MECHANISTIC UNDERSTANDING OF AMMONIA REMOVAL KINETICS IN FLOATING TREATMENT WETLANDS

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ABSTRACT

The role of Floating Treatment wetlands (FTWs) in nitrogen removal from natural water is reasonably well documented, however their function as a polishing technique in domestic wastewater treatment systems is poorly defined. Specifically, develop design criteria for optimal system performance in removing ammonia from sewage is not well addressed. The contribution of this research is to develop a mechanistic understanding of ammonia dynamics in FTWs to improve design and operation. A scaled-up methodology of different experimental FTW systems associated with modelling based-approach were employed to investigate kinetics of ammonia removal under different design criteria. Effects of surface area of mat material, plant density and water depth on ammonia removal kinetics were investigated using microcosm, mesocosm and field pilot-scale systems. The results revealed that ammonia removal was enhanced in FTWs and the magnitude of removal was controlled by the design factors examined. Removal by nitrification was directly proportional to mat surface area. Vegetated treatments with higher plant density exhibited higher ammonia removal by uptake. Field observations showed that ammonia removal was inversely proportional to water depth. Findings presented in this thesis suggest that a design code of full coverage of water surface with mat material, high plant density, and shallow water depth can be considered a critical design for FTW system to remove ammonia from domestic wastewater. This design promoted nitrification as principal ammonia removal process even when plants were present. The contribution of nitrification to overall ammonia removal in the vegetated treatments was estimated to be between 59–83 % in mesocosms and between 81 - 85% in the pilot-scale system. Plant uptake contributed to 16–40% of the total N loss in mesocosms and 19-14 % in the pilot system. Ammonia loss via volatilization was determined to be negligible in all examined systems. Estimated kinetics parameters in the examined systems revealed close agreement between microcosm and pilot-scale system performance in treating ammonia (0.03 and 0.14 day^{-1}). The results suggest that supplementing field study with a well-controlled laboratory microcosm study was useful for confirming kinetics parameters derived from field data. The use of kinetics parameters obtained from such approach could be useful to estimate ammonia removal and establish design criteria in a broader perspective including full-scale wastewater treatment systems.

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Acronyms and Abbreviations

1	FTW	Floating Treatment wetland
2	CW	Constructed Wetland
3	FSCWs	Free Surface Constructed Wetlands
4	NH_x	Total ammonia nitrogen
5	NO_x	Total oxidized nitrogen
6	ON	Organic nitrogen
7	NO	Nitric Oxide
8	N_2O	Nitrous oxide
9	TIN	Total inorganic nitrogen
10	TKN	Total Kjeldahl Nitrogen
11	TN	Total nitrogen
12	DO	Dissolved oxygen
13	EC	Electrical conductivity
14	mg L^{-1}	milligram per liter
15	mS cm^{-1}	milli Siemens per centimeter
16	°C	Degree Celsius
17	MDL	Minimum Detection Limit
18	AOB	Ammonium oxidizing Bacteria
19	NOB	Nitrite Oxidizing Bacteria
20	CFU	Colony Forming Unit
21	C:N	Carbon-Nitrogen ratio
22	CSTR	continuously-stirred tank reactor
23	*ET*	Evapotranspiration
24	HRT	Hydraulic Retention Time
25	*Q*	Flow rate
26	*J*	Flux rate
27	*k*	Rate constant
28	$T_{1/2}$	Degradation half-life
29	*RE*	Removal efficiency
30	STELLA	Structural Thinking and Learning Laboratory with Animation
31	RMSE	Root Mean Squared Error
32	ANOVA	Analysis of variance
33	EPA	U.S. Environmental Protection Agency
34	APHA	American Public Health Association
35	WHO	U.N. World Health Organization

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Chapter One – Introduction & Literature Review

1.1 The Nitrogen Cycle

Nitrogen (N) is one of the key elements for life on Earth. It is an essential part of DNA, proteins, and enzymes, and is thus a fundamental nutrient for biota (Zerkle and Mikhail, 2017). N often limits the function of ecological systems by controlling the rate of primary production; it's forms and abundance can change the competition between species and hence biological diversity (Gruber and Galloway, 2008). Cycling of nitrogen is of central interest to global biogeochemistry (Galloway et al., 2004b). Of approximately 6×10^{15} kg N in the atmosphere, hydrosphere, and crust, about two-thirds are in the atmosphere as molecular N (N_2) with most of the remainder present in the crust (Schlesinger, 2005). Atmospheric N_2 is relatively chemically inert and does not readily combine to form other compounds due to the strong triple bond of the N_2 molecule that requires a significant amount of energy to break (Levine and Allario, 1982). Therefore, most of this N is metabolically unavailable to biosystems, making this element often a limiting nutrient (Burt, 2013).

The conversion of N_2 to reactive N directly or indirectly supports ecosystem functions (Herman et al., 2006). Biologically-available N is naturally derived either by lightning and atmospheric deposition or by biological reduction of N_2 by symbiotic and asymbiotic microorganisms (Nieder and Benbi, 2008). Fixed N includes inorganic reduced N forms (e.g., ammonia and ammonium), and inorganic oxidized forms (e.g., nitrite, nitrate, nitrogen oxides) and organic forms (e.g., urea, amines, and proteins) (Denk et al., 2017).

The existence of these forms in biosphere compartments provides a cascade of biogeochemical transformations including sequences of oxidation and reduction reactions which are often mediated by enzymes produced by microbes (Galloway et al., 2003; Schlesinger, 2005; Housecroft, 2010; Vymazal, 2010; Stüeken et al., 2016).

The five principal biochemical processes in the N cycle are nitrogen fixation, ammonification, nitrification, denitrification, and assimilation. The main transport processes are Advection with flowing water, volatilization, particulate settling and resuspension, diffusion of dissolved forms. Biological transfer processes include plant

translocation, litterfall, and sorption of soluble N to a substrate (Kadlec and Wallace, 2009b) (Figure 1.1).

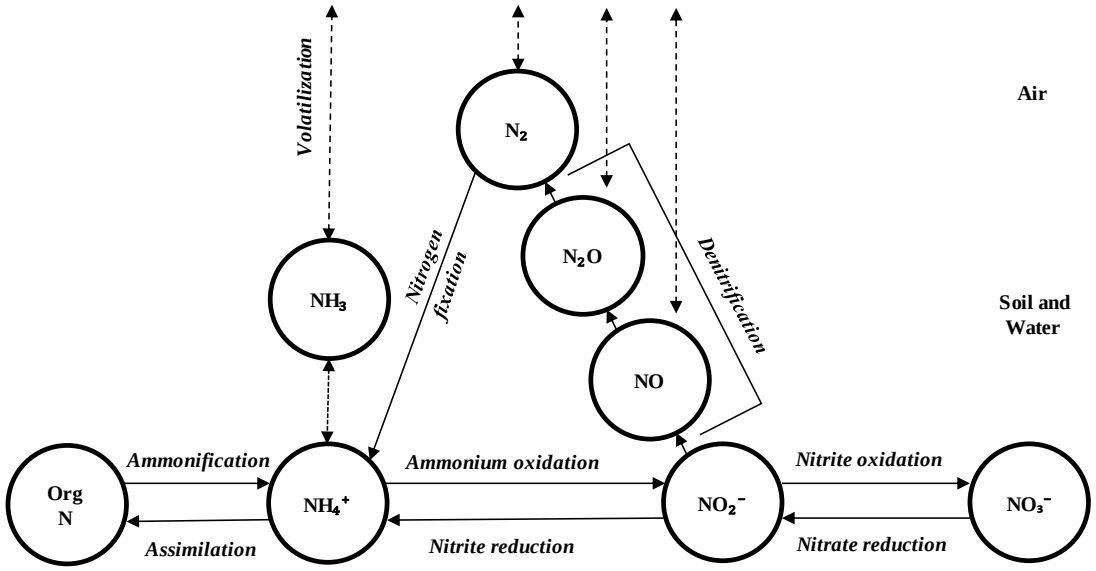

Figure 1. 1 The nitrogen cycle. Org N is organic-N, NH_3+NH_4^+ is reduced-N, and NO_2^-+NO_3^- is oxidised-N (Schlesinger, 2005).

Once nitrogen is fixed, organic and inorganic nitrogen are interconverted by *ammonification* and *assimilation* (Boyd, 2001). During *ammonification* (*mineralization*), ammonium is converted from organic-N through the hydrolysis of complex nitrogen-containing compounds (Vymazal, 2007). The reverse processes, *assimilation*, and *immobilisation* occur when the produced mineral-N is incorporated into organisms during growth (Zerkle and Mikhail, 2017). Ammonium can be utilized by some specialized microbes to gain energy via *nitrification* which produces nitrate as a final product. In nitrification, ammonia is oxidized into nitrite and then nitrate. Nitrate can be lost under the anaerobic process of *denitrification* in which nitrate is converted into dinitrogen gas (Fernández et al., 2011). During nitrate ammonification, nitrate is first reduced to nitrite and then reduced to ammonium by specialized fermentative bacteria (Vymazal, 2007; Stein and Klotz, 2016; Denk et al., 2017).

