Ammonia Removal in Free-Surface Constructed Wetlands Employing Synthetic Islands

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Abstract:

Free water surface constructed wetlands (FSCWs) can be used to complement conventional waste water treatment but removal efficiencies are often limited by a high ratio of water volume to biofilm surface area (i.e. high water depth). Floating treatment wetlands (FTWs) consist of floating matrices which can enhance the surface area available for the development of fixed microbial biofilms and provide a platform for plant growth (which can remove pollutants by uptake). In this study the potential of FTWs for ammoniacal nitrogen (AN) removal was evaluated using experimental mesocosms operated under steady-state flow conditions with ten different treatments (two water depths, two levels of FTW mat coverage, two different plant densities and a control, all replicated three times). A simple model was constructed as a framework for understanding N dynamics in each treatment. The model was calibrated using data obtained from one treatment and validated independently for the other treatments. Specifically, we hypothesized that the nitrification and volatilization rate constants are inversely proportional to water depth and proportional to mat surface area. This allowed the relative magnitude of different removal mechanisms to be estimated. The model was able to predict steady-state concentrations of AN and total oxidized nitrogen (TON) across the different treatments well (values for correlation in the regression between measured and predicted steady-state concentrations and RMSE were 0.88 and 0.40 mg N L−1 for AN, and 0.63 and 1.75 mg N L−1 for TON). The results confirm that nitrification is the principal AN removal process, with maximum removal occurring in shallow systems with high matrix cover (i.e. a high ratio of biofilm surface area to water volume). Plant uptake was a relatively minor loss process compared to nitrification. Integrated experimental and model-based approach was found to be a useful tool to improve mechanistic understanding AN dynamics in FSCWs and system performance.

Key words: Ammonia, Continuously stirred tank reactor, FTW, csRemoval kineti, System dynamics modelling

Introduction:

Constructed wetlands are being increasingly used for water quality improvement during wastewater treatment (1-3). This is, in part, a result of increasingly strict water quality standards which mean that conventional secondary treatment may not always be sufficient to comply with targets in receiving waters. However, it also reflects an increasing desire to replicate natural systems, minimize energy and resource consumption and enhance treatment sustainability. Free surface constructed wetlands (FSCWs) are the simplest constructed wetland design and basically consist of one or more shallow basins, which usually contain plants, through which wastewater is directed.
However, limited surface area for growth of fixed microbial biofilms and the lack of direct contact between plant roots in the sediment and the water column often limit FSCW performance (4, 5). In order to overcome these issues, floating treatment wetlands (FTWs) have been proposed (6, 7). These consist of floating islands constructed from synthetic or natural materials which possess a high submerged surface area that can facilitate the development of microbial biofilms (8). They also provide a platform for the growth of plants which can enhance nutrient removal via uptake and introduce carbon-rich exudates and oxygen via their roots which can facilitate microbiologically-mediated transformations of pollutants. Their performance has been evaluated in a number of experimental (9-11) and operational (12-14) settings and they have been shown to effectively enhance the removal of total suspended solids (TSS), zinc and copper by 41, 40 and 39% (15); total phosphorus and orthophosphate by 47 and 79% (16) and total nitrogen by 72% (17). However, most of these studies have adopted “black-box” (input – output) approaches, in which inherent system complexity and the relative contributions of different potential removal mechanisms have been neglected (18). FSCWs containing FTWs are complex multimedia environments in which a number of physical, chemical and biological processes interact (19). It is, therefore, critical to understand and evaluate these interactions, in order to understand and optimize system performance. This can be done most effectively via the application of numerical models which can provide a useful framework for integrating the combined effects of several different interacting processes. This study aims to investigate the dynamics of inorganic nitrogen in experimental FSCW mesocosms operated under different design criteria, with special emphasis on the removal of ammoniacal nitrogen (AN). Ammoniacal nitrogen (AN) is used here to denote the total nitrogen present as either ammonium (NH₄⁺) or free (unionized) ammonia (NH₃). The ratio of the two forms is pH- and temperature-dependent (e.g. (20). Only NH₃ is considered to have toxic effects on organisms living in receiving waters (21). For reference, a predicted no-effect concentration (PNEC) for NH₃ of 10 mg L⁻¹ was reported by (22) based on a species sensitivity distribution and a value of 25 mg L⁻¹ was reported by Alabaster and Lloyd (23) for the protection of freshwater salmonids. Specifically, we looked at the role of the following factors under steady-state flow conditions:

- Water depth (z). We assume that reactions occur principally at the interfaces between water and solids (24, 25). Reaction rate constants should, therefore, be inversely proportional to the water volume to solid surface area ratio (which can often be approximated by the water depth);
- Fraction of water surface covered with FTW matrix (fₘ). The synthetic matrix from which FTWs are typically constructed is assumed to function as a habitat for nitrifying bacteria in mixed biofilms which convert NH₄⁺ to NO₂⁻ and NO₃⁻ (26, 27). We hypothesise that the overall reaction rate constant will increase with increasing fₘ;
- Plant density. Uptake of mineral nitrogen (as both NH₄⁺ and NO₃⁻) should be proportional to the number of plants per unit area of wetland.

A simple model was constructed in order to disentangle the relative contribution of different processes to overall treatment performance. Such approaches have been employed previously in agriculture (28-30) and stormwater management (31-33).

