Nitrogen transformations and mass balance in an integrated constructed wetland treating domestic wastewater

Mawuli Dzakpasu, Miklas Scholz, Valerie McCarthy and Siobhán Jordan

ABSTRACT

Nitrogen (N) transformations and removal in integrated constructed wetlands (ICWs) are often high, but the contributions of various pathways, including nitrification/denitrification, assimilation by plants and sediment storage, remain unclear. This study quantified the contributions of different N removal pathways in a typical multi-celled ICW system treating domestic wastewater. Findings showed near complete average total N retention of circa 95% at 102.3 g m⁻² yr⁻¹ during the 4-year period of operation. Variations in total N and NH₄–N removal rates were associated with effluent flow volume rates and seasons. According to the mass balance estimation, assimilation by plants and sediment/soil storage accounted for approximately 23% and 20%, respectively, of the total N load removal. These were the major N removal route besides microbial transformations. Thus, the combination of plants with high biomass production offer valuable opportunities for improving ICW performance. The retrieval and use of sequestered N in the ICW sediment/soils require coherent management and provide innovative and valuable opportunities.

Key words | constructed wetlands, domestic wastewater, mass balance, nitrogen transformation, plant assimilation

INTRODUCTION

Nitrogen (N) removal in constructed wetlands (CWs) is extremely complex and includes various physical, chemical, and biological interactions, which occur sequentially or simultaneously (Vymazal 2007). In free water surface wetlands, the N transformation and removal processes include microbial conversion, plant uptake and biomass assimilation, sedimentation, and volatilization. The removal rates vary considerably and are associated with the species of plants, wetland media, wastewater types, retention time, loading rates, climatic conditions, and system design (Kadlec & Wallace 2000).

Current estimates indicate that nutrient uptake by macrophytes in CWs accounts for 3–47% of the N removal, depending on the species used, type of wastewater treated and nutrient loading rates (Gottschall et al. 2007). Nonetheless, some recent studies argue that N removal in CWs through macrophyte uptake was significant only in systems with low N loadings. Mostly, less than 1% of the N removal was attributed to uptake by macrophyte (Brix 1994, 1997; Mustafa & Scholz 2011). However, many such studies were conducted at microcosm, mesocosm and pilot scales (Ko et al. 2011).

The concept of integrated constructed wetlands (ICWs) (Scholz et al. 2007), which has been pioneered in Ireland over the last two decades, incorporates a combination of several different species of macrophyte to harness their cumulative nutrient uptake capacities, and also to take advantage of their inherent different responses to seasonal changes. The complex combination of plant diversity and habitat characteristics could, most likely, increase the size of the microbial community (Zhang et al. 2013) and, subsequently, the N removal efficiency in ICWs. In addition, the diversity of macrophytes could be an important factor, with respect to their cumulative N uptake capacities. Furthermore, necromass and associated nutrient-containing residuals (detritus) can accumulate in sediment/soils, remaining in situ for many years.

Whereas the N removal in ICWs is known to be high (Dzakpasu et al. 2011), the contribution of various transformation and removal pathways (nitrification/denitrification,
assimilation by plants and sediment storage) remains unclear. The objectives of this paper, therefore, were to assess the N removal in a typical multi-celled ICW system treating primary domestic wastewater, and to quantify the contribution of different N removal pathways. An estimate of the N mass balance is compiled by comparing the input and output N loadings and the assimilation and storage of N in ICW plants and sediments/soils. By using this mass balance approach to quantify the contributions of the different N removal pathways, the results from this study may be helpful in providing guidelines for the design and management of ICW systems for domestic wastewater treatment.

MATERIALS AND METHODS

Study site description

The ICW treatment system at the centre of the study is located within the walls of the Castle Leslie Estate at Glaslough in County Monaghan, Ireland (06°53′37.94″ W, 54°19′6.01″ N). Mean seasonal temperatures for the area were: spring (10.7 °C), summer (14.9 °C), autumn (7.9 °C), and winter (2.9 °C). The mean annual rainfall was approximately 970 mm. Hydraulic characteristics of the wetland cells are presented in Table 1. The ICW system (Figure 1) was commissioned in October 2007, to treat sewage from Glaslough.

