Effects of intermittent loading on nitrogen removal in horizontal subsurface flow wetlands
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ABSTRACT
Removal of CBOD$_5$ and nitrogen from septic tank effluent was evaluated in four horizontal subsurface flow (HSSF) wetlands. An intermittently loaded cell was compared to a continuously loaded control cell, with both treatments receiving the same weekly volume. The intermittently loaded cell was rapidly drained and "rested" for 24-hr, then refilled in steps, twice weekly. Two media with different particle sizes but similar porosities were also compared. The two media, light weight expanded shale and gravel, were both continuously loaded. As hypothesized, the wetland cell that was intermittently loaded had higher dissolved oxygen, greater ammonia removal, and greater nitrate production than the continuously loaded cells. Areal NH$_3$-N removal for the intermittently loaded cell was 0.90 g m$^{-2}$ d$^{-1}$ compared to 0.47 g m$^{-2}$ d$^{-1}$ for the control. Ammonia removal was also higher in continuously loaded gravel cells than in cells with expanded shale. Ammonia-N removal was an order of magnitude lower in a similar SSF wetland that had been in operation for 3 years. However, CBOD$_5$, total suspended solids, and total nitrogen did not vary substantially among the treatments.

Key words | CBOD, intermittent loading, nitrification, nitrogen removal, subsurface flow, treatment wetlands

INTRODUCTION
Domestic wastewaters contribute to nitrogen pollution that threatens drinking water supplies and aquatic environments. On-site wastewater treatment comprises 33% of new home construction in the USA and 10–20% of existing on-site systems are believed to be malfunctioning (EPA 2005). Constructed wetlands are a cost-effective, low maintenance solution for secondary treatment of on-site septage. Subsurface flow (SSF) wetlands are more appropriate for on-site treatment than surface water wetlands because they reduce human exposure to pathogens (EPA 2000a); however, the low oxygen environment presents a challenge for efficient nitrogen removal through nitrification (Brown et al. 2000).

Increasingly, experimental designs have employed strategies to increase oxygen levels in SSF wetlands. Examples include use of porous media (Albuquerque et al. 2009), active aeration of the SSF beds (Chazarenc et al. 2009), recirculation (Sikora et al. 1995), cascading type systems (Tanner et al. 2002), and various combinations, or hybrids, of these techniques (Ye & Li 2009). Higher nitrification efficiency has been observed in wetland systems known as Seidel models (Seidel 1966) which are operated continuously for 1–2 days, then drained and rested for 2–8 days. The volume of effluent drained is passively replaced with fresh air for oxidation of carbon and nitrogen. Modifications of this design have further improved ammonia removal (Green et al. 1999; Langergraber et al. 2008). Additionally, the porosity and nature of the surface available for colonization can affect biofilm development and thus treatment rates (Akratos & Tsihrintzis 2007).
For example, wetlands with an expanded clay aggregate had better ammonia removal than gravel wetlands (Albuquerque et al. 2009).

This research investigated design and operation strategies to improve the CBOD$_5$ and ammonia removal of SSF wetlands receiving septic tank effluent. An intermittent (fill and draw) hydraulic loading regime was compared to a control that had the same weekly hydraulic load. Two types of media, light weight expanded shale (Texas Industries, Inc.) and gravel, that have different particle sizes but similar external porosity, were also compared. Both cells were operated under continuous loading. Because the hydraulics and nutrient removal efficiency of treatment wetlands can change over time (Batchelor & Loots 1997) the treatment efficiency of an existing 3-year-old wetland system with similar components was compared to the neophyte cells.

**METHODS**

Research was conducted at the Baylor Wastewater Research Program (BWRP) facility located in Waco, Texas, USA (Figure 1). The BWRP receives raw municipal wastewater which is dosed to a two-chamber septic tank using a design loading protocol that simulates household daily water use patterns. The dosing protocol has 33% of the total daily load delivered in the morning between 6–9 am, 25% at mid-day between 11 am–2 pm, and 40% in the evening between 5–8 pm (NSF/ANSI 40 2005). Top and front loaded, HSSF wetland cells (Figure 1) were lined with 0.45-mm Firestone Pond Gard Rubber Liner®. The control (CONT) and intermittent (INTR) cells were filled with alternating sections of expanded shale and grade 3 concrete rock (gravel), whereas the gravel (GRAV) and AGED cells contained only gravel. Median particle size of gravel and expanded shale were 1.3 and 1.0 cm respectively. Substrate porosities of the units were determined by fill and drain to be 40% in GRAV and 39% in units with gravel and shale (INTR, CONT).