Material and Methods:
Experimental
Thirty experimental mesocosms were established at a campus location (52.3814° N, 1.0754° W) in Leicestershire, UK. Each mesocosm consisted of an 80 L polyethylene tank (length 58 cm × width 38 cm × height 48.5 cm) which was placed uncovered outdoors (i.e. open to rainfall and evapotranspiration). The experimental design is summarized in Table 1. Briefly, half the mesocosms had shallow water depths (0.2 m, volume 36 L) and half were deep (0.4 m, volume 72 L). For each depth (shallow or deep) there were two control treatments which contained no floating island (C₁ and C₂). All remaining treatments contained floating mats at either full coverage of the water surface (100%) or 50% coverage. The mats were constructed from an open matrix of extruded plastic injected with polystyrene foam to support buoyancy and were obtained from Frog Environmental, UK. The mats were either vegetated with soft rush (Juncus effuses) or unvegetated. Vegetated mats were drilled with 7 cm diameter holes to accommodate pots containing plant seedlings in a bed of sawdust to support plant establishment. Plant roots were washed carefully to remove all attached soil before insertion. All the macrophytes had the same growth history and maturity and originated...
from the same batch at a local nursery. Vegetated mats contained two plants per mat for 50% coverage or four plants per mat for 100% coverage. Each treatment was replicated three times and organized using a randomized block design (Fig. 1).

Table 1. Summary of experimental treatment characteristics and treatment codes. In all cases three replicates were used.

| Treatment Description       | Treatment Code | Water depth (m) | Mat cover (%) | Plants per mat |
|-----------------------------|----------------|----------------|---------------|----------------|
| Shallow Control             | C1             | 0.2            | 0             | 0              |
| Deep Control                | C2             | 0.4            | 0             | 0              |
| Shallow Mat 50%             | M1             | 0.2            | 50            | 0              |
| Deep Mat 50%                | M2             | 0.4            | 50            | 0              |
| Shallow Mat 100%            | M3             | 0.2            | 100           | 0              |
| Deep Mat 100%               | M4             | 0.4            | 100           | 0              |
| Shallow Veg Mat 50%         | V1             | 0.2            | 50            | 2              |
| Deep Veg Mat 50%            | V2             | 0.4            | 50            | 2              |
| Shallow Veg Mat 100%        | V3             | 0.2            | 100           | 4              |
| Deep Veg Mat 100%           | V4             | 0.4            | 100           | 4              |

Figure 1. Illustration of the three types of treatment. Top: Control (C), Middle: Mat only (M) and Bottom: Mat + vegetation (V) for Left shallow and Right deep mesocosms. Not show are variations in mat cover and number of plants.

All treatments were subjected to a steady state continuous flow regime in which an influent containing a relatively high concentration of AN was pumped into each mesocosm at a rate of 5.1 ± 0.2 L d⁻¹ for the shallow treatments and 10.3 ± 0.5 L d⁻¹ for the deep treatments. Each mesocosm was allowed to overflow via an outlet tube which could be sampled periodically. This established a nominal hydraulic residence time of approximately 7 days in each mesocosm. Since the concentration of the influent water was always the same (approximately 10 mg N L⁻¹) for each treatment the mass loading rate (MLR) of AN was 48.2 ± 0.5 mg N d⁻¹ for the shallow treatments and 96.4 ± 1.1 mg N d⁻¹ for the deep treatments.

Prior to the experimental phase, each mesocosm was operated under steady state conditions for two months using water supplied continuously from an on-site stormwater retention pond, in order for biofilms to be established and for plants to take root in the FTW matrices. During the experimental phase, influent water was obtained...
from the domestic supply and de-chlorinated using an activated carbon filter prior to pumping into a central holding tank (~210 L) where it was spiked with an ammonium stock solution (6.2 g NH₄Cl L⁻¹) daily to create a constant initial concentration of 9.4 mg AN L⁻¹. This was routed to each mesocosm via three intermediate reservoirs using a system of eight dosing pumps, calibrated to achieve the required flow rates. The average air temperature at the research site during the experimental phase was 13.3 °C, the average rainfall rate was 89 mm month⁻¹ (local rain gauge), and the average evapotranspiration (ET) rate was 79 mm month⁻¹ (estimated using the Penman-Monteith model: (34)). Since the rainfall rate exceeded the estimated ET rate by approximately 0.33 mm d⁻¹, net water losses due to ET were assumed to be negligible and the inflow was assumed to be equal to the outflow for the majority of the time.

During the experimental phase, aqueous samples (50 mL) were periodically collected (every 3 days) from the outflow of each tank over a six week period. Samples were transported to the laboratory in a dark cool box and stored in a cold room at 4 °C. The following day they were filtered into clean plastic containers using syringe-mounted 0.45 μm disk filters. Samples for AN and NO₃-N analyses were preserved by acidifying to pH < 2 using concentrated sulfuric acid (H₂SO₄) and cooling to 4 °C (35). Samples for NO₂-N analysis were frozen at -20 °C until analysis (36). All analyses were performed within two weeks of sampling. Concentrations of AN and NO₂-N were analyzed according to established protocols using an automated discrete colorimetric instrument (AQ2: SEAL Analytical, UK). Concentrations of NO₃-N were determined on the AQ2 using an ultra-violet spectrophotometric screen method (37).

Plant uptake was calculated by measuring the total N concentration and dry mass in plant tissue at the start and end of the experimental phase. Root and shoot tissues of random samples at the start and end of the study were dried and weighed for dry biomass. Dried tissues were ground in a ball mill, weighed (3 mg per sample) and analysed for TN content using an elemental analyzer: SERCON ANCA GSL according to established protocol. Reference samples (wheat flour standard-OAS-SERCON Ltd.) were analysed in parallel to ensure quality control.