In addition to the biochemical reactions, some physical processes transfer nitrogen compounds from one point to another within ecosystems without resulting in a molecular

transformation (Kadlec and Wallace, 2009b). During ammonia volatilization, the function of the ammonia nitrogen (NH_4^++NH_3) which is present in the unionized form (NH_3) can be lost from solution to the atmosphere through diffusion across the water-air interface (Poach et al., 2004). Ammonium ions can be adsorbed to both organic and inorganic substrates by electrostatic attraction to negatively charged microsites, and therefore be removed from water through exchange with detritus and bed substrate (McCarthy et al., 2007; Vymazal, 2007).

Although an understanding of the N cycle has developed over the last few decades, it is still very complex (Karl and Michaels, 2013). A better understanding of the unprecedented environmental degradation associated with an acceleration of N fluxes which has occurred as a consequence of anthropogenic inputs, therefore demands a reconstruction of the biogeochemical N cycle over time (Anbar et al., 2007).

1.2 Anthropogenic Nitrogen and Environmental consequences

Over the last century, our understanding of the N cycle at different scales has improved dramatically. It is now well known that the reactive N in ecological systems is the result of a number of natural processes, and that the function of these systems is often controlled by N bioavailability (Galloway et al., 2004b). During the last decades, the production of N by man-made activity has exceeded the production from all natural systems (Stein and Klotz, 2016). Anthropogenic activities from food production, fossil-fuel combustion, mineralization of animal manure, along with miscellaneous other sources have increased the global N production over the last century from approximately 203×10^9 kg N yr^{-1} to 413×10^9 kg N yr^{-1} (Erisman et al., 2008). Further, the notable increase of world population since 1970 (78%) accompanied with the accelerated growth of various industrial activities have inevitably caused an increased cascade of N fluxes through wastewater discharges (Galloway et al., 2008).

Freshwaters comprise a small fraction (2.5%) of global water (Gleick et al., 2014). Discharge of large volumes of untreated wastewater into surface waters is common practice in most low income developing countries (UN Water, 2008), which can cause a number of environmental and human health problems (Galloway et al., 2008). The magnitude of the consequences depends on the magnitude of the rates of N releases and the degree of dilution in receiving waters (Galloway et al., 2004b).

In EU countries, approximately 18% of anthropogenic N ends up in wastewater in the form of ammonia or organic nitrogen (Mulder, 2003). Ammonia is a hazard for natural ecosystems because it is toxic to a number of aquatic taxa at high concentrations (Vymazal, 2010). It can make up more than 50% of the total nitrogen in municipal discharges, at concentrations of 20-60 mg L^{-1} (Kadlec and Wallace, 2009b). Official estimations reported that ammonia might be present in some surface waters up to 12 mg N L^{-1} (WHO, 1986).

Naturally, ammonia is a colorless gas with a characteristic smell. It is corrosive and an irritant (Housecroft and Constable, 2010). The large-scale commercial production of ammonia began after the development of the Haber-Bosch process, which uses high temperature and pressure with a metallic catalyst to combine atmospheric N_2 and H_2 produced from fossil fuels (Schlesinger, 2005).

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad (1.1)$$

Ammonia is widely used in fertilizers and food production, in synthetic fibers as well as various chemical and pharmaceutical manufacturing (WHO, 2003). It has also been used in drinking water treatment (0.4 mg N L^{-1}) to reduce the formation of chlorination by-products which may be carcinogenic (US EPA, 2013). It may find its way to surface waters through discharge of industrial process wastes, municipal wastewater effluent, and agricultural drainage. Ammonia is very soluble in water (421 g L^{-1} at 20 ºC) (WHO, 2003). The high solubility is due to the extensive hydrogen bonding that occurs between H_2O and NH_3 molecules (Housecroft, 2010). Ammonia exists in water solutions as either un-ionized ammonia or the ammonium ion with one oxidation state (-3) (Schlesinger and Bernhardt, 2013). The equilibrium of these chemical species can be expressed by the following equation (Housecroft and Constable, 2010):

$$NH_3 + H_2O \leftrightarrow NH_4OH \leftrightarrow NH_4^+ + OH^+ \qquad (1.2)$$

The equilibrium constant of this reaction is mainly controlled by temperature, pH, and the concentration of dissolved ammonia in the water (Poach et al., 2004). At higher temperature and pH, the balance between NH_3 and NH_4^+ tends to shift towards the

formation of un-ionized NH_3 (Vymazal, 2007). For a typical environmental condition of 25 °C and a pH of 7, free or unionized ammonia makes up only 0.6% of the total ammonia present. However, at pH 9.5 and 30 °C NH_3 makes up 72% of total ammonia (Kadlec and Wallace, 2009b). Un-ionized NH_3 can be volatilized (i.e., converted from the liquid to the atmosphere) based on the difference in the partial pressures between water and atmosphere (Schlesinger, 2005). Ammonia in the atmosphere can be deposited by dry and wet deposition, which can be regarded as a further source of ammonia (Stüeken et al., 2016). Ammonium (NH_4^+) cation is less mobile in soil and water than un-ionized ammonia because it is attracted to the negative charges of clay minerals and organic matters (Nieder and Benbi, 2008). Although ammonia can have a beneficial role in the augmenting plant growth, if concentrations are high, it can trigger eutrophication (Richmond and Hu, 2013). Eutrophication is a condition of nutrient excess characterized by proliferation of aquatic plants and algae, which can lead to substantial impacts on ecosystem function and composition (Serediak et al., 2013). Excessive quantities of these contaminants can reduce oxygen content in the water bodies (anoxia and hypoxia) when the plants and algae decompose and can cause toxic effects in some aquatic organisms (Camargo and Alonso, 2006).

High concentrations of NH_3 can be inhibitory for the growth and activity of ammonia-oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), key contributors of nitrification, through influencing the metabolism of these microbes (Kim et al., 2006). It has been reported that NH_3 has a significant inhibitory effect on the cellular respiration of NOB (Vadivelu et al., 2007). NH_3 can induce oxidative stress at the cellular level through elevating the concentration of reactive oxygen species, which could damage the biomacromolecules including DNA fragmentation, lipid peroxidation, and eventually cause to the cell dysfunction (Livingstone, 2001; Liang et al., 2016). Likewise, NH_4^+ can contribute to acidification in the freshwater environment through nitrification that produces two hydrogen ions for every ion of NH_4^+ converted to NO_2^-. Anthropogenic acidification of water ecosystems is also associated with a number of adverse effects on key microbial processes essential for nutrients cycling and ecosystem functioning (Cervantes, 2009). Although evidence is very inconclusive, methemoglobinaemia (blue-baby syndrome) (Daalkhaijav and Nemati, 2014), mutagenicity and congenital disabilities have been associated with high nitrate concentrations in drinking water (Powlson et al., 2008). During chlorination of drinking water, carcinogenic nitrosamines

may be formed by the interaction of nitrite with compounds containing organic nitrogen (WHO, 2003). The sections below describe the processes and environmental factors that regulate nitrogen transformations with special emphasis on ammonia removal.

1.3 Nitrogen Removal Processes

1.3.1 *Ammonification*

Under aerobic conditions, organic nitrogen is decomposed to ammonia via the process of *ammonification* (or *mineralization*); a set of energy-releasing processes mediated by heterotrophic microbial communities (Kadlec and Wallace, 2009b). *Mineralization* is the deamination of amino acids (Romillac, 2019). Reaction rates of *mineralization* are faster under aerobic conditions but decrease under anaerobic conditions (Reddy and Patrick, 1984).

Two typical urea and amino acid breakdown reactions are (Kadlec and Wallace, 2009b):

$$NH_2CONH_2 + H_2O \rightarrow 2NH_3 + CO_2 \qquad (1.3)$$

$$RCH(NH_2)COOH + H_2O \rightarrow NH_3 + CO_2 \qquad (1.4)$$

Since *mineralization* is temperature and pH-dependent, the optimal reaction rate is reported to be at 40–60 °C, and in the pH range of 6.5–8.5 (Coban et al., 2015b). Simultaneously, *mineralization* is associated with the *immobilization* reaction, when the mineral-N produced by the former is converted to organic N for biomass growth (Kadlec and Wallace, 2009b). The net balance between mineralization and immobilization depends on the C:N ratio of the substrate to the relative microbial biomass. This immobilized N can be remobilized again when organisms die, and their biomass is decomposed (Paul et al., 2000). However, some fractions of the organic nitrogen incorporated in detritus may eventually become unavailable for additional nutrient cycling through the process of humification (e.g., peat formation) and burial on land and sedimentation in water (Hietz et al., 2011).

1.3.2 *Nitrification*

Nitrification is biological oxidation of ammonium (NH_4^+) to nitrate (NO_3^-) with nitrite (NO_2^-) as an intermediate in the reaction sequence under aerobic condition using oxygen

as the terminal electron acceptor (Vymazal, 2007). *Nitrification* is typically associated with the chemoautotrophic bacteria, although it is now recognized that heterotrophic *nitrification* also occurs and can be significant (Kadlec and Wallace, 2009b). Autotrophic nitrification mainly consists of two successive aerobic reactions, the conversion of NH_4^+ to NO_2^- by ammonium oxidizing bacteria, and the conversion of NO_2^- to NO_3^- by nitrite oxidizing bacteria (Faulwetter et al., 2009). The first reaction, nitritation, is comprised of two steps, using ammonia monooxygenase (*amo*) and hydroxylamine oxidoreductase (*hao*) as catalysts to obtain energy for growth (Cervantes, 2009). These reactions are executed by strictly chemolithotrophic bacteria belonging to β-proteobacteria that include three genera, *Nitrosomonas, Nitrosospira* and *Nitrosococcus* (Faulwetter et al., 2009).