The daily hydraulic loading rate was 76 L cell$^{-1}$ (4.2 cm d$^{-1}$) yielding a hydraulic retention time of approximately 3 d. The intermittent cell was drained, rested, and refilled twice weekly but received the same weekly volume of septic tank effluent as the other cells. Water depths were kept at approximately 30 cm with standpipes. All cells were vegetated with Schoenoplectus californicus; AGED also contained Typha latifolia, Sagittaria lancifolia, and Pontederia cordata. We evaluated the following hypotheses:

**Hypothesis 1.** Intermittent loading provides higher dissolved oxygen, greater CBOD$_5$ removal, and greater ammonia removal than continuous loading (CONT v INTR).

**Hypothesis 2.** Cells with expanded shale provided greater CBOD$_5$ and ammonia removal than cells with gravel (CONT v GRAV).

**Hypothesis 3.** Aged wetlands provided poorer CBOD$_5$ and ammonia removal than newly constructed wetland cells (GRAV v AGED).
Septic tank and wetland effluents were sampled weekly (24-hour composite) from December 2007 through December 2008. For dissolved nutrients (NH₃ and NO₂ + NO₃) samples were filtered through a 0.45-micron glass fiber filter. Duplicate filters were then used to determine total suspended solids by drying in a 103–105°C oven. Total and dissolved nutrients were measured with a Lachat Quickchem 8500 Flow Injection Autoanalyzer using standard colorimetric techniques (APHA 1998). Carbonaceous BOD₅ analyses were determined in triplicate using a nitrification inhibitor. Temperature and pH were measured using an YSI XLM 600 multiparameter datasonde.

Mass removal rates for CBOD₅ ($r_{\text{CBOD}_5}$), total nitrogen ($r_{\text{TN}_5}$), ammonia ($r_{\text{NH}_3}$), and nitrite + nitrate ($r_{\text{NO}_3}$) were calculated from differences of influent (septic) and effluent concentrations (Equation (1), EPA 1999).

$$r = (C_i - C_e)Q/A$$  

where $r$ is the removal rate (g m⁻² d⁻¹), $C_i$ and $C_e$ are the influent and effluent concentrations (g m⁻³), $Q$ is the average flow rate (m³ d⁻¹) and $A$ is the total effective area of the wetland bed (m²).

Hypotheses were evaluated with two-sample t-tests after log transformation, where necessary, to meet assumptions of normality. Differences among multiple cells were modeled using two-factor analyses of variance (ANOVA) followed by Tukey-Kramer multiple comparison test ($\alpha = 0.05$, JMP 7.0). Effects of sampling date were accounted for in two-way ANOVAs.

**RESULTS**

Means and distributions of turbidity, dissolved oxygen, and CBOD₅ data are shown in Figure 2. Mean turbidity was lowest in the INTR cell, followed by GRAV and CONT. The AGED effluent had the highest turbidity with a removal efficiency (RE) of only 45% compared to 94% by the INTR cell. Removal of total suspended solids (TSS) followed a similar trend but the large variability in TSS data precluded statistical significance among treatment cells (data not shown). Mean TSS of septic tank effluent was 57.1 mg l⁻¹ while the mean of the treatment cells was 11.7 mg l⁻¹ yielding a mass removal of 45.4 mg l⁻¹ (1.9 g m⁻² d⁻¹; RE = 80%).

Dissolved oxygen was not significantly different among cells based on a multiple range test (due to bonferroni correction $\alpha = 0.008$), however a t-test between CONT and INTR supported the hypothesis that INTR has higher oxygen levels ($p = 0.0139$). While INTR mean dissolved oxygen was approximately 1 mg l⁻¹ higher than in CONT, INTR had only 6 (of 50) samples with dissolved oxygen values less than 2.0 mg l⁻¹ and only 18 that were less than 4.0 mg l⁻¹ (Figure 2). In contrast, CONT had 12 samples with dissolved oxygen less than 2.0 mg l⁻¹ and 32 samples that were less than 4.0 mg l⁻¹. Mean pH was slightly higher (7.62) in INTR and lower in GRAV (7.25); pH generally decreased over time (data not shown). Temperatures ranged from 4.1°C to 26.2°C (mean 15.7°C) with no significant differences among cells.