Model

A conceptual model was constructed to represent mineral N dynamics in the experimental systems. Each mesocosm was assumed to behave as a continuously stirred tank reactor (CSTR) with loss processes occurring via first-order kinetics (31). The AN MLR (mg N d⁻¹) in the influent was assumed to be the product of the inflow rate (Qin; L day⁻¹) and the influent AN concentration (mg N L⁻¹). The mass balance for AN can be written as:

\[
\frac{dC_{AN}}{dt} = J_{IN} - k_{pool} f_{FREE} C_{AN} V - k_{Nit} C_{AN} V - k_{up} C_{AN} V - Q_{out} C_{AN} (1)
\]

where \( C_{AN} \) is the AN concentration (mg N L⁻¹), \( k_{pool} \) is a first order rate constant for the mass transfer of free ammonia across the air-liquid interface by volatilization (day⁻¹), \( f_{FREE} \) is the fraction of AN which is present as free NH₃ (dependent on pH and temperature), \( V \) is the operational volume of liquid in the mesocosm (L), \( k_{Nit} \) is a rate constant for nitrification (day⁻¹), \( k_{up} \) is a rate constant for AN uptake by plants (day⁻¹), and \( Q_{out} \) is the discharge in the outflow (L day⁻¹). Since ET was assumed to have a negligible effect on the net water balance, \( Q_{out} \) was assumed to be equal to the inflow rate (Qin). Nitrification was represented as a single step process (i.e. no distinction was made between the oxidation of ammonium and the oxidation of nitrite, which implicitly assumes that the rate constant for nitrite oxidation is higher than that for ammonium oxidation, such that nitrite does not accumulate). Note that mineralisation of organic nitrogen to AN and immobilization of AN and TON in the microbial biomass were assumed to be zero here. This is because organic N was not introduced in the influent, although clearly some ON could have built up in the system from root litter and exudates, from autotrophic microbial biomass and from photosynthate derived from algae. Since there was no explicit consideration of organic N, there was also no representation of the sedimentation process.

The NH₃:NH₄⁺ ratio at equilibrium was calculated from

\[
f_{FREE} = \frac{1}{1 + 10^{(pK_a - pH)}} \quad (2)
\]

where \( pK_a \) was estimated to be 9.56 at a mean system temperature of 15 °C. For a mean pH over the course of the experiment of 6.4, \( f_{FREE} \) was calculated to be 0.00068.

The rate constant for volatilization (\( k_{vol} \)) was estimated as the combined mass transfer coefficient for volatilization derived using two-film resistance theory (27) divided by the water depth (20, 38):

\[
k_{vol} = \frac{v_{aw}}{z} \quad (3)
\]

where \( v_{aw} \) is the combined mass transfer across the air-water interface (m d⁻¹) and \( z \) is the water depth (m) which is simply a surrogate for the
ratio of water volume to the surface area of the air-water interface. \( v_{aw} \) is calculated as

\[
\frac{1}{v_w} + \frac{1}{v_a K_{AW}} = \left( \frac{1}{v_w} + \frac{1}{v_a k_{AW}} \right)^{-1}
\]

(4)

where \( v_w \) and \( v_a \) are the partial mass transfer coefficients for water and air (assumed to be 0.01 and 1 m h\(^{-1}\), respectively; Mackay, 2001) and \( K_{AW} \) is the dimensionless air: water partition coefficient for ammonia (assumed to be 0.00071 which is equivalent to a Henry’s law constant of 1.76 Pa m\(^3\) mol\(^{-1}\); (39)). These assumptions result in a value of \( v_{aw} \) of 0.016 m d\(^{-1}\) and \( k_{vol} \) values equivalent to 0.08 and 0.04 d\(^{-1}\) for the shallow and deep mesocosms, respectively. It is assumed that floating mats do not interfere with volatilization. Although this is probably not realistic, the overall rate constant for AN is very low in any case because \( f_{FREE} \) is low (even for the highest pH of 8.2 observed in one of the control mesocosms \( f_{FREE} \)) is only 0.4 which gives an overall AN rate constant for the shallow systems of 0.003 d\(^{-1}\) equivalent to a dissipation half-life of 213 days.

The mass balance for nitrate can be written as

\[
\frac{dC_{TON}}{dt} = V_{\text{out}} k_{\text{up}} C_{\text{TON}} - k_{\text{up}} C_{\text{TON}} V - Q_{\text{out}} C_{\text{TON}}
\]

(5)

where \( C_{\text{TON}} \) is the concentration of total oxides of nitrogen (mg N L\(^{-1}\)) and \( k_{\text{up}} \) is a first order rate constant for nitrate and nitrite uptake by plants (day\(^{-1}\)). Plant uptake is assumed to occur for both AN and NO\(_3\). Opinions vary as to which is preferentially utilized and this will undoubtedly depend on plant species. In soil, reference DeKock (40) observed short term uptake of ammonium in excess of that of nitrate in tobacco plants, but equal amounts at the end of the growing season. Reference Barraclough, Geens (41) suggest that ammonium is preferentially utilized but that the rate of nitrate uptake exceeds that of ammonium when soil ammonium concentrations are high – possibly due to the fact that high surplus ammonium concentrations can be toxic to plant cells whereas excess nitrate can simply be stored in cell tissue. Note that denitrification and nitrate ammonification were also assumed to be zero in the application described here. Since these processes occur predominantly under anaerobic conditions, they are assumed to be of negligible importance in the experimental system which is open to the atmosphere and flowing continuously.