The *nitritation* reaction can be written as (Kadlec and Wallace, 2009b):

$$NH_4^+ + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \qquad (1.5)$$

$$NH_2OH + H_2O \rightarrow NO_2 + 5H^+ + 4e^- \qquad (1.6)$$

In this reaction, AOB uses ammonium as an electron donor and produce acidity through generating a proton motive force that is used as an energy source for ATP production (Cervantes, 2009).

The second reaction in nitrification is executed by facultative chemolithotrophic bacteria, using nitrite as the electron donor and nitrite oxidoreductase (*nor*) as a catalyst (Cervantes, 2009). Nitrite is oxidized by the genus *Nitrobacter* as well as *Nitrospira* (Kadlec and Wallace, 2009b).

The *nitrification* reaction can be written as:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad (1.7)$$

Both AOB and NOB use CO_2 and bicarbonate for cell synthesis and ammonium or nitrite as the energy source (Faulwetter et al., 2009). Furthermore, heterotrophic bacteria and fungi are also capable of oxidizing NH_4^+ to NO_3^- (Alzate Marin et al., 2016). However, the reactions occurring in heterotrophic nitrifiers are not ATP-coupled and, thus, do not provide energy (Nieder and Benbi, 2008).

Nitrification is influenced by different factors including dissolved O_2, temperature, pH, the alkalinity of water, and the concentration of free ammonia (Vymazal, 2007). Based on the stoichiometric relationship, complete *nitrification* reaction requires 4.6 kg oxygen per kg NH_4^+-N. A dissolved oxygen concentration of 1 mg L^{-1} thought to be sufficient for complete oxidation of ammonium (Faulwetter et al., 2009). One gram of ammonia can be utilized for microbial growth to produce microbial biomass of 0.17 g of dry weight (EPA, 1993). Nitrifying bacteria are in most cases mesophilic and have an optimum temperature for growth between 28–36 °C, but *nitrification* is still possible at a temperature as low as 5 °C (Cervantes, 2009). The optimal pH range observed for *nitrification* is between 6.6 – 8.0, however, acclimatized systems can be operated to nitrify at much lower pH (Faulwetter et al., 2009). During nitrification, oxidation of each gram of NH_4^+ to NO_3^- requires the consumption of approximately 7.1 g of alkalinity (as $CaCO_3$), as two moles of H^+ are released for each mole of ammonia. (Kadlec and Wallace, 2009b). Thus, nitrification lowers alkalinity and pH of the water. Free ammonia and free nitrous acid (FNA) are known to inhibit *nitrification,* and their concentrations depend on total ammonia and nitrite nitrogen concentrations, respectively, but also pH (Cervantes, 2009). *Nitrification* rates in natural ecosystems have been reported to be in the range of 0.01 – 2.15 g m^{-2} day^{-1} with the mean value of 0.048 g m^{-2} day^{-1} (Vymazal, 2007).

1.3.3 *Ammonia Volatilization*

Ammonia volatilization is a physicochemical process where free NH_3 is transferred from water to the atmosphere through diffusion (Kadlec and Wallace, 2009b). Based on the medium pH and temperature, the equilibrium ratio between NH_4^+ and NH_3 varies as discussed above. *Volatilization* of free NH_3 can be significant in a system with high pH and temperature (Marimon et al., 2013). Proliferated algal biomass and submerged macrophytes in aquatic ecosystems often create high pH values, therefore pushing the balance between NH_4^+ and NH_3 towards the production of NH_3 resulting in more *volatilization* (Castro et al., 2017). However, this process is only indirectly mediated by microbes, as they contribute in the NH_4^+ production (Nieder and Benbi, 2008).

Ammonia volatilization is more important in open water systems because of direct contact between contaminants and the atmosphere (Kadlec and Wallace, 2009b). NH_3 *volatilization* is generally regarded as an undesirable process because NH_3 gas is an atmospheric pollutant that can adversely affect off-site ecosystems through dry and wet

deposition (Poach et al., 2004). In general, *ammonia volatilization* rates in wetlands have been reported to be as high as 2.2 g N m^{-2} day^{-1} (Vymazal, 2007).

1.3.4 *Denitrification*

Denitrification is the process in which NO_3^- is converted into N_2 via the intermediates nitrite (NO_2^-), nitric acid (NO), and nitrous oxide (N_2O), where every reaction is catalyzed by a specific enzyme under anoxic conditions (Kadlec and Wallace, 2009b). Biochemically, *denitrification* takes place as follows: (i) NO_3^- is reduced to NO_2^- by nitrate reductase, (ii) a subsequent reduction of NO_2^- to NO is carried out by nitrite reductase, (iii) NO is reduced to N_2O by nitric oxide reductase, (iv) finally, N_2O is reduced to N_2 by nitrous oxide reductase (Cervantes, 2009). Partial anaerobiosis results in the production of NO and N_2O, while complete reduction of nitrogenous oxides to N_2 is an anaerobic process (Nieder and Benbi, 2008). Most denitrifying bacteria are facultative anaerobic chemoheterotrophs using organic compounds as electron donors and as a source of cellular carbon and oxygen as a terminal electron acceptor under aerobic conditions. Under anaerobic conditions, nitrogen oxides are used as terminal electron acceptors (Faulwetter et al., 2009). The biochemical reaction comprises the transfer of electrons from donors (carbon) to acceptors (nitrogen oxides) through several carriers systems in the respiratory chain (Vymazal, 2007).

The *denitrification* reaction can be written as (Vymazal, 2007):

$$6(CH_2O) + 4NO_3^- \rightarrow 6CO_2 + 2N_2 + 6H_2O \qquad (1.8)$$

Different microbial groups can function as denitrifiers including organotrophs (e.g., *Pseudomonads*), chemolithotrophs (e.g., *Nitrosomonas*), archaea (e.g., *Halobacterium*) and others (Kadlec and Wallace, 2009b). Environmental factors that influence *denitrification* include the absence of oxygen, availability of organic matter and nitrate, temperature, and pH (Faulwetter et al., 2009). *Denitrification* activity is inhibited reversibly under aerobic conditions (Cervantes, 2009). Elevated C:N ratios can lead to increased *denitrification* rates (Wu et al., 2007). Literature reports a range of pH values (6 – 9), where *denitrification* operates effectively. However, denitrification becomes inhibited or ceases below pH 4 (Cervantes, 2009). *Denitrification* activity generally increases with increasing temperature (with all other factors non-limiting) up to

60 °C, but the rates of reactions proceed very slowly at or below 5 °C (Gerardi, 2010). The estimation of *denitrification* rates in the natural wetlands varies widely in the literature between 0.003 – 1.02 g N m^{-2} day^{-1} (Vymazal, 2007).

1.3.5 *Assimilation*

Plant uptake of mineral N is an important sink for N in ecosystems. Nitrogen uptake refers to a variety of biological processes that convert inorganic N forms into organic compounds that serve as building blocks for cells and tissues (Vymazal, 2007). Many plant species utilize ammonium and nitrate as nitrogen sources (Hammer, 1989). Ammonium is regarded as the preferable N form to be assimilated into plant tissues due to the lower energy required for storage compared to NO_3^- (Kadlec and Wallace, 2009b). Nitrogen reaches the subsurface root system via diffusion, but more importantly by mass flow under the effect of the transpiration flux (in vascular plants). Then it is taken up by the root and transported to the vascular tissues via cross plasma membranes using cellular transport systems (Glass et al., 2002). Uptake is thought to depend on root morphology architecture as well as root surface area (Yamauchi et al., 1996; Samal et al., 2003). Root absorptive capacities are sensitive to photosynthetic rate and can be affected by environmental stress (Hammer, 1989). The potential rate of N *uptake* by the plant is limited by its net productivity and the concentration of N in the plant tissue (Vymazal, 2007). However, several investigations have revealed that nitrogen *uptake* by plants is of minor significance for long-term nutrient removal (e.g., from wetland) unless plants are harvested, and biomass is removed from the system (Bastviken, 2006). This is because assimilated nitrogen is re-released to the system during decomposition of litter (Johnston, 1991).

Although knowledge of the N cycle, at different scales, has been improved over the last two decades, it is still incomplete (Galloway et al., 2004b). A better understanding of nitrogen transformation and transfer processes is important for optimizing the performance of treatment systems, particularly those with high nitrogen concentrations.