The design capacity of the ICW system is 1,750 p.e. The functional area (open water and vegetated) of the ICW cells is 3.25 ha within a curtilage area of 6.74 ha. Influent primary domestic wastewater from Glaslough is pumped directly into a receiving sedimentation pond. From there, the wastewater subsequently flows by gravity sequentially through five wetland cells. The effluent from the last wetland cell discharges directly into the adjacent Mountain Water River (Figure 1).

The wetland cells were planted in a club pattern, and the main ones were Carex riparia Curtis, Phragmites australis (Cav.) Trin. ex Steud., Typha latifolia L., Iris pseudacorus L., and Glyceria maxima (Hartm.) Holmb. The wetland plants currently includes a complex mixture of Glyceria fluitans (L.) R.Br., Juncus effusus L., Sparganium erectum L. emend Rchb, Elisma natans (L.) Raf., and Scirpus pendulus Muhl.

Wetland wastewater sampling

Sampling was carried out weekly from February 2008 to March 2012. Sampling points were fixed at the inlet of each ICW cell (Figure 1). Composite influent water samples were collected during the monitoring period by using a suite of ISCO 4700 automatic wastewater samplers (Teledyne Isco, Inc., USA). All collected samples were stored in the refrigerated chambers of the auto-samplers at 2 °C and then transported to the laboratory in Ireland for analysis.
Hydrological monitoring

Wastewater inflows and outflows

The volumes of water flowing into and out of each ICW cell were measured and recorded with Siemens Electromagnetic Flow Meters FM MAGFLO and MAG5000 (Siemens Flow Instruments A/S, Nordborgrej, Nordborg, Demark) and their allied computer-linked data loggers. Mean flows were recorded at one-minute interval frequencies.

Meteorological data

A weather station (WeatherLink®, Davis Instruments Corp., Hayward, CA, USA) is located beside the inlet pump sump, which measured elements of the local weather, including air temperature, precipitation and reference (potential) evapotranspiration \((E_{T_0})\). All meteorological variables were measured and recorded at 15-minute intervals. The WeatherLink® uses air temperature, relative humidity, average wind speed, and solar radiation data to estimate the \(E_{T_0}\). The daily evapotranspiration rate was then calculated as shown in our previous paper (Dong et al. 2011).

Water quality analyses

The water samples collected from the different sampling sites were analysed weekly at the Monaghan County Council wastewater laboratory in Ireland, using kits supplied by HACH Lange (HACH Company, Loveland, CO, USA), and by following the standard operating procedures for the HACH DR/2010 portable data logging spectrophotometer (HACH Company 2000). \(NH_4-N\) and \(NO_3-N\) were determined by the Nessler method (HACH Method 8038) and the cadmium reduction method (HACH Method 8171), respectively. The low range total N (HACH Method 10071) and high range total N (HACH Method 10072) were determined following persulfate digestion.

Nitrogen loading and removal rates

The mass loading for N in the ICW cells was calculated by using the dynamic water budget approach (Kadlec & Knight 1996; Kadlec & Reddy 2001; Kadlec & Wallace 2009).

Nitrogen mass budget

The annual N mass budget was compiled for the entire ICW system. Nitrogen input to the ICW and its compartments was domestic wastewater effluent input from the village, and atmospheric deposition. The corresponding outputs were surface water output, harvest of emergent macrophytes, accumulation in the sediment/soils, groundwater recharge (from Dzakpasu et al. 2012), and microbial transformations.

Harvest of emergent macrophytes

Samples were collected during the macrophyte life cycle in 2011. The above-ground biomass of emergent macrophytes was sampled on 29 July 2011 to coincide with the time of maximum growth, where nutrient uptake is highest, and prior to autumn senescence, where above-ground nutrients are translocated to roots and rhizomes (Kadlec & Knight 1996; Vymazal 2007). No macrophytes were harvested in the years before, but they naturally die off during the autumn and winter. Macrophyte samples were collected from 30 × 30 cm quadrats that were established at five randomly selected locations along the full length of each ICW cell. The ICW cells contained a mixed set of macrophyte species and, therefore, the individual nutrient uptake rates of different plants could not be determined. The samples were oven dried at 60 °C until constant weight was achieved, to determine dry weight biomass. The amounts of N removed by harvesting were determined as a product of dry weight biomass (standing stock) and the concentration of N in macrophyte tissue.