The measured average net N uptake in the \( V_4 \) treatment (full mat cover with four plants) was calculated to be 25 mg N day\(^{-1}\) (112 mg N m\(^2\) water surface day\(^{-1}\)). This is within the range of plant uptake rates of 0.0015 - 2.8 g N m\(^2\) day\(^{-1}\) reported by (42) and with experimental data obtained by Lynch, Fox (43) and by McAndrew and Ahn (33), suggesting N uptake rates between 0.011 and 0.1 g N m\(^2\) day\(^{-1}\) in mesocosm-based FTWs. In our system, the shoot system accounted for most of the plant biomass with a shoot: root ratio of 0.69±0.05. Since there was no analytical indication of the preference of the plants for either NH\(_3\) or NO\(_3\), the assimilation rate constants (\( k_{up} \)) for NH\(_3\) or NO\(_3\) were assumed to be equal. This assumption allows \( k_{up} \) to be derived independently by solving the following simultaneous equations for average uptake:

\[
\begin{align*}
U_T - U_{TON} &= k_{up} V C_{AN} \\
U_{TON} &= k_{up} V C_{TON}
\end{align*}
\]

(6)

(7)

where \( U_T (=U_{TON} + U_{AN}) \) is the average total N uptake rate over the course of the experiment (25 mg N day\(^{-1}\)), \( U_{TON} \) is the average daily uptake of TON, \( C_{AN} \) is the average observed concentration of AN over the course of the experiment (mg N L\(^{-1}\)) and \( C_{TON} \) is the average observed concentration of TON over the course of the experiment (mg N L\(^{-1}\)). Here, there are two unknowns \( U_{TON} \) and \( k_{up} \) and two equations. By substitution we can eliminate \( C_{TON} \) and derive a value for \( k_{up} = 0.0603 \) d\(^{-1}\).

At steady state, Equation (1) can be rearranged to give

\[
C_{AN} = \frac{J_{IN}}{(k_{vol} f_{FREE} V + k_{nit} V + k_{up} V + Q_{out})}
\]

(8)

Similarly, at steady state Equation (5) yields

\[
C_{TON} = \frac{k_{nit} V C_{AN}}{(k_{up} + Q_{out})}
\]

(9)

Except for \( k_{nit} \), all terms in Equation (8) can be estimated independently for the “calibration” treatment \( V_4 \) (see above). We can, therefore, use the measured steady state concentrations of \( C_{AN} \) and \( C_{TON} \) in the \( V_4 \) treatment to give an estimate for \( k_{nit} \) as follows:

\[
k_{nit} = \frac{C_{TON} (k_{up} V + Q_{out})}{V C_{AN}}
\]

(10)

In the other treatments values of \( k_{nit} \) were then adjusted via simple scaling, based on the hypothesised effects of water depth and mat surface area. The adjustments were as follows: (1) \( k_{nit} \) was assumed to be inversely proportional to water depth (27) where depth is a surrogate for the ratio of water volume to biofilm surface area. Specifically, for shallow systems, \( k_{nit} \) was assumed to be twice \( k_{nit} \) for deep systems based on the assumption that nitrification only occurs on the bed of the mesocosm and in the FTW matrix i.e. at the surface); (2) \( k_{nit} \) was assumed to be proportional to mat area (i.e. \( k_{nit} \) for full mat cover was assumed to be twice \( k_{nit} \) for 50% cover, based on a similar rationale that most nitrifiers inhabit the mat material). Uptake was also scaled in proportion to
the number of plants present, i.e. \( k_{up} \) was assumed to be proportional to the number of plants in the system such that uptake rates for systems containing four plants were twice those in systems containing two. Following these simple adjustments, the model predictions were compared with the observed concentrations in the independent treatments. This can be regarded as a validation of the model since no further parameter optimisation was performed for these treatments.

**Results:**

**Experimental Data**

Changes in mean AN and TON concentrations over time in each treatment are shown in Fig. 2, grouped by control data (top panels), data for mesocosms with mats but without vegetation and data for mesocosms with mats and vegetation. For most treatments, the concentrations of both AN and TON remain relatively constant over time, confirming that the systems were approximately in steady state. Two notable exceptions are the control treatments (without mats). At the start of the monitoring period, AN concentrations were initially high (ANOVA, \( F_{9,120} = 41.018; \ P < 0.05 \)) and TON concentrations were initially low (ANOVA, \( F_{10,132} = 42.790, \ P < 0.05 \)) in both the shallow and deep mesocosms. However, after Day 10 there was a systematic decrease in AN concentrations and a simultaneous increase in TON. This is consistent with conversion of AN to TON via nitrification and suggests that a competent nitrifier community developed in these vessels between Days 10 and 20. After approximately Day 20 for the shallow systems (C1) and Day 30 for the deep systems (C2) AN and TON concentrations appear to reach a steady state. AN appears to be removed more effectively in the shallow (\( r^2 = 0.60; \ p = 0.05 \)) vessels which is consistent with our hypothesis that microbial processing is primarily occurring in fixed biofilms on solid surfaces. Data on changes in dissolved oxygen (DO) concentrations and pH over time are shown in Fig. 3. These data support the explanation that changes in AN and TON in the control plots are driven by the development of a competent nitrifying population. The decline in DO concentrations to approximately Day 20 for the shallow system (C1) and Day 28 for the deep system (Fig. 3a) (\( r^2 = 0.28; \ p = 0.05 \)) is consistent with the utilization of oxygen in the microbially-mediated oxidation of AN to TON. According to Guisasola, Jubany (44), 4.57 mg O\(_2\) is required to oxidise each mg of NH\(_4^+\)-N to NO\(_3^-\), so nitrification represents a very powerful DO sink. The higher steady state DO concentration in C1 is expected as DO is only replenished at the surface and hence the rate of reaeration will be proportional to depth as well as to the oxygen deficit. The depression in pH seen in the control systems (Fig. 3d) is also likely to be related to the release of protons as ammonium is oxidized (2 moles of H\(^+\) are generated for every mole of NH\(_4^+\) converted to NO\(_3^-\); e.g. Alzate Marin, Caravelli (45) and Thakur and Medhi (46).