1.4 Nitrogen in Wastewater and Environment

Prior to 1800, the degradation of water quality caused by nitrogen contamination from point and non-point sources was not a big concern because human populations were small, and the majority of the people lived in scattered rural communities (Mason, 1996). Therefore, the self-purification of natural water bodies could cope with the wastes

released into them (Galloway et al., 2004a). Sharply since 1960s, N removal appeared strongly on the agenda. This was mainly due to the fact that man-made eutrophication was causing an obvious increasing and undesirable deterioration of water quality in lakes and reservoirs (Zhu et al., 2008). Discharge from wastewater works has been shown to be an important source of N forms into the environment which is of major concern because some are toxic and disruptors (Weedon, 2017). As a result of its environmental problems, N removal from wastewater became a concern in water management worldwide. This concern led to the establishment of several national and international agencies in order to issuing different regulations and directives with the aim of developing an environmental policy. For instance, the European Community Urban Wastewater Directive (UWWD) has set criteria and standards for N that implemented throughout the Community by 1999. The Urban Waste Water Treatment Directive of the EU91/271/EEC (2002) requires wastewater treatment plants (WWTPs) discharging to environmentally-*sensitive areas* to remove nitrogen and other contaminants (Table 1.1). *Sensitive areas* are defined as natural freshwater lakes, estuaries or coastal waters, which are, or may become eutrophic, or surface waters used for the abstraction of drinking water which may potentially contain more than 0.5 mg NO_3^- N L^{-1} which constitute the limit set by European Community. Von Sperling (2007) reported that for wastewater works discharging into such waters and serving greater than 10,000 P.E. (*Population Equivalent*), effluents must comply with the values for concentration or percentage reduction of total N given in Table 1.1.

Table 1. 1 European Community requirments for discharges from urban wastewater treatment plants (Von Sperling, 2007).

Parameter	Concentration	Minimum percentage of reduction [2]	Population Equivalent
BOD	25 mg O_2 L^{-1}	70-90%	-
COD	125 mg O_2 L^{-1}	75%	-
Total suspended solids	35 mg L^{-1}	90%	P.E. > 10.000 inhab.
	60 mg L^{-1}	70%	P.E. between 2,000 and 10,000 inhab.
	150 mg L^{-1}	-	For ponds effluents
Total nitrogen [1]	10 mg L^{-1}	70-80	P.E. > 10.000 inhab.
	15 mg L^{-1}	70-80	P.E. between 10,000 and 10,000 inhab.
Total phosphorus	1 mg L^{-1}	80	P.E. > 10.000 inhab.
	2 mg L^{-1}	80	P.E. between 10,000 and 10,000 inhab.

[1] Total N: requirement for discharge in sensitive water bodies. Values are annual.

[2] Removal in relation to the load of the influent.

In the UK, ammonia standards applied to the rivers based on the implementation of the Water Framework Directive (WFD) and the Environmental Quality Standards Directive (EQS) are presented in Table 1.2.

Table 1. 2 Ammonia standards for rivers (DEFRA, 2015).

Total ammonia as nitrogen (mg N L^{-1}) (*90 percentile*)			
High*	Good	Moderate	Poor
0.2-0.3	0.3-0.6	0.75-1.1	1.1-2.5

*Type of standard.

The Water Framework Directive 90th percentile standards are used for managing the risk posed by continuous discharges of ammonia on freshwater ecosystems. 90th percentile standards are standards that are failed if the concentration of the pollutant is greater than the standard for 10% or more of the time. These standards are intended to be used in classifying the status of water bodies. However, the 99th percentile standards in Table 1.3 were derived from Water Framework Directive 90th percentile to managing the impacts of intermittent discharges such as that resulted from combined sewer overflows and discharges from storm tanks. 99th percentile standards are standards that are failed if the concentration of the pollutant is greater than the standard for 1% or more of the time. These standards are intended to assist the agencies in setting appropriate operating requirements for (1) proposed new intermittent discharges, and (2) existing intermittent discharges where such requirements are considered the most cos-effective and proportionate means of improving the status of water bodies. These standards are not intended to be used in classifying the status of water bodies.

Table 1. 3 The 99th percentile standards for ammonia in rivers (DEFRA, 2014).

Type of standard	Total ammonia N (mg N L^{-1})	Un-ionised ammonia (mg N L^{-1})
	99th percentile	
High	0.5-0.7	0.04
Good	0.7-1.5	0.04
Moderate	1.8-2.6	0.04
Poor	2.6-6.0	0.04

1.4.1 *Domestic Sewage and Treatment Processes*

Sewage is a dilute mixture of domestic waste, trade wastes, infiltration from the subsoil, and runoff of surface water (Englande et al., 2013). Sewage composition is varying considerably depending on the amount of water used per head of population, the degree of infiltration of subsoil water and the nature and proportion of trade effluent present. In general, Sewage impurities can be classified into: (a) suspended particles in the liquid; (b) dissolved forms in the liquid; (c) fine-colloidal substances (Mason, 1996). Domestic sewage contains a large variety of organic pollutants and nutrients. Nitrogen is mainly present as ammonia (60-70%), soluble and particulate organic nitrogen (urea, proteins) and seldom as nitrate and nitrite (Table 1.4) (Cervantes, 2009).

Table 1. 4 Characteristics of typical domestic wastewaters (concentration in mg L^{-1}) (Cervantes, 2009).

Parameter	Concentrated	Moderated	Diluted	Very diluted
COD	740	530	320	210
BOD_5	350	250	150	100
TKN	80	50	30	20
NH_4^+-N	50	30	18	12
NO_3^--N	0.5	0.5	0.5	0.5
NO_2^--N	0.1	0.1	0.1	0.1
SS (COD)	440	320	190	130
TP	14	10	6	4

TKN: Total Kjeldhal nitrogen, NH_4^+-N: Ammonia nitrogen, NO_x-N: Nitrite and Nitrate nitrogen, COD: Chemical Oxygen Demand, SS: Suspended Solids, TP: Total Phosphorus, BOD: Biochemical Oxygen Demand.

In most modern WWTPs, treatment of sewage is usually performed by four different stages (e.g. preliminary, primary, secondary and tertiary), depending on the quality of the effluent required (Englande et al., 2013). The objective of preliminary treatment is only the removal of coarse solids, while primary treatment aims at removing settleable solids and part of the organic matter. Physical pollutant removal mechanisms are predominant in both levels (e.g. screening, mixing, flocculation, sedimentation, floatation, filtration) (Von Sperling, 2007). Secondary treatment is the process where dissolved and colloidal substances are oxidised in the presence microorganisms, and tertiary treatment (often referred to as "polishing") is used when a very high effluent is required that might involve further reduction in different contaminants (e.g. BOD, TSS, N, P and other toxic

compounds). Biological mechanisms are predominant in both levels (e.g. carbonaceous organic matter removal, nitrification, denitrification) (Zhu et al., 2008). The main objective of sewage treatment is to reduce the extent of contaminants (strength) to sufficient degree to allow safe discharge into natural waters without causing a nuisance (Popple et al., 2016). The UWWD summarised the main requirements for effluent of sewage treatment plants (STPs) in Europe (EU91/271/EEC, 2002). Limit concentrations for N and P apply only when the discharge is to sensitive water bodies (Table 1.5).

Table 1. 5 Possible discharge standards, according to different levels of stringency, for the main pollutants in domestic sewage (Von Sperling, 2007).

		Discharge standard (mg L^{-1})		
Parameter	Discharge to	Less stringent	Stringent	Very stringent
COD	Any water body	200	100-150	50
BOD	Any water body	60	20-30	10
SS	Any water body	60	20-30	10
TN	Sensitive water body	-	10-15	10
TP	Sensitive water body	-	1-2	1

The treatment method and level to be implemented for wastewater purification is depending on the objectives for the quality of the receiving water and the extent of dilution which is available for that water. For instance, treatment method in which physical forces are predominant (e.g. preliminary stage) only may be applied for effluents being discharged in the sea, while treatment method in which the removal of the contaminants occurs by means of biological activity (e.g. tertiary treatment) could be required if water quality is needed for potable supply downstream of a discharge (Englande et al., 2013). Nitrogen removal is usually achieved in tertiary treatment level (Weedon, 2017). Conventional biological systems aim to remove nitrogen by means of hydrolysis of organic nitrogen, nitrification of ammonia, and denitrification of nitrate. In the following sub-sections, a brief overview of the conventional systems for removal of nitrogen from domestic wastewater is presented, followed by an outline of constructed wetlands as alterative systems for the wastewater treatment.

1.4.2 *Nitrogen Treatment in Conventional Systems*

In raw domestic wastewater, the predominant nitrogen forms are organic nitrogen and ammonia, with nitrites and nitrates rarely being present (Horan, 1989). Ammonia is mainly derived from urea, which is rapidly hydrolysed and rarely found in raw sewage (Karri et al., 2018). The total nitrogen content of wastewater is often referred to as the Total Kjeldahl Nitrogen (TKN), according to the technique by which it is determined (Von Sperling, 2007). However, it is important to pointed out that the TKN measurements gives the amount of organic nitrogen plus ammonia nitrogen but exclude most of the oxidised forms which is lost during digestion (APHA, 2005a). Most of the TKN in domestic sewage has physiological origin (80% of TKN is urine from households) (Karri et al., 2018).