The mean (± SD) areal loading rate of CBOD₅ to the wetlands was 2.41 ± 1.87 g m⁻² d⁻¹. Mean CBOD₅ ranged from 56 mg l⁻¹ in septic tank effluent to 8.6 mg l⁻¹ in INTR, an 85% reduction (Figure 2). However, the INTR cell was not statistically different than the CONT (two-sample t-test, $p = 0.079$), thus the CBOD₅ hypothesis was not supported. The second lowest mean was in GRAV (13.8 mg l⁻¹). CBOD₅ was strongly influenced by sample date ($p < 0.0001$), and removal generally improved as the cells...
matured. Areal CBOD$_5$ removal rates ($r_{\text{CBOD}_5}$) ranged from 1.9 g m$^{-2}$ d$^{-1}$ in CONT, GRAV and AGED cells, to 2.0 g m$^{-2}$ d$^{-1}$ in INTR (Table 1).

The mean areal loading rate of NH$_3$-N was 1.36 ± 0.39 g m$^{-2}$ d$^{-1}$. All of the cells removed NH$_3$-N; however, INTR and GRAV produced lower NH$_3$-N effluents than CONT (Figure 3). INTR and GRAV had mean effluent NH$_3$-N of 10.9 mg l$^{-1}$ and 15.2 mg l$^{-1}$ respectively compared to 21.4 mg l$^{-1}$ in the control. The INTR cell had the largest areal NH$_3$-N removal rate ($r_{\text{NH}_3\text{-N}}$) at 0.90 g m$^{-2}$ d$^{-1}$ and the AGED cell the lowest at 0.29 g m$^{-2}$ d$^{-1}$ (Table 1). Ammonia concentrations dropped sharply in INTR beginning in March 2008 (Figure 3). A similar decline occurred in GRAV in April–May, 2008 and in CONT in August, 2008. Ammonia levels in influent from the septic tank were more consistent over time.

Only in the INTR and GRAV cells did evidence of nitrification coincide with decreased ammonia; although the appearance of nitrate (NO$_3$-N) was not observed in GRAV until July (Figure 2). Mean NO$_3$-N levels were well below 1.0 mg l$^{-1}$ in septic tank effluent and generally less than 1.0 mg l$^{-1}$ in CONT and AGED. Mean NO$_3$-N in INTR was 10.9 mg l$^{-1}$ and in GRAV mean NO$_3$-N was 3.3 mg l$^{-1}$. The maximum NO$_3$-N concentration of 28.8 mg l$^{-1}$ occurred in INTR on June 27th. These results support the hypothesis predicting better ammonia removal and higher NO$_3$-N concentrations in an intermittently loaded wetland. The improvement in nitrification and CBOD$_5$ removal over time did not appear to be directly related to temperature (Figure 3). Hypothesis 2 was not supported with respect to ammonia because GRAV had greater ammonia removal than CONT.

Total nitrogen (TN) concentrations in septic tank effluent averaged 43 mg l$^{-1}$. The poorest TN removal occurred in AGED, which reduced TN by only 41% while the INTR cell reduced TN by 55% to a mean of approximately 19 mg l$^{-1}$; however, this difference was not statistically significant (Figure 4).

**DISCUSSION**

**Intermittent loading**

As hypothesized, intermittent loading increased nitrification compared to continual loading. The continuously loaded gravel cell also showed an increasing trend toward nitrification; however, since the AGED gravel wetland had the poorest ammonia removal, it is unclear whether nitrification in GRAV would be sustainable. Areal ammonia removal and removal efficiency in INTR (0.9 g m$^{-2}$ d$^{-1}$; 66%) were higher than many values reported in the literature for SSF wetlands. For example, Vymazal &

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**Table 1** | Means (± standard deviations) of removal rate constants for CBOD$_5$, NH$_3$-N, NO$_3$-N, and total nitrogen (TN) by wetland unit