Concentrations of AN for the other treatments range between approximately 0.5 and 3 mg N L\(^{-1}\). For both the vegetated and unvegetated treatments, concentrations were systematically lowest in the shallow vessels (dashed lines in Fig. 2). TON concentrations in the treatments with unvegetated mats (Fig. 2e) were all similar (approximately ranging between 7 and 9 mg N L\(^{-1}\)). These were higher than the steady state TON concentrations in the treatments containing vegetation (Fig. 2f) which ranged between approximately 1 and 6 mg N L\(^{-1}\). This suggests that a TON sink process was operating in the vegetated systems – probably plant uptake, although denitrification is also a possible nitrate loss process. Anomalously low TON concentrations were observed in all the mesocosms in treatment V3 (shallow water depth with full mat cover and four plants). This may have been due to a high rate of plant uptake or denitrification.
Figure 2. Changes in mean observed AN concentrations (left panels) and TON concentrations (right panels) over time in each treatment. (a) and (d) data for control mesocosms; (b) and (e) data for mesocosms with mats without vegetation; (c) and (f) data for mesocosms with mats and vegetation. Error bars show standard deviations.

Mean DO concentrations and pH in the M and V treatments (Fig. 3) changed relatively little over most of the experiment, which is consistent with the idea of a system in steady state. DO concentrations were slightly higher in the shallower treatments compared with the deeper ones which, again, is consistent with physical expectations. DO concentrations were lowest in the deep unvegetated mesocosms (M2 and M3) with mean concentrations only about 1.1 and 1.5 mg L\(^{-1}\), respectively, over the period between Day 20 and Day 40. In the vegetated treatments (Fig. 3c) DO concentrations were generally between 2 and 3 mg L\(^{-1}\) with concentrations in the deeper treatments consistently lower than in the shallow treatments. Higher DO concentrations in the vegetated treatments may have been maintained by the radial release of photosynthetic oxygen via the rhizosphere (47, 48). Nitrification is a strong sink for DO but can itself be limited by low DO concentrations (49). Reference Guisasola, Jubany (44) showed that the second step in nitrification (NO\(_2\) to NO\(_3\)) is more sensitive to depressed DO concentrations and reported a half-saturation constant for this process of 1.75 mg O\(_2\) L\(^{-1}\). This means that nitrification may be partially inhibited below about 2 mg O\(_2\) L\(^{-1}\), although this was not obviously the case in the TON data reported here (Fig. 2e). Mean pH ranged between approximately 6.5 and 6.6 in the unvegetated treatments (Fig. 3e) and between approximately 6.3 and 6.4 in the vegetated systems (Fig. 3f), between Days 20 and 40. The slightly lower pH in the vegetated system may have been due to the release of organic acids by the plants or due to a slight elevation in CO\(_2\) concentration caused by either CO\(_2\) diffusion through the roots or by the degradation of organic matter introduced into the water column.
Steady state model performance

Values of \( k_{\text{vol}} \) and \( f_{\text{FREE}} \) were estimated independently from the two film resistance model (Equations 3 and 4) and the influence of pH and temperature on the theoretical partitioning of AN into \( \text{NH}_4^+ \) and \( \text{NH}_3 \) (Equation 2). A value of \( k_{\text{up}} \) (assumed to be the same for both AN and TON) was derived from the uptake data in treatment V4. The main unknown is, therefore, \( k_{\text{nit}} \), which was derived from Equation 10 using the average measured AN and TON data from treatment V4 during steady state (i.e. between Days 20 and 40). The steady state solutions for AN and TON concentrations (Equations 8 and 9) were then used to estimate the AN and TON concentrations for the other treatments with \( k_{\text{nit}}, k_{\text{vol}} \) and \( k_{\text{up}} \) adjusted from their calibrated (V4) values a priori by factors reflecting the predictive hypotheses outlined in the introduction. In the case of the mat-free controls, there is clear evidence that a nitrifier community developed in the system over the first half of the monitoring period with both the deep and shallow systems appearing to reach an approximate steady state between Days 30 and 40. This means that \( k_{\text{nit}} \) could not be simply scaled from the V4 data. Instead a value of 0.3 d\(^{-1}\) was obtained by trial and error optimization for AN in C2 and the value of \( k_{\text{nit}} \) for C1 was assumed to be 1.64 x 0.3 = 0.49 d\(^{-1}\), where 1.64 is the ratio of the submerged container surface area in the deep mesocosm to that in the shallow systems. The values for the rate constants adopted in each treatment and the measured and predicted steady state concentrations are shown in Table 2. The correlation coefficients between measured and modelled steady state concentrations were 0.88 and 0.63 for AN and TON respectively. The RMSE for AN was 0.4 mg N L\(^{-1}\) and the RMSE for TON was 1.75 mg N L\(^{-1}\).
Table 2. Main N process parameters used in the steady state model. Also shown are the mean measured (Days 20 - 40) and modelled AN and TON concentrations (mg N L\(^{-1}\)).