The nitrogen abatement approaches are categorically divided into physicochemical (e.g. ammonia stripping, ion-exchange) and biological methods (e.g. nitrification, denitrification) (Karri et al., 2018). Ammonia stripping is a process in which un-ionised ammonia is transferred from the waste stream into the air, then absorbed from the air into a strong acid solution (typically sulphuric acid), thereby generating an ammonium-salt, which can be crystallised (Bonmatí and Flotats, 2003). Ion exchange process can be accomplished by passing the wastewater through the bed of the ion-exchanger which exhibits a high selectivity for the ammonium ion over other cations commonly present in wastewater. Zeolites are used as an ion-exchange medium and found to be very useful since they possess high sorption and ion exchange selectivity (Jorgensen and Weatherley, 2003).

Nitrogen is treated biologically by two different methods after initial separation of solids in the primary sedimentation tanks. These methods include suspended and attached growth systems. The most widely used nitrogen treatments are conventional activated sludge (CAS) and trickle filters. They are used in their own or in combination depending on the size of the treatment plants. Conventional Biological removal of nitrogen involves three basic processes: (a) synthesis – incorporation of nitrogen into the microbial biomass as a result of cell growth; (b) nitrification – conversion of the ammonia, derived from the hydrolysis of organic nitrogen, to nitrate through oxidation by nitrifying organisms; (c) denitrification – conversion of the nitrate to nitrogen gas by denitrifying organisms (Hammer, 1989; Zhu et al., 2008). Both autotrophic nitrification and heterotrophic denitrification are described in details in section 1.3. In biological removal system, these

processes may be accomblished in a separate unit process, referred as sparate stage process, or (b) may be achieved by combined processes, which referred to as the single-sluge process (Figure 1.2). Separate stage nitrification involves the use of two biological processes in series, whereby the first one removes carbonaceous biological oxygen demand (BOD) and the second one is used to nitrify the low BOD effluent from the first process. When a two sluge process is desired, combined carbon removal and nitrification, the first stage accomblishes BOD oxidation and nitrification, while the second stage denitrifies the nitrate generated from the first stage. However, denitrification could take place in a separate unit process following BOD removal and nitrification (Zhu et al., 2008; Englande et al., 2013).

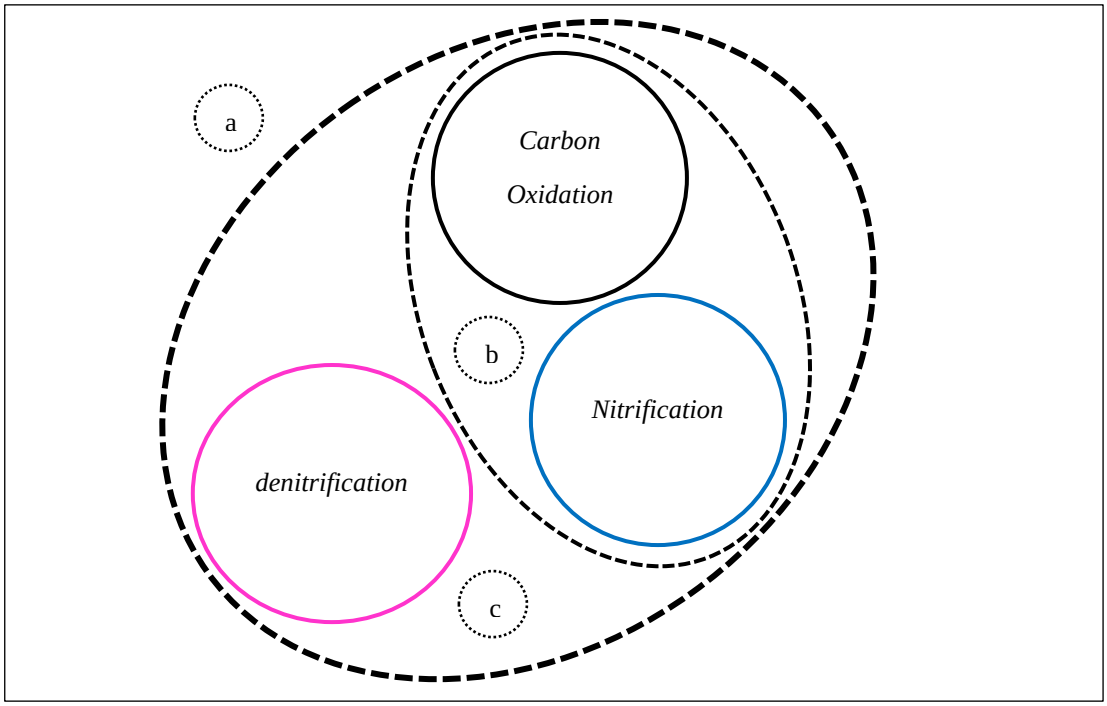

Figure 1. 2 Conventional Biological removal of nitrogen: (a) Separate stage process; (b) Two sludge process; (c) Single sludge process.

1.4.3 *Limitation of Conventional Processes for N Treatment*

The common feature of conventional systems for nitrogen removal is that wastewater can be treated rapidly within short residence time, the systems can be operated at a range of organic and hydraulic loading rates, and can be modified to meet specific discharge limits (Ludwig and Mohit, 2000). These systems are also characterised by high capital and

operating coast, and highly trained staff is required for maintenance and truble-shooting. Requires expert design and construction. Also, high energy consumption, a constant source of electricity power is required (Carty et al., 2008). Conventional systems are prone to complicated chemical and microbiological problems. For example, a high level of external carbon sources (e.g. methanol, acetate) is normally added in the denitrification process when treating wastewater with high nitrogen concentration of low C/N ration, which increases the operational coast (Zhu et al., 2008). Although, biological treatment systems require less control of the equipment than chemical ones, the influent and effluent must be constantly monitrored and the controlled parameters adjusted, specially for activated sludge units, to avoid abnormalities that could influence the active biomass and the development of deterimental organisms which could impair the process (e.g. filamentous bacteria) (Safoniuk, 2004). Sludge and possibly effluent require further treatment and/or appropriate dischsrge. The limitations of operational costs, size and and structural configuration of the conventional treatment systems, associated with the more stringent standards of the effluent dicharge (<10 mg N L^{-1}), are the driving forces for developing new low-cost biological treatment processes for complete nitrogen removal (Khin and Annachhatre, 2004).

During the last decades, number of alternative options to the conventional treatment systems were investigated with the aim of minimise the environmental and economical burdens onto small-communities. Most of these alternatives are based on the utilisation of the ecosystems service for wastewater treatment, such as constructed treatment wetlands.

1.5 Constructed Wetland Technology

Constructed wetlands (CWs) are engineered ecosystems that has been designed to utilize the natural processes from biotic and abiotic components for water quality improvement (Vymazal, 2007; Andersson et al., 2005; Matamoros et al., 2017; Weedon, 2017; He et al., 2018). Historically, the emphasis on the use of constructed wetlands as low-energy technology to improve water quality and the promotion of this technique was started in the seventies after the first energy crisis in 1973 (Hammer, 1989). At the primary stage, constructed wetlands were applied as a complement to wastewater treatment with varied success. The growing popularity of these systems is largely since these systems offer the advantages of providing a relatively passive, low-maintenance and operationally simple

treatment solution whilst potentially enhancing habitat and aesthetic values within the urban landscape (Nahlik and Mitsch, 2006; Mitsch et al., 2008; Borne et al., 2013; Rahman et al., 2014; Lu et al., 2015; Masters, 2012). Besides providing storage capacity to control peak runoff and increase residence time for treatment performance, the high biomass production makes CWs among the most biologically active ecosystems in the planet (Bastviken et al., 2009). Therefore, CWs transform different contaminants via a combination of biogeochemical mechanisms (Kadlec and Wallace, 2009a). In particular, biological activity potentially influences contaminant removal, thank to microbial biofilm development within root zone, which offer attractive degradation zone for a wide range of contaminants such as nitrogen (Vymazal, 2010). While vegetation is contributing in N removal via direct uptake, plants can boost biofilm establishment with bioactive exudates by promoting mosaic structure of aerobic/anaerobic zones within rhizosphere (Coban et al., 2015b). Nowadays, CWs have many applications, including water and wastewater treatment from different sources such as domestic sewage, urban stormwater, agricultural wastes, industrial effluents and landfill leachate (Nahlik and Mitsch, 2006; Mitsch et al., 2008; Borne et al., 2013; Rahman et al., 2014; Lu et al., 2015). Removal capacities of these systems for different contaminants such as total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), heavey metals, nitrogen (N), and phosphorus (P) have been reported (Vymazal, 2007; Kadlec and Wallace, 2009a; Ilyas and Masih, 2017; Wang et al., 2017). For instance, the removal of N varied between 40 – 55 % with removed load ranging between 250 and 630 g N m^{-2} yr^{-1} depending on CWs type and inflow loading (Ilyas and Masih, 2017).