<table>
<thead>
<tr>
<th>Unit</th>
<th>$r_{\text{CBOD}_5}$ (g m$^{-2}$ d$^{-1}$)</th>
<th>$r_{\text{NH}_3\text{-N}}$ (g m$^{-2}$ d$^{-1}$)</th>
<th>$r_{\text{NO}_3\text{-N}}$ (g m$^{-2}$ d$^{-1}$)</th>
<th>$r_{\text{TN}}$ (g m$^{-2}$ d$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.9 ± 1.5</td>
<td>0.47 ± 0.62</td>
<td>−0.01 ± 0.08</td>
<td>0.96 ± 0.83</td>
</tr>
<tr>
<td>Intermittent</td>
<td>2.0 ± 1.6</td>
<td>0.90 ± 0.56</td>
<td>−0.30 ± 0.29</td>
<td>1.02 ± 0.69</td>
</tr>
<tr>
<td>Gravel</td>
<td>1.9 ± 1.5</td>
<td>0.73 ± 0.65</td>
<td>−0.12 ± 0.25</td>
<td>0.99 ± 0.97</td>
</tr>
<tr>
<td>Aged</td>
<td>1.9 ± 1.6</td>
<td>0.29 ± 0.40</td>
<td>−0.01 ± 0.05</td>
<td>0.74 ± 0.62</td>
</tr>
</tbody>
</table>
Kröpfelová (2009) calculated a median of 0.27 g m$^{-2}$ d$^{-1}$ (RE 30.4%) for 254 SSF wetlands treating municipal wastewaters. Removal rates tend to be lower in free surface water (0.01 to 0.16 g N m$^{-2}$ d$^{-1}$; Reddy & D’Angelo 1997) and natural wetlands (0.1 g m$^{-2}$ d$^{-1}$; Reddy et al. 1989). Based on mean in and out concentrations, first order volumetric rate constants ($k$) for our cells ranged from 0.04 d$^{-1}$ in AGED to 0.38 d$^{-1}$ in INTR. Reddy & Patrick (1984) found a mean $k$ of 0.29 d$^{-1}$ for a range of flooded soils. Sikora et al. (1995) found that $k$ ranged from 0.06–0.24 d$^{-1}$ for a recirculating SSF wetland.

A variety of passive or low energy designs for increasing nitrification in SSF systems have been evaluated; including systems that facilitate oxygen exchange with pumps, fill and draw systems, cascades, and hybrids of various designs connected in series (Vymazal 2007). Cascading designs have yielded nitrification rates of 0.56 to 2.15 g N m$^{-2}$ d$^{-1}$ (Tanner et al. 2002) and high removal rates of TN with simultaneous nitrification and denitrification (Ye & Li 2009). Fill and draw experiments with dairy wastewater had higher ammonia removal rates with increasing frequency of water level fluctuations in the range of 0–16 d$^{-1}$ (Tanner et al. 1999). Langergraber et al. (2008) achieved ammonia removal of 3.9 g N m$^{-2}$ d$^{-1}$ with a 3-hr fill and draw cycle. Green et al. (1999) measured ammonia removal as high as 19 g N m$^{-2}$ d$^{-1}$ in laboratory experiments simulating a vertical-flow fill and draw system; however the artificial wastewater did not have organic carbon or organic nitrogen.

The missing N problem

“Missing nitrogen” is common in wetland studies (Wallace & Austin 2008), particularly in black box studies such as ours where the difference between TN and TIN (NH$_3$-N + NO$_3$-N) removal is substantial (Figure 4). Conventional pathways of NH$_3$-N in wetlands include volatilization, sorption, plant uptake, and nitrification, with the latter accounting for the bulk of removal in enriched systems. The removal of particulate N by TSS at removal rates of 1.9 g m$^{-2}$ d$^{-1}$ and 0.005% nitrogen content (Heukelekian & Balmat 1959), results in less than 1 mg N m$^{-2}$ d$^{-1}$ removal by TSS filtering. Vymazal & Kröpfelová (2009) found a range for above-ground plant-bound N of 5–59 g N m$^{-2}$. Doubling these estimates to account for below ground biomass, a neophyte wetland could remove from 0.03 to 0.32 N m$^{-2}$ d$^{-1}$ during initial plant development. Total nitrogen is continually converted by mineralization to NH$_3$ at rates of 0.22–0.53 g N m$^{-2}$ d$^{-1}$ (Tanner et al. 2002) which could account for a large portion of our TN removal. These crude estimates suggest that our gross NH$_3$-N removal may be underestimated because we did not account for the continual production of NH$_3$. This underestimation may be greater in the continuously loaded wetlands, where simultaneous nitrification-denitrification could occur.