| Treatment | \(k\text{vol}\times f\text{FREE} \text{(d}\text{\(^{-1}\)}) | \(k\text{up} \text{(d}\text{\(^{-1}\)}) | \(k\text{nit} \text{(d}\text{\(^{-1}\)}) | Measured \(C\text{AN} | Measured \(C\text{TON} | Modelled \(C\text{AN} | Modelled \(C\text{TON} |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| C1             | 5.4 x 10\(^{-5}\) | 0.49          | 1.26          | 7.42          | 7.60          | 1.80          | 6.36          |
| C2             | 2.7 x 10\(^{-5}\) | 0.3           | 3.04          | 7.79          | 6.56          | 3.03          | 8.19          |
| M1             | 5.4 x 10\(^{-5}\) | 1.55          | 0.46          | 8.19          | 8.61          | 0.79          | 7.94          |
| M2             | 2.7 x 10\(^{-5}\) | 0.78          | 2.21          | 8.03          | 7.94          | 1.46          | 7.94          |
| M3             | 5.4 x 10\(^{-5}\) | 3.11          | 0.44          | 7.97          | 9.99          | 0.41          | 6.35          |
| M4             | 2.7 x 10\(^{-5}\) | 1.55          | 1.27          | 7.51          | 6.35          | 0.79          | 6.19          |
| V1             | 5.4 x 10\(^{-5}\) | 0.0302        | 0.67          | 5.66          | 6.98          | 0.77          | 6.35          |
| V2             | 2.7 x 10\(^{-5}\) | 0.0302        | 1.36          | 5.61          | 6.35          | 1.42          | 6.35          |
| V3             | 5.4 x 10\(^{-5}\) | 0.0603        | 0.38          | 5.61          | 6.35          | 0.40          | 6.19          |
| V4             | 2.7 x 10\(^{-5}\) | 0.0603        | 0.77          | 5.36          | 5.36          | 0.70          | 6.19          |

The steady state measured and modelled AN and TON concentrations are compared in Fig. 4. Also shown in Fig. 4 are the 1:1 lines and the best fit regression lines constrained to go through the origin and the associated equations and \(r^2\) values. For AN (Fig. 4a) the data group well along the 1:1 line (with an \(r^2\) value of 0.75) and the slope of the best fit line is close to unity. There are two noticeable outliers for treatments C1 and M2 (highlighted). For TON (Fig. 4b) the data also sit close to the 1:1 line for most treatments but the linear fit is less strong with an insignificant \(r^2\) value (in part, caused by the constraint to fit the regression line through the origin). However, the slope of the regression is still close to unity, despite the obvious outlier V3, which is highlighted.

![Figure 4. Measured versus modelled steady state concentrations for (a) AN and (b) TON. The dashed lines show the 1:1 relationship. Solid grey lines show the best fit linear regression constrained to fit through the origin.](image)

There are four principal AN loss processes: volatilization, nitrification, plant uptake and advection. The relative contribution of each processes to AN loss (\(f\text{PROCESS}\)) was quantified by

\[
f\text{PROCESS} = \frac{G\text{PROCESS}}{(k\text{vol}\times f\text{FREE} + k\text{nit}V + k\text{up}V + Q\text{out})}
\]

where \(G\text{PROCESS}\) (L d\(^{-1}\)) is the respective volumetric loss term for each process i.e. \((k\text{vol}\times f\text{FREE})V\) for volatilization; \((k\text{nit}V)\) for nitrification; \((k\text{up}V)\) for uptake and \(Q\text{out}\) for advection.

Nitrification is made by far the largest contribution to total AN loss in the experimental system (68 – 96%), followed by advection (4 – 32%) (Table 3). There appeared to be a substantial conversion of AN to TON even in the absence of floating mats, which resulted in an estimated 68% contribution in the deep control (C2) and 78% contribution in the shallow control (C1). This was confirmed by systematic decreases in pH and DO concentrations which were consistent with the development of a nitrification capacity in these vessels. This is likely to have been in fixed biofilms rather than in freely suspended microbial colonies. All other things being equal, the overall contribution of nitrification to AN loss was higher in the shallow treatments than the equivalent deep ones (Table 3). Similarly, in the presence of mat material, the contribution of nitrification was higher when more mat material was present (cet. par.).
This confirms the postulate that nitrification is occurring in microbial biofilms attached to the mat material. The good agreement for outlet TON concentrations confirms the plausibility of this explanation (which is also supported by studies by Marimon, Xuan (31) and Pavlineri, Skoulidakis (50), who reported increased TN reduction in FTWs when surface coverage increased). All of these observations support our central hypothesis, that treatment performance will be proportional to the solid surface area submerged which is available for biofilm formation. Volatilisation made a negligible contribution in all cases. Ammonia volatilization is generally insignificant below a pH of 7.3, but can account for nearly 10% of the total AN loss in aquatic systems with higher pH (51, 52). Uptake was also a fairly insignificant loss process when plants were present (1.8 – 3.4 %). That said, cet. par. total removal did increase with plant density and we should point out that the planting density used here was quite low. Reference García-Lledó, Ruiz-Rueda (53) reported that removal rate constants for AN and TON were higher in the presence of dense vegetation than when vegetation was sparse. In operational FTWs, the number of plants utilized per unit area will vary. We might expect uptake to be approximately proportional to the number of plants grown, although this will only be an effective sink during the growing season.