1.5.1 *Designs and Operational Conditions of CWs*

Based on the water flow regime and the type of macrophytic growth, CWs can be classified to two main types: free water surface constructed wetlands (FWS CWs) and subsurface flow constructed wetlands (SSF CWs) (Figure 1.3) (Hammer, 1989). FWS CW is an open basin characterized with the shallow flow of water (less than 0.4 m) over saturated substrate similar to natural wetlands. In SSF CW, an excavated basin or impermeable plastic container, where the water is transported within the porous medium of planted bed (Fonder and Headley, 2011). Based on the feeding mode of water flow, SSF CWs are typically subdivided to horizontal flow (HSSF) and vertical flow (VSSF) (Chang et al., 2014b). Some CWs are hybrids of the above designs that comprise a combination of different wetland systems for treatment of wastewater. This model usually

includes more than one stage of different parallel constructed wetlands in series (Vymazal, 2013). Enhanced CWs represent the most recent design of constructed wetlands proposed to improve the performance of wastewater treatment system (Wu et al., 2015a). In the next sub-sections, the focus will be given to describe the basic designs of the CWs with particular emphasis on the application of FWS CWs in wastewater treatment.

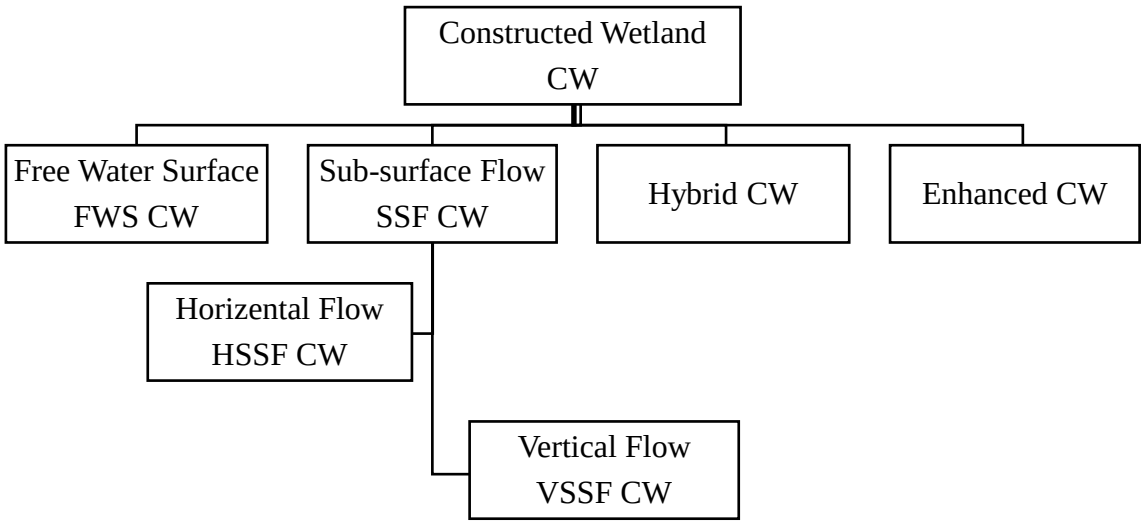

Figure 1. 3 Classification of constructed wetlands, (Kadlec and Wallace, 2009b).

1.5.1.1 Free Water Surface Constructed Wetlands (FWS CWs)

Free water surface wetlands are the simplest design and the most commonly type of the constructed wetlands applied around the world (Hammer, 1989). The FWS CWs have been created using different construction designs and with different operational characteristics, i.e. with different depths, surface areas, water quality, hydraulic and mass loads, and with or without vegetation (Bastviken et al., 2009). Typically, FWS CWs comprise of a shallow sealed basin or series of basins with water depth ranges between 0.2-0.4 m, where the water flow is passing through horizontally across the wetland surface similarly to a natural wetland (Figure 1.4). The most common application for FWS wetlands is for advanced treatment of effluent from secondary or tertiary treatment processes (e.g. lagoons, trickling filters, activated sludge systems, etc.) (Kadlec and Wallace, 2009a). FWS wetlands are the nearly exclusive choice for the treatment of urban, agricultural, and industrial stormwaters, because of their ability to deal with pulse

flows and changing water levels (Kadlec and Wallace, 2009a). Generally, FWS CWs have lower N removal efficiency than other types, however these systems promote sedimentation, filtration, oxidation, reduction, and assimilation as principal mechanisms for contaminant removal using surface re-aeration as O_2 source for biological reactions (Zhou et al., 2010). The role of microbial population setteled in these systems regards the most important factor influencing N removal. Most bacteria are associated with solid surfaces, where biofilms responsible for microbial processes, rather than free-floating within the water column (Hammer, 1989). Nitrification and denitrification are regard the main NH_4^+ removal processes, while volatilization at high pH values represents the principal sink for NH_3. Direct uptake of N by plants is considered as temporal storage as the assimilated amounts could be released to the water after the plant decay (Vymazal, 2010).

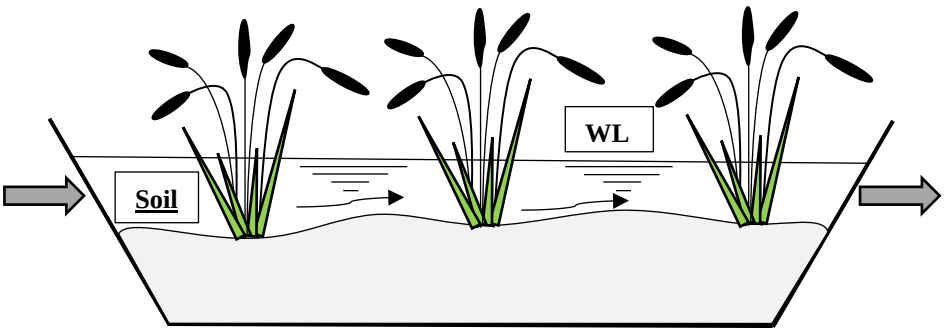

Figure 1. 4 Schematic representation of Free Water Surface Constructed Wetland, (Von Sperling, 2007). WL: Water level.

1.5.1.2 Sub-Surface Flow Constructed Wetlands (SSF CWs)

Horizontal sub-surface flow wetlands are comprising of a shallow system (0.5-0.8 m), where the water continuously flow from the inlet horizontally through filtration bed of the wetland to the outlet (Figure 1.5a) (Vymazal, 2013). Typically, the design includes sealed system with soil or gravel-based bed planted to support emergent macrophytes (usually *Phragmites australis*). The system design allows to the flow to pass through entire bed medium in a plug-flow mode (Kadlec and Wallace, 2009a). Compared to the FWS CWs, biochemical reactions in the HSSF CWs are limited by the oxygen influxes from the plant roots (Brix, 1997). Since the majority of the transferred O_2 into root zone

is used by roots and rhizomes themselves for respiration, ammonium oxidation via nitrifying bacteria is limited (Wu et al., 2014). However, switching of aerobic and anaerobic activity by facultative heterotrophic bacteria could explain the robust removal efficiency of organic-N within HSSF root zones (Vymazal, 2005).

In vertical flow wetlands (Figure 1.5b), the system consists one or more filter layers of porous media with total depth ranged between 0.6-1.0 m, where the flow percolates down as intermittent batch fed (Vymazal, 2010). Typically, porous layers are consisting of sand or gravel bed planted with wetland vegetation. Compared to HSSF CWs, vertical systems were designed to provide higher levels of oxygen transfer, thus promoting higher rates of nitrification, but provide only partial denitrification (Kadlec and Wallace, 2009a).

The baic types of CWs are not necessarily operate as stand-alone treatment systems, but they can combined with each other as hybrid systems or even with other high-tech or low-tech wastewater treatment units to achieve specific performance advantages.

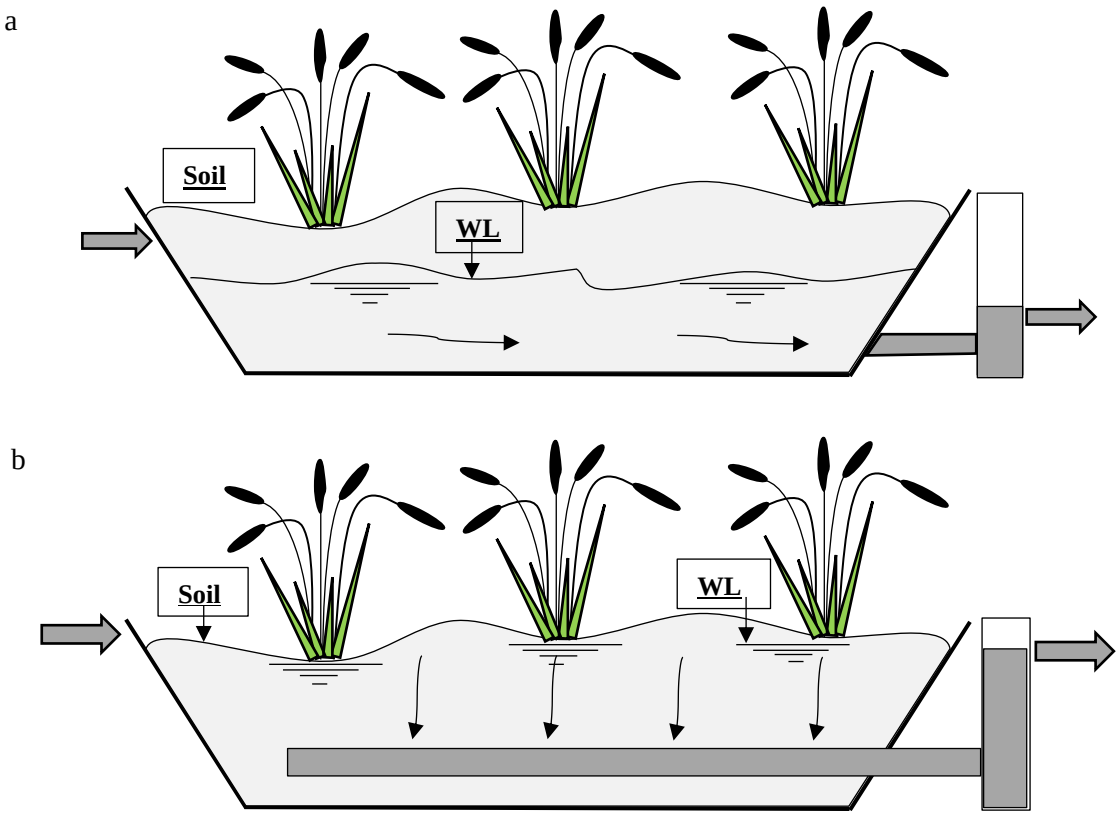

Figure 1. 5 Schematic representation showing variants of Sub-Surface Constructed Wetlands: (a) Horizontal Flow Sub-Surface Constructed Wetland; (b) Vertical Flow Sub-Surface Constructed Wetland, (Von Sperling, 2007). WL: Water level.