Accumulation in the sediment/soil

Annual storage of N in the sediment/soil was determined in the ICW cells between March 2011 and 2012. There was no sediment/soil removal from the ICW cells during the experimental period. Therefore, the amount of N included in the mass balance was assumed to have accumulated over this period, which was calculated as the difference between the initial concentration of N measured before the start of the ICW operation and the final concentration, which was measured between March 2011 and 2012. During this time, the sediment/soil layers at 0–15 cm depth (no N accumulation was observed in the deeper layers underneath) were sampled quarterly at sampling locations distributed over the full length of the ICW system. Three locations, one near the inflow, one near the middle and one near the outflow of each ICW cell were sampled. At each of these locations, samples were collected in triplicate along vertical transects across each cell, which were then pooled to form one sample. Sediment samples were oven
dried at 60 °C until constant weight was achieved. The sediment/soil nitrogen content (kg m⁻²) of each ICW cell over the experimental period was calculated as a product of the nitrogen concentration in the sediment/soil (g kg⁻¹) and the sediment weight per unit area (kg m⁻²).

**Analyses of nitrogen in macrophytes and sediments**

Macrophytes and sediment/soil samples were milled (Pomix® PX–MFC 90D, Kinematica AG, Luzern) to a fine mesh size and subsequently analysed for N. Total N concentrations were determined by the dynamic flash combustion method performed with an elemental analyser, vario EL cube (Elementar Analysensysteme GmbH, Hanau, Germany).

**Statistical analyses**

Data distributions were tested for normality using the Shapiro–Wilk U-test. Statistically significant differences were determined at α = 0.05, unless otherwise stated. Comparisons of means were by paired samples t-test and one-factor analysis of variance. All statistical analyses were performed using Minitab 16 (Minitab Inc., State College, PA, USA) and IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY, USA).

**RESULTS AND DISCUSSION**

**Influent and effluent nitrogen concentrations**

Overall, NH₄-N was recorded as the dominant species of N contained in the influent wastewater received by the ICW (Table 2). Annual influent concentrations for total N, NH₄-N and NO₃-N, indicated a slight variability of the influent domestic wastewater composition. Individual values of N concentrations in the ICW effluent were mostly less than 1.0 mg L⁻¹, but values greater than 5.0 mg L⁻¹ were recorded during winter. The annual mean values are shown in Table 2.

Overall, the ICW system reduced total N and NH₄-N concentration by 95% and NO₃-N by 86%. Most of the removal was recorded within cells 1–3 (Figure 2). Furthermore, influent concentrations of the N species showed some seasonal variations. Nevertheless, whereas the seasonal variations in concentrations of the influent NO₃-N were significant (p < 0.01), variations of the influent total N and NH₄-N were not.

The highest seasonal influent concentrations of total N and NH₄-N (69 ± 26.3 mg N L⁻¹ and 42 ± 10.0 mg N L⁻¹, respectively) and NO₃-N (8 ± 5.9 mg N L⁻¹) were recorded in summer and spring, respectively. The highest removal rate for total N and NH₄-N occurred during the summer, and that for NO₃-N occurred during spring. The lower N removal performance of the ICW during winter was reflected in the effluent total N and NH₄-N concentrations, which were significantly higher compared to the other seasons (p < 0.05). No significant seasonal variations in the concentrations of NO₃-N in the effluent were observed and were typically in the region of 0.5 mg N L⁻¹. Factors affecting the seasonal variation of contaminants including N in this ICW system were previously evaluated in detail by Dong et al. (2011) and Dzakpasu et al. (2011).

**Nitrogen loading and removal rates**

In general, average annual mass removal efficiencies were relatively high for the ICW. Approximately 93.4 and 91.0% removal were recorded, respectively, for total N and NH₄-N, whereas 77.5% was recorded for NO₃-N. Over the study period, surface inflows carried a total load of 0.54 kg m⁻² and 0.41 kg m⁻² of total N and NH₄-N, respectively, into the ICW system and nearly 95% for both total N and NH₄-N were retained (Table 2). Similarly, a total load of 0.04 kg m⁻² NO₃-N had been received by the ICW, and about 90% retention had been recorded. Overall, nitrogen

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration (mg L⁻¹)</th>
<th>Total load (kg)</th>
<th>Total mass retained (%)</th>
<th>(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Influent*</td>
<td>Effluent*</td>
<td>Influent</td>
<td>Effluent</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>62 ± 3.54</td>
<td>3.3 ± 0.43</td>
<td>17,520</td>
<td>927</td>
</tr>
<tr>
<td>Ammonia-nitrogen</td>
<td>37 ± 1.60</td>
<td>2.2 ± 0.45</td>
<td>13,017</td>
<td>688</td>
</tr>
<tr>
<td>Nitrate-nitrogen</td>
<td>4.7 ± 0.57</td>
<td>0.5 ± 0.05</td>
<td>1,191</td>
<td>131</td>
</tr>
</tbody>
</table>

*Average ± standard error.
was effectively removed from the influent wastewater throughout the study period.