Despite the paradigm that nitrification is inhibited when oxygen levels fall below 2.0 mg l$^{-1}$, (EPA 2000b) more recent studies confirm ammonia oxidation proceeds at DO levels as low as 0.5 mg l$^{-1}$ with sharp increases in rates between 0.5–1.5 mg l$^{-1}$ DO (Jianlong & Ning 2004). An alternative pathway for ammonia removal, anaerobic ammonia oxidation (Anammox) has been documented in many low oxygen systems including SSF wetlands (Paredes et al. 2007). Heterotrophic nitrification has also been demonstrated with heterotrophic nitrifiers capable of both nitrification and denitrification (Paul & Clark 1996). The process occurs via Paracoccus denitrificans, a slow-growing bacteria that simultaneously use both oxygen and nitrate as terminal electron acceptors. Both Anammox and heterotrophic nitrification have been documented in fill and drain treatment wetlands (Wallace & Austin 2008).

Figure 4 | Mean areal removal (g m$^{-2}$ d$^{-1}$) of ammonia-N, nitrate-N, and TN. Dashed line and adjacent values show difference between TN and TIN.
Media porosity

Media characteristics such as surface area and porosity have been shown to affect nitrification rates. For example, fine gravel reduced TKN by 82.5% compared to 54.8% removal using medium gravel (Akratos & Tsihrintzis 2007). Similarly, SSF wetlands with a LECA (light expanded clay aggregate, porosity 0.45) media had better ammonia removal than gravel (porosity 0.40) wetlands (Albuquerque et al. 2009). Surfaces such as twigs yielded higher nitrification rates than Eurasian watermilfoil or sediment (Bastviken et al. 2003). Greater ammonia removal in this study’s GRAV cell (relative to CONT which contained 50% shale) may be due to gravel’s larger pore size, which could facilitate atmospheric gas exchange. Thus larger particle size may enhance nitrification despite smaller surface areas that reduce the total biomass of attached nitrifiers and Anammox bacteria.

Aged wetland

The 3-year-old wetland (AGED) had the poorest nitrogen removal and showed no conversion of NH₃ to NO₃. The AGED cell also had the lowest dissolved oxygen levels, as well as higher turbidity. The accumulation of solids was observed when removing samples of AGED gravel media and wastewater was observed backing up near the front of the AGED wetland. Thus reduced efficiency may be attributable to the accumulation of solid material in the substrate pore spaces, which could then exert additional oxygen demand. Others (Tanner et al. 1998) have observed substantial organic matter accumulation and decreased effective wastewater retention times in gravel-bed constructed wetlands over a 5-year period. The formation of a detritus-soil layer on the surface of the bed may also limit oxygen exchange from the atmosphere. Although TN removal was not statistically significant among treatments, the newly constructed cells removed 53–57% of applied TN while the AGED cell removed only 41%. Thus age, as well as differences in cell size and configuration, probably contributed to the differences in TN removal between GRAV and AGED cells. It has been shown that periodic draining wetlands can reduce solids buildup in wetland media (Chazarenc et al. 2009), thus draining or an intermittent loading approach may prolong the useful life of SSF wetlands.

CONCLUSIONS

Removal efficiencies for suspended solids (80%) and CBOD₅ (92%) were similar for the four treatments and within the range of those reported for many SSF systems (Vymazal 2002). Our intermittently loaded cell had an areal NH₃-N removal rate (0.9 g m⁻² d⁻¹) which was nearly twice that of the continuously loaded control and higher than average removal rates reported for treatment wetlands. The higher DO levels and consistent production of nitrate suggests that aerobic nitrifiers were probably responsible for most of the ammonia processing; however, redox conditions were unfavorable for complete denitrification. In contrast, ammonia removal in continuously loaded cells may have been due to a combination of conventional nitrification-denitrification and Anammox processes. At the NH₃-N k rate of 0.38 d⁻¹, producing an EPA recommended effluent concentration of 12.3 mg l⁻¹ (EPA 1999) from an inflow of 50 mg l⁻¹ would require a detention time of approximately 4 days. In addition, greater ammonia removal may be achieved by increasing the frequency of the drain and fill cycle.

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