1.5.2 *Role of Key Design Criteria*

Probably the most important design and operational parameter for optimising performance of a wetland system is Hydraulic Retention Time (HRT). The typical values of HRT for free water surface ranging from 5 to 14 days and for subsurface flow systems between 2 and 7 days (Kadlec and Wallace, 2009b). In UK systems, it was estimated that the surface area required for secondary sewage treatment is usually 5 m^2 per P.E., while it was 1 m^2 $P.E^{-1}$ for tertiary treatment (Cooper and Green, 1995). Therefore, in order to optimise systems performance in treating wastewater efficiently, the configuration of the systems should be such that it minimises channeling and short-circuiting and maximises contact between the wastewater and substrate (Annamraju, 2000). In this regard, aspect ratio (length to width) plays a potential role. Although early designs were based on larger aspect ratios (4:1 to 10:1) as they promote longer HRT for the processes to take place, it is now though that aspect ratios of 1:1 could be more appropriate because they offer reduced construction costs and improved hydraulic control (Buchberger and Shaw, 1995; Parkinson and Tayler, 2003). Although, porosity criteria of the systems usually range from 30% for coarse gravels to 45% for clay and silt, however isolation from the groundwater by means of plastic linear is necessary to prevent groundwater contamination on the one hand, and to avoid groundwater infiltration on the other hand (Vymazal, 2013). Both fluxes can substantially influence the HRT and therefore the treatment performance. Most UK and European subsurface systems are built to a depth of 0.6 m at the inlet, with outlet being slightly deeper to fit the slope (Vymazal, 2005). Such established criterion was based on a believe that beyond this depth plant roots may start to weaken, whereas shallow systems may suffer from freezing under such cold climate (Brix, 1997). The cross-sectional area of the bed for a given flow is determined by the bed hydraulic gradient and hydraulic conductivity, both being essential parameters in the design of the subsurface constructed systems. An appropriate choice of the filter material is important to avoid clogging, to ensure a sufficient hydraulic conductivity and to provide enough sorptive capacity. The European Guidelines suggest that hydraulic conductivity should be around 10^{-3} m s^{-1} (Brix et al., 2007). The slope of the bed is usually between 0.5 and 2% (Hammer, 1989). In addition to the hydraulic parameters and geometric configurations, the plant species is based on range of criteria. They should firstly be able to flourish under the local climate conditions. A high biomass production is preferable when one intends to export nutrients from the system by harvesting. The

more extensive the root system, the better the filtrative capacities and the more surface is available for biofilm development. Finally, they should be able to withstand hydraulic and pollutant shock loads (Kadlec and Wallace, 2009b).

1.5.3 *Constructed Wetlands for Wastewater Treatment*

Constructed wetlands are being increasingly used for water quality improvement during wastewater treatment (Andersson et al., 2005; Matamoros et al., 2017; Weedon, 2017; He et al., 2018). This is, in part, a result of stricter water quality standards which mean that conventional secondary treatment may not always be sufficient to comply with targets in receiving waters (Mulder, 2003; Wu et al., 2015b). Several hundred wastewater systems employ treatment wetland technology in the UK (Weedon, 2003), where they are recognised by the Building Regulations (Ministry of Housing, 2010) and are under consideration as the main wetland approach acceptable to the government pollution regulator (Weedon et al., 2017). Thousands now exist across Europe (Brix et al., 2007). Free water surface wetlands were widely used to complement conventional wastewater treatment worldwide (Matamoros and Salvado, 2012; Tuszynska et al., 2013). In the UK, tertiary sewage treatment by wetlands has used subsurface horizontal flow treatments, first introduced in the mid-1980s by the statutory water companies (Jewell, 1999). The vertical flow approach was introduced by private individual to serve a small community (Burka and Lawrence, 1990). However, ease of maintenance and lower capital cost have been asserted worldwide as favouring free water surface wetlands over subsurface systems. These systems provides a potentially effective buffer between tertiary wastewater treatment plants and natural waterways (Kadlec and Wallace, 2009a). Such function can be traced back to the complex combination of physical, chemical, and biological processes for contaminant removal brought about by the setteled microorganisms, vegetation, and soil matrix, as well as their interactions with each other (Zhang et al., 2015a). According to Zhou et al. (2010), there are four main functions of these systems which make them potentially attractive for wastewater treatment: dispersion of surface waters over a large area; physical sorption of pollutants onto surface areas of the wetlands soils and organic litter; uptake and metabolic utlisation by plants; transformation and utilisation of the elements by microorganisms. Cumulative results of several decades of experiences with these systems have confirmed relative capabilities in remediating different natural and anthropogenic contaminants, e.g. nutrients, haeavy metals, organic maters, suspended solids, etc (Andersson, 2005; Svedin et al., 2008;

Chen, 2011). FWS CWs can remove different contaminants such as TSS (86 %), BOD (82 %), and COD (70 %) through settling, filteration, and more importantly microbial pathways (Kadlec and Wallace, 2009a; Wang et al., 2017). However, these systems often show limited capacity for N, which typically ranged between 40 – 50 % with ammonia removal of 57 % (Vymazal, 2007).

1.5.4 *Disadvantage of Conventional Constructed wetland Systems*

The accumulated experience from the work with the conventional constructed wetlands has revealed an inherent limitations. There is still lack of understanding of the extent to which different treatment proccesses (e.g. microbial transforamtions, plant related processes, algal uptake, and physicochemical processes) influence the pollutant removal, and therefore the optimal design and operating criteria have not been established (Hammer, 1989; Brix et al., 2007; Kadlec and Wallace, 2009b; Vymazal, 2013). For instance, removal efficiencies of FWS CWs are often limited by a high ratio of water volume to biofilm surface area. In another word, limited surface area for growth of fixed microbial biofilms as well as the lack of direct contact between plant roots in the sediment and the water column often limit system performance (Stewart et al., 2008; García-Lledó et al., 2011). The majority (81 – 95%) of microbial biomass in wetland systems is located near root zones and solid surfaces that serve as habitats of microorganisms, rather than in the water column (Truu et al., 2009). The development of a microbial biofilm is therefore fundamental to many pollutant removal processes including organic compounds subject to biodegradation (Imfeld et al., 2009) and ammonia which is converted to nitrite and nitrate by specialized bacteria (Truu et al., 2009). One way in which this can be solved is to increase the biofilm-water column contact area and plant root densities in the water column. Floating Treatment Wetlands (FTWs) are designed to overcome these limitations by providing additional surface area for microbial growth and the establishment of plants, and thereby promoting pollutant removal.

1.6 Floating Treatment Wetlands (FTWs)

Floating Treatment Wetlands (FTWs), also called Constructed Floating Wetlands (CFWs) are variants of constructed wetlands (Benvenuti et al., 2018). FTWs share, with conventional soil-based CWs, the use of helophytes and are similar to treatment ponds as they have an extended water body without phytoplankton, which is dominating ponds (Karstens et al., 2018).

FTW consist of a water system with a buoyant structure called floating island, which is usually constructed from synthetic or natural materials and which has a high submerged surface area upon which microbial biofilms can develop (Headley and Tanner, 2008; Chang et al., 2013) (Figure 1.6). They also provide a platform for the hydroponic growth of plants, which can enhance nutrient removal via uptake directly from the water column (Tanner and Headley, 2011; Borne et al., 2013; Lynch et al., 2015). The platfrom typically comprised of materials such as wood, plastic, inorganic matting, or fiberglass (Lucke et al., 2019). FTWs commonly use rooted, emergent macrophytes including various sprcies of *Carex*, *Phragmites*, *Juncus*, *Vetiveria* and *Baumea* plants (Headley and Tanner, 2012; Pavlineri et al., 2017b). The upper parts of the vegetation grow and remain primarly above the water level, while the roots extend down in the water column, developing an extensive beneath water-level root systems (Hubbard, 2010; Fonder and Headley, 2013). The development of an extensive and dense root system is crucial for the performance of the system. Biofilm is attached on the roots, rhizomes and raft, and as physical and biochemical processes take place, the system functions as a natural filter (Li and Li, 2009).