Nitrogen assimilation by plants

Over the study period, the highest average standing biomass production of 1.4 kg m$^{-2}$, as determined by the dry weight method, was recorded in cell 3. The biomass production yields in the entire ICW system averaged to 1.2 kg m$^{-2}$. Standing biomass in cell 3 accounted for approximately 43% of the total biomass in the ICW system (Table 3), whereas the other cells accounted for approximately 57%. The difference in biomass production yields in the different cells of the ICW were not statistically significant ($p > 0.05$), suggesting that fairly similar growing conditions prevail in all the wetland cells.

The biomass production yield in this ICW system is similar to other free water surface wetlands in Ireland and elsewhere (Karunaratne et al. 2014; Healy et al. 2007; Borin & Salvato 2012). Biomass levels greater than those in the ICW have also been reported (Meuleman et al. 2002; Kadlec & Wallace 2000).

The total N contents of the above-ground biomass in the ICW cells showed very little variation (Figure 3). An overall average of 21.6 g N kg$^{-1}$ dry weight (DW) was recorded, which rose from a minimum value of 17.8 g N kg$^{-1}$ DW in cell 5 to a maximum value of 23 g N kg$^{-1}$ DW in cell 3 and cell 4. The results obtained in this current study are comparable to similar studies under similar climatic conditions in western Ireland (Healy et al. 2007) and northeast Italy (Bragato et al. 2006).

The corresponding macrophyte total N uptake rates in the individual ICW cells are illustrated in Figure 4. An overall average uptake rate of approximately $28 \pm 4.4$ g N m$^{-2}$ yr$^{-1}$ was recorded for the entire ICW system. The highest uptake rate of $32 \pm 6.3$ g N m$^{-2}$ yr$^{-1}$ was recorded in cell 3, whereas the lowest uptake rate of $22 \pm 1.6$ g N m$^{-2}$ yr$^{-1}$ was recorded in cell 5.

Nitrogen accumulation in sediment/soils

The total N contents in sediment/soils in each ICW cell are shown in Figure 3. After 4 years of operation, the average concentration of total N in ICW sediment/soil was found to be $6 \pm 0.8$ g N kg$^{-1}$ DW. The variation of total N concentrations in the ICW sediment/soil was found to be quite pronounced. The highest concentration of $14 \pm 1.3$ g N kg$^{-1}$ DW was

<table>
<thead>
<tr>
<th>ICW section</th>
<th>Biomass (kg m$^{-2}$)</th>
<th>Total biomass per ICW cell (kg)</th>
<th>Total area covered by plants (m$^2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell 1</td>
<td>1.1</td>
<td>5,130</td>
<td>4,664</td>
</tr>
<tr>
<td>Cell 2</td>
<td>1.2</td>
<td>5,400</td>
<td>4,500</td>
</tr>
<tr>
<td>Cell 3</td>
<td>1.4</td>
<td>17,724</td>
<td>12,660</td>
</tr>
<tr>
<td>Cell 4</td>
<td>1.3</td>
<td>11,921</td>
<td>9,170</td>
</tr>
<tr>
<td>Cell 5</td>
<td>1.2</td>
<td>1,489</td>
<td>1,241</td>
</tr>
</tbody>
</table>

Figure 2 | Profiles of nitrogen concentrations within individual cells of the integrated constructed wetland. Vertical bars are standard errors of the mean, $n = 50$ months from February 2008 to March 2012.

Figure 3 | Average total nitrogen concentrations in above-ground macrophyte biomass and sediment from the different wetland cells. Vertical bars are standard errors of the mean, macrophyte samples $n = 5$, sediment samples $n = 4$.