### Table 3. Relative contribution of the different loss processes to the total steady state AN loss rate. All values expressed as percentages.

| Treatment | Volatilisation | Uptake | Nitrification | Advection |
|-----------|----------------|--------|---------------|-----------|
| C1        | <0.01          | 0      | 77.6          | 22.4      |
| C2        | <0.01          | 0      | 67.7          | 32.3      |
| M1        | <0.01          | 0      | 91.6          | 8.4       |
| M2        | <0.01          | 0      | 84.5          | 15.5      |
| M3        | <0.01          | 0      | 95.6          | 4.4       |
| M4        | <0.01          | 0      | 91.5          | 8.4       |
| V1        | <0.01          | 1.8    | 90.0          | 8.2       |
| V2        | <0.01          | 3.2    | 81.8          | 15.0      |
| V3        | <0.01          | 1.8    | 93.9          | 4.3       |
| V4        | <0.01          | 3.4    | 88.4          | 8.2       |

Although the experimental system appeared to be in approximate physical and biogeochemical steady state (especially in the last ten days of the experiment), there were fluctuation in the monitored variables (e.g. an apparent increase in AN concentrations for several treatments on Day 14) which could reflect variations in treatment performance, sample treatment or analytical errors. Variations in treatment performance may have been due to variations in pumping rate, variations in temperature, evaporation rates, rainfall and the physical stability of the attached biofilms.

### Discussion:

In this study, nitrification rates were the highest in treatments with shallow water depths supporting our initial hypothesis. An inverse relationship between the nitrification rate coefficient and water depth was also proposed by Kadlec and Wallace (54). Observed overall ammonia removal efficiencies were 57, 93, 88, 93 and 94 % for shallow treatments: C1, M1, V1, M3 and V3, and 39, 77, 83, 85 and 91 % for deep treatments C2, M2, V2, M4 and V4. Where C series represents controls (without mats), M series are treatments with mats only, and V series are treatments with mats plus vegetation. Nitrification is a surface-limited process, e.g., the organisms which mediate it live predominantly in fixed biofilms on solid surfaces (55, 56). Shallow systems are characterized by a higher surface area of bed (and, where present, of mat material) per unit volume of water. These surfaces are available for microbial biofilm growth and hence can contribute to increase reaction rates. In addition, redox potential may be higher in shallower systems because the ratio of the air-water interface area to water volume is higher, which facilitate reaeration by diffusion exchange with the air (57, 58). Here, the dissolved oxygen concentrations were higher in the shallower systems than in the deeper ones (by typically 1 mg O₂ L⁻¹) over the course of the study. Differences in DO between two systems may be also be explained due to the competition between O₂ consumption by microbial activity and O₂ supplement by atmospheric diffusion. However, this is unlikely to have affected ammonia removal by nitrification because DO concentrations were always > the threshold for nitrification inhibition (0.2-0.5 mg O₂ L⁻¹) (59, 60). Ammonia volatilization is also likely to be more important in shallow water systems because of the higher ratio of air:water interface to volume. Volatilization is also a diffusion process across the air:water interface.
Nitrification rates were higher in treatments where mat area was 100% surface area coverage compared to 50% and no mat treatments. This reinforces the idea that biofilm development (onto the floating mat material and roots) enhances nitrification (8, 61). Observed NH₄ removal efficiencies increased from 93 and 77% in M₁ and M₂ to 93 and 85% in M₃ and M₄. Reference Pavlineri, Skoulikidis (50), also reported increased N oxidation in FTWs when surface coverage increased. The idea that the enhanced reduction of ammonia concentrations observed when mat area increased was due to increased microbial activity was also supported by the microbial biomass analysis. Bacterial biomass and growth rates on floating mat underwater surfaces were significantly higher than in control treatments. Growth rates of bacterial population was 0.18±0.01 Log10 CFU g⁻¹ day⁻¹ compared to the unplanted treatments and controls (0.13±0.01 and 0.015±0.002 Log10 CFU g⁻¹ day⁻¹, respectively). High surface area available for microbial growth in the FTWs (e.g., submerged mat material and hydroponic roots) could explain high production of microbial biomass, however microbial biomass in the controls reflected microbial density in the water column only as there is no mat or plant were introduced. Physicochemical changes observed in the mesocosms such as a decline in pH and dissolved oxygen can also be attributed to nitrification in the FTW treatments. Overall losses of NH₃ via volatilization are believed to have had relatively little effect on ammoniacal-N losses, due to the relatively low pH in the experimental system and, hence, the low fraction of free ammonia.

Ammonia removal was the highest in treatments with vegetation than without and increased with increasing plant density. The hypothesis posed was that a linear relationship of ammonia removal would be observed with plant density due to direct uptake of NH₄⁺. When plant density increased from 2 individual plants to 4, removal efficiencies increased from 88 and 83% in V₁ and V₂ cells to 94 and 91% in V₃ and V₄ cells, respectively.

As well as increased uptake, plants can influence the removal of ammonia via nitrification by providing additional surfaces for biofilm development (62, 63). Some wetland plants can also enhance dissolved oxygen levels by transferring air through their root systems (64). Finally, plants can enhance microbial activity via root exudates. Plants roots can release a variety of dissolved organic compounds (DOC) to the rhizosphere, which can be used by microbial populations as a carbon source for their activity (65).