FTWs simulate the naturally occuring water treatment processes that take place in natural floating wetlands islands (Christopher et al., 2017). Natural floating wetlands develop when large quantities of floating organic matter, sediment and wetland plants combine to form a buoyant island (Yeh et al., 2015). This organic layer is an ideal medium in which rooted macrophyte plant species can grow. Natural Floating Wetlands occur in a variety of water bodies. Along the shorelines of lakes, ponds, and estuaries, terresterial vegetation may proliferate and eventually begin to extend to the water surface by means of floatation. Eventually, the un-rooted vegetation loses the connection with their terresterial communities and becomes a free floating wetland (Headley and Tanner, 2006).

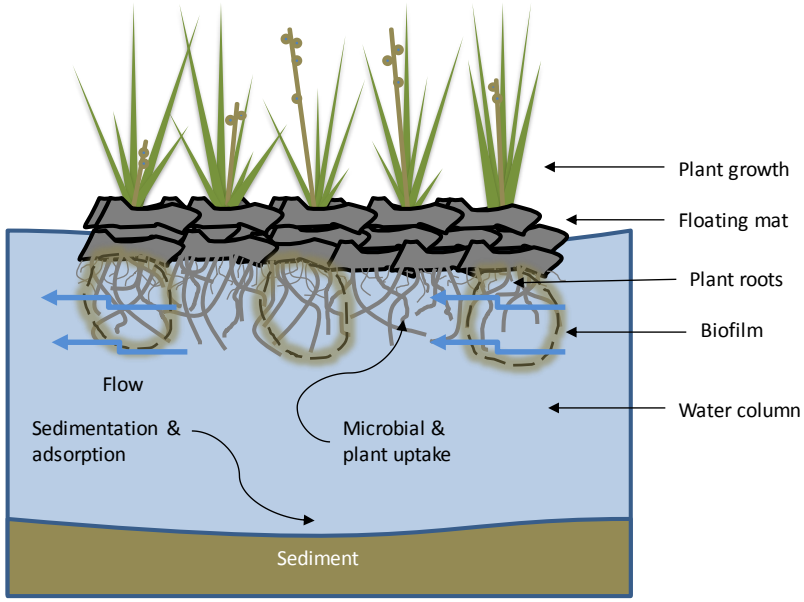

Figure 1. 6 Cross section of Floating treatment wetland.

Given the vast surface area available for biofilm growth on the plant roots and floating mat material, FTWs have the potential to provide significantly greater pollution removal rates per unit area compared to other treatment systems such as conventional constructed wetlands and bioretention basins (Nichols et al., 2016). In contrast to FTWs, bioretention basins and constructed wetlands generally function best when they are located "offline". This means that flows above a certain rainfall event are bypassed, or only minor flows are diverted into a system (Lucke et al., 2019). The design objective of offline systems is to ensure that extended detention depths within the pond (i.e. flood storage) are minimised and high runoff flow volumes are directed away from (bypass) the systems to avoid potential damage (Nunes et al., 2012). This often requires the construction of additional detention or retention basins, or large bypass channels which are separate from the treatment systems. The construction of this additional infrastructure can have significant costs and land requirements (Lu et al., 2015). Further, macrophytes in conventional constructed wetlands are susceptible to submerged condition during flood events. Such damaged plants may experience chronic dieback and release nutrients into water bodies (Headley and Tanner, 2006). FTWs, from another hand, are not affected by increases in extended detention levels as the buoyancy of these systems allows them to rise and fall to compensate for any changes in water levels that may occur, e.g. during large storm events or high inflow rates (Headley and Tanner, 2006). This important and distinguishing feature of FTWs can significantly reduce the area required by CWs in

urban areas with space limitation, as well as being aesthetically pleasant (Burgess and Hirons, 1992; Yao et al., 2011; Lu et al., 2015).

Most conventional treatment wetland systems can only be installed and made operational once the urban developments are nearing completion. This is to ensure that they are not damaged during construction activities, including being inundated by sediment-laden construction runoff, In contrast, FTWs can be installed at the start of the construction phase which means they have the ability to treat the initial construction runoff as well as urban inflow (Nunes et al., 2012; Lucke et al., 2019). The maintenance of FTWs is generally easier to accomplish than conventional constructed wetlands. First, macrophytes may be harvested annually to enhance pollutant removal efficiency and avoid releasing nutrients back into the environment due to plant senescence (Chen et al., 2009). Second, sediments stored in the system can be easily degrade without excessive damage to the plants (Wang and Sample, 2014).

While nutrient removal performance and cost are obviously key bottom line considerations, as we look to the future, much greater consideration of the energy sources would be a major concern for scientists and engineers (Shilton, 2005). Energy expenses of FTWs are significantly lower than conventional engineering-based practices, because FTWs are driven by solar power (Todd et al., 2003). Todd et al. (2003) reported that energy requirements of a poultry processing plant dropped 74% after converting a sequencing batch reactor to an FTW system. However, there is still lack of cost data for FTWs in the literature.

As well as improving performance, FTWs also have the potential as a source for renewable energy and a as tool to reduced climate change. Harvested vegetation grown on FTWs can be used as a source for biofuel production (Cohen et al., 2012; Soda et al., 2013), and may act as a sink for greenhouse gases such as CO_2 via photosynthesis (de Freitas et al., 2015; Lazar et al., 2014).

1.6.1 *Applications of FTWs*

Constructed Floating Wetlands have been used internationally for over two decades in a variety of aquatic enhancement projects. For examples, FTWs can be applied to habitat restoration projects because well-functioning floating wetlands are isolated, secure, and attractive to many species and thus help increase the biodiversity of local ecosystems (Kato et al., 2009). Kerr-Upal et al. (2000) reported a variety of macroinvertebrate

including shredders, grazers, and collectors have been found in artificial floating islands. More fish and shrimp were found under the floating islands than in nearby open water locations. Cherry and Gough (2006) stated that floating islands may replenish seed banks and maintain populations of otherwise uncommon plant species. However, the main purpose of FTWs application is to improve water/wastewater quality. FTWs are considered as Best Management Practices (BMPs) or Low Impact Development (LID) that are testing at different scales and gaining increased popularity across the world (Chang et al., 2014a). These uses include improving the quality of airport runway runoff (Revitt et al., 1997); treating wastewater and sewage discharges(Van de Moortel et al., 2010; Headley and Tanner, 2012; Ijaz et al., 2015); improving agricultural and farming runoff (Stewart et al., 2008; Hubbard, 2010); improving the quality of mine tailings water (Yang et al., 2006); and reducing algal blooms (Song et al., 2009).

In terms of its potential in removing different contaminants during water purification, FTW performance has been evaluated in a number of experimental (Keizer-Vlek et al., 2014; Saad et al., 2016; Abed et al., 2017) and operational (Chang et al., 2013; White and Cousins, 2013; Nichols et al., 2016) settings and they have been shown to effectively enhance the removal of different contaminants such as TSS, zinc, and copper by 41, 40, 39%, respectively (Borne et al., 2013); phosphorus by 47% (Chang et al., 2013); nitrogen by 72% (Lu et al., 2015).

Due to the differing experimental approaches adopted in previous research studies, as well as variation in climate and selected pollutant analytes, a direct comparison of the performance of FTW systems is challenging. Most of the research focused on the removal efficiency of different contaminants, including nitrogen. For instance, Hartshorn et al. (2016) investigated the nutrient, microcystin and chlorophyll interactions with three stormwater detention ponds fitted as FTWs in North America. This study analysed water quality to estimate the nutrient removal efficiencies of FTWs. The coverage of the three systems was between 5 and 7 %. The contributing catchments for the three FTWs comprised of a combination of residential, commercial and forest areas. Manual water sampling from the inlet and outlet was undertaken both during the inter-event dry periods. Although, there was improvement in nitrogen removal within the FTWs, the findings have not determined how much of the measured nutrient removal could be directly attributed to the performance of the floating structures rather than water bodies themselves. A further Australian field study was undertaken by Christopher et al. (2017),

who assessed the treatment performance of FTWs in treating stormwater runoff from an existing urban development. The contributing catchment of the development site treated by FTW was 7.46 hectare (2% coverage area) containing residential development under construction. The two-years field study used automatic water samplers installed at the inlet and outlet of the FTWs to enable the stormwater treatment performance of the FTWs to be evaluated during storm events. A range of analytes were measured for all water samples, including TSS, TN, TP, TKN, NH_4^+, NO_2^-, and NO_3^-. Additionally, particle size range, Particle size Distribution for each water sample were also undertaken in order to determine whether the FTWs removed more of any particle sediment. A clear reduction in contaminants concentrations, particularly for nitrogen, was observed in the FTWs. Although the findings allowed the treatments performance of the buoyant infrastructures of the FTW to be differentiated from the overall detention basin performance, the experimental design could have been improved having an identical control treatment area without floating material. Again, this could allow the treatment performance of both systems to be accurately measured and quantified.

It is appearing from the literatures that the most researches adopted a black box approach to quantify treatment performance of the examined systems through