Figure 4 | Total nitrogen uptake/accumulation rates in macrophytes and sediments in each wetland cell. Vertical bars are standard errors of the mean, macrophyte samples $n = 5$, sediment samples $n = 4$. 
recorded in cell 1 (Figure 3). Subsequent cells had significantly lower total N concentrations, which were typically less than 5 g N kg\(^{-1}\) DW. The relatively high concentrations found in cell 1 can predominantly be explained by long-term deposition of suspended solids and organic constituents within the wastewater, and accumulation of detritus from dead biota (Mustafa & Scholz 2011). In addition, cell 1 received a higher concentration of nutrients and organic matter compared to the other cells. It has been previously shown (Dzakpasu et al. 2012) that the highest removal rate also occurred in cell 1, which also results in enhanced accumulation rate of approximately 25 g N m\(^{-2}\) yr\(^{-1}\) was recorded for the entire ICW system. The highest uptake rate of 39 g N m\(^{-2}\) yr\(^{-1}\) was recorded in cell 1, whereas the lowest uptake rate of 13 g N m\(^{-2}\) yr\(^{-1}\) was recorded in cell 5. Overall, total N accumulation in ICW sediment/soils exhibited a spatial differential pattern and tended to decrease from the proximal cells to the distal ones. This differential pattern may have been influenced by factors such as hydrology and hydraulics (Reddy & DeLaune 2008; Mustafa & Scholz 2011), which caused N and other suspended organic materials within the influent wastewater to settle near the influent point of the proximal cells.

**Nitrogen mass balance**

The N budget of the ICW system (Table 4) illustrates that the total N load to the ICW system was approximately 4,205 kg N yr\(^{-1}\). Surface outflow from the ICW contained circa 221 kg N yr\(^{-1}\), accounting for about 5% of the total N losses from the system. The average uptake in biomass by the different macrophyte species was found to be 954 kg N yr\(^{-1}\) and accounted for nearly 25% of the removed N load. This uptake rate by macrophytes within the ICW was quite high in comparison with some results in earlier studies involving different wastewater types (e.g., Toet et al. 2005; Gottschall et al. 2007; Chung et al. 2008; Mustafa & Scholz 2011, Lee et al. 2014), where the macrophyte nutrient uptake rate was <1% of the removed N. In other studies, slightly higher plant nutrient uptake rates have also been reported (Greenway & Woolley 2001; Tao et al. 2012); the higher uptake rates were reported to be associated with lower influent loadings. Given the lower influent total N loadings in the ICW, as a result of its comparatively larger footprint, the relative proportion of total N removed that can be attributed to plant uptake is higher. Circa 839 kg N yr\(^{-1}\) was accumulated in the ICW sediment/soil, accounting for about 20% of the N loss from the ICW system. The sum of N assimilation by macrophyte and sediment/soil accumulation formed the second major removal pathway.

The application of the N mass balance also allowed the gaseous losses through microbial transformations and subsequent release to the atmosphere to be estimated. The sum of this process and the other processes that were not independently measured was the largest and most important N loss pathway from the ICW system. This accounted for approximately 51% of the total N retention in the ICW.

### Table 4

<table>
<thead>
<tr>
<th>N mass fluxes</th>
<th>Input/output rate (kg N yr(^{-1}))</th>
<th>Input/output rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wastewater inflow</td>
<td>4,205</td>
<td>99.6</td>
</tr>
<tr>
<td>Atmospheric deposition(^a)</td>
<td>15</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ICW effluent</td>
<td>221</td>
<td>5.2</td>
</tr>
<tr>
<td>Groundwater recharge</td>
<td>59</td>
<td>1.4</td>
</tr>
<tr>
<td>Macrophyte uptake</td>
<td>954</td>
<td>22.6</td>
</tr>
<tr>
<td>Accumulation in sediment</td>
<td>839</td>
<td>19.9</td>
</tr>
<tr>
<td>Microbial transformation and other processes</td>
<td>2,147</td>
<td>50.9</td>
</tr>
</tbody>
</table>

\(^a\)Estimated from Jordan (1997).

**CONCLUSIONS**

The average N removal rate was 102.3 g m\(^{-2}\) yr\(^{-1}\), of which approximately 23% was attributed to assimilation by macrophytes. Some 839 kg N yr\(^{-1}\) (20%) was accumulated into wetland sediment/soil, the bulk of which was achieved in the first wetland cell. Findings indicate that the assimilation by plants, and storage in sediment/soil were the primary N removal routes besides microbial transformations, which accounted for circa 51% N removal in ICWs treating domestic wastewater. Thus, the combination of plants with high biomass production offer valuable opportunities for improving ICW performance. The retrieval and use of sequestered N require coherent management and provide innovative and valuable opportunities.
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