Overall results support the idea that shallow depth, full mat coverage and a higher plant density promote optimal operational ammonia removal. Application of the numerical model to the mesocosms suggested that nitrification in fixed biofilms is the principal ammonia removal process (responsible for 59-95%). Losses of NH₄ via volatilization is estimated to be negligible of removed NH₄. Where plants were present their contribution was estimated to be in the range (16 - 40 %) of overall removal.

The model was useful and was able to make good quantitative predictions of effluent concentrations in all treatments, after calibration on one treatment and making adjustments for water depth, mat coverage area, and plant density based on a priori hypotheses (e.g., simple linear adjustment for rate constants as depth or mat area changed). The deviation between the measured and predicted concentrations of for ammonia and total oxidized N was low in general, although there were occasional samples and treatments replicates could have been better. For instance, a poor model performance for NO₃ dynamic was observed in V₁ and to a lesser extent in the V₁ treatment. This could be explained as a result of some competitive processes such as denitrification, and nitrate immobilization, which they are assumed to be of negligible importance as the system was open to the atmosphere and flowing continuously and, therefore should be aerobic as well as because organic N was not introduced in the influent. Sensitivity analysis confirmed that the most important loss process was nitrification associated with fixed microbial biofilms on mat surfaces. Good model performance suggests that this type of modeling approach is useful as a framework to improve understanding of N dynamics in experimental wetland systems.

Overall, experimental-based data associated with modelling approach findings indicated that a treatment system operated under shallow water depth with high surface area for microbial biofilm and high plant density is a critical design for ammonia removal from wastewater. Therefore, such design could be applicable at full scale to improve ammonia removal from domestic sewage in wastewater treatment system.

Conclusions:

In this paper, we explored the factors controlling AN removal from experimental FSCW mesocosms under steady state conditions. In
particular, we investigated the potential of FTWs to enhance treatment performance for AN. Findings presented in this research suggest that FTWs have the potential to enhance AN treatment performance in FSCWs, principally via the promotion of nitrification fixed biofilms. Treatment should also be improved by shallow depth and higher plant abundance, although this will depend on planting density and growth stage. Model-based analysis indicates reasonable agreement between the modelled steady state AN and TON concentrations and the measured data suggests that the model provides a good description of the experimental system and that our hypotheses, expressed quantitatively via the a priori adjustment of the rate constants are valid. This is particularly remarkable considering the deliberately simplistic nature of process representation and the fact that some processes (like organic N mineralization, denitrification and anaerobic ammonia oxidation) are not represented. The most important loss process in all treatments is nitrification, followed by advection associated with fixed microbial biofilms on mat surfaces.

The work demonstrates the value of a systems modelling framework for understanding complex wetland systems with multiple loss processes, which could be used for design purposes. Future developments could attempt to explore the potential role of omitted processes (e.g. mineralization and immobilization, denitrification and ANAMOX) and explicitly represent interactions with environmental variables (e.g. temperature, pH and DO concentrations).

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- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for republication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Technology.

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إزالة الأمونيا في الأنظمة ذات الجريان السطحي باستخدام نظم المعالجة العائمة

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الخلاصة:
تعتبر أنظمة الأراضي الرطبة ذات الجريان السطحي أحد أنظمة المعالجة التقليدية المستخدمة في معالجة ملوثات المياه على الرغم من محدودية كفتها جراء النسبة الحجمية العالية للسعة المائية إلى المساحة السطحية المتبقي للنشاط الميكروبي في تحلل الملوثات البيئية. تميز أنظمة المعالجة العائمة (بما تتضمن من أنظم طافية فوق سطح الماء) بالقدرة على زيادة المساحة السطحية الضرورية لتشريحة وتنوع المجتمعات الميكروبيوية المختلفة على إختلاف أنواعها فضلاً عن إعادة منصة عامة لنمو النباتات فوق سطح الماء وبالتالي زيادة معدلات العمليات البيئية المحلة للملوثات المختلفة. يهدف هذا البحث إلى تقديم فاعلية أنظمة المعالجة العائمة في إزالة تراكيز الأمونيا الكلية من مياه الصرف الصحي المصنف، باستخدام أنظمة تجريبيّة تعمل بطريقة الدفق المستمر-الثابت المؤلفة من 10 عاملات مكررة وبتصاميم تشغيلية مختلفة تتم تضمين إختبار مستويات مختلفة لعمود ماء ومساحات سطحية مختلفة للأطوار الطافية فوق الماء. وتشمل هذه وقودية بكافحة مختلفة ضعاف عن عوامل الماء البيئيًة لمقاومة النترات الناتجة في النظام التجريبي. تم تطبيق هذه الموديلات الرياضية باستخدام بيانات أحد المعاملات من خلال تقدير النماذج الرياضية لنتائج الضغط الشريحي على النباتات، واستنتاج النتائج من حجم النتائج الجزيئية في معالجة الأمونيا. تقدم النتائج مشاركةً قويةً في الحلول الأساسية لاستخدام أنظمة المعالجة العائمة في إزالة الأمونيا. OUTCOME المائي. وتشير النتائج إلى أن إزالة

الكلمات المفتاحية: الأمونيا، مفاعل الخزان ذي المزج المستمر، أنظمة المعالجة العائمة، أنظمة معالجة المياه