils (72–988 mg C kg^{-1} soil; Grierson et al., 1999), or other denitrification walls (260–445 mg C kg^{-1} soil; Long et al., 2011 and 92–357 mg C kg^{-1} soil; Schipper et al., 2004) but were greater than those collected in a riparian aquifer soil (39.2–41.5 mg C kg^{-1}) collected at a comparable depth (0.6–1.55 m) (Jacinthe et al., 2003). The low background microbial biomass C of native soils collected adjacent to the wall attests to the limitations on biological activity from continuously inundated soils at this depth. The addition of sawdust and inert sand increased the microbial biomass four-fold, which while still low is sufficient for rapid denitrification.

The total C concentration in the wall declined by 5% from 74 ± 7.4 g kg^{-1} to 70 ± 10.9 g kg^{-1} ($n = 9$) 540 days after wall installation (Table 5), although the differences were not statistically significant. Schipper and Vojvodic-Vukovic (1998) also found no significant difference in total C after one-year of operation in a denitrification wall with a lower nitrate-N loading rate. This implies that the C consumption rate is still low in groundwater with high N loading rates.

The lignocellulose index (LCI) is the ratio of lignin content to lignin + cellulose which can be used to infer carbon decomposition in anaerobic soils. Leaf litter in wetland soils stabilizes at an LCI of 0.8, at which point the organic matter is highly resistant to decomposition under continued anaerobic conditions (DeBusk and Reddy, 1998). Within the wall, the LCI was initially 0.25 and increased to 0.4 ± 0.04 in the 540 day duration of the study. This indicates that although there is a decline in C quality, the C is still available for decomposition. Although the C quality has declined, there were no detectable reductions in denitrification rate within 540 days. Longer time-frames will be necessary to discern if this reduction in C quality will influence denitrification rates.

3.9. Total carbon mass balance

A mass balance of C losses can aid with determining major C export processes (aerobic respiration, denitrification, DNRA, DOC export) and projections of wall longevity. The total C mass at the beginning of the study was $1.4 \times 10^4 \pm 1.4 \times 10^3$ kg. Oxygen declined from the upgradient well (3.7–3.9 mg L^{-1}) to the denitrification wall wells (0.56–0.72 mg L^{-1}), which would consume approximately 34–38 kg of C y^{-1} based on the stoichiometry of the aerobic respiration reaction ($C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$). Assuming the likely scenario (Section 3.3) that nitrate-N concentrations are completely

Table 5
Properties of the media within the denitrification wall at the beginning (Day 0) and end of the study (Day 540). Sampling locations at the end of the study are shown in Fig. 1b.

	Distance along transect (cm)	Microbial biomass C		Total C (%)		Total N (%)		NDF (%)		Hemicell (%)		Cellulose (%)		Lignin (%)	
		Day		Day		Day		Day		Day		Day		Day	
		0	540	0	540	0	540	0	540	0	540	0	540	0	540
T1	0	NA	18	$7.42 \pm 0.7n = 4$	5.01	$0.032 \pm 0.01n = 4$	0.057	$2.3 \pm 0.6n = 4$	3.3	$1.8 \pm 0.0n = 4$	0.7	$5.5 \pm 0.6n = 4$	2.5	$1.8 \pm 0.2n = 4$	2.3
	37		46		7.67		0.059		2.9		2.0		7.3		4.4
	74		55		6.74		0.045		2.3		1.1		3.7		2.4
T2	0		50		6.83		0.057		3.6		2.2		7.9		4.7
	37		82		7.88		0.044		1.7		2.3		7.4		4.4
	74		40		8.79		0.054		4.6		1.6		6.2		3.9
T3	0		27		6.99		0.056		3.4		1.3		3.7		2.8
	37		32		6.76		0.041		2.3		1.4		5.2		3.1
	74		43		6.00		0.043		2.3		1.2		4.4		2.8
Ave. ± st. dev.			44 ± 18		6.96 ± 1.0		0.05 ± 0.00		2.9 ± 0.9		1.5 ± 0.5		5.4 ± 1.9		3.4 ± 0.9

depleted within the wall, stoichiometry of the denitrification reaction ($5\,C_6H_{12}O_6 + 24NO_3^- + 24H^+ \rightarrow 12\,N_2 + 42H_2O + 30CO_2$) indicates that the C lost as CO_2 by the denitrification process is approximately $315 \pm 65\,kg\,y^{-1}$. Presuming that the TKN increase is due solely to the DNRA process, C lost from this reaction would be $48 \pm 29\,kg\,y^{-1}$.

To determine C export as a result of DOC leaching, a first-order exponential decay curve was fit to the DOC concentrations over time (Fig. 3)($n = 29$) in the downgradient well with the use of an iterative fit model. Based on this model, combined with volumetric treatment rate estimates from head gradients, the total mass loss attributed to DOC export in the 540 day duration of the study is approximately 990 ± 257 kg. At this stage of the denitrification wall, DOC export has been the dominant process exporting C. The majority of this DOC export occurred in the first few months with export rates as high as $14.5\,kg\,d^{-1}$, although the current DOC export rate of approximately $48\,kg\,y^{-1}$ is much lower than other C-utilizing processes. Long-term field studies have indicated that C consumption follows an exponential decay curve, possibly due to the high leaching of DOC after initial installation (Long et al., 2011; Moorman et al., 2010).

Based on all these estimates, the C loss rate in the wall is approximately $450 \pm 71\,kg\,y^{-}1$. The nitrate-N reduction rates within the denitrification wall will likely decline before the C has been completely depleted, particularly because the lignin fraction ($26 \pm 1.1\%$) of the total C pool is recalcitrant to decomposition in anaerobic conditions. Removing the lignin fraction from the total C pool, the estimated longevity of the denitrification wall is 23 ± 5.9 years. Although this linear valuation provides an initial lifespan estimate, it is likely that C consumption will decline as an exponential decay function (Long et al., 2011; Moorman et al., 2010).

4. Conclusions and recommendations

Previous denitrification walls were installed in aquifers with low groundwater velocities and subsequently volumetric treatment rates were low, and N-limitations occurred in a fraction of the wall width. The present study demonstrates that at least in groundwater with high temperatures, denitrification walls can maintain high nitrate-N removal rates ($Max = 5.5\,g\text{-}N\,m^{-3}\,day^{-1}$) even with short detention times ($1.7–1.9\,d^{-1}$) and rapid groundwater velocities ($1.7\,m\,d^{-1}$). Locating this wall in a rapid-flow aquifer allowed for a large total N load reduction of approximately $228 \pm 155\,kg\,y^{-1}$.

A rapid decline in denitrifying enzymes with short distances and nitrate-N removal rates in the well transect study indicates that nitrate-N was generally depleted in a fraction of the wall width. The rapid N depletion in the wall indicates that the denitrification wall was oversized. A greater treatment volume could be achieved with the same media volume by installing a thinner wall over a longer length. Techniques should be developed, such as utilizing trenching equipment to allow for the construction of thin walls over the edges of agricultural properties and along ditches/streams.

Contrastingly, because C is lost over time, the large size of this wall makes available a larger pool of C which could increase longevity. This conclusion rests on the assumption that C loss rates would be greater at the leading edge, where nitrate is still available for denitrification. In the present study, there were no significant differences in total C or fiber content (Table 5) between samples collected at the leading edge of the wall (0 cm) and two samples located 37 and 74 cm horizontally in to the wall (Fig. 1b). This is possibly due to the fact that the leaching of soluble C (DOC) was the greatest source of C loss in the duration of the study, which could be expected to affect the denitrification wall evenly in space. Because DOC leaching has declined, any spatial differences in C consumption should become more pronounced over time and further monitoring will be necessary to confirm this conclusion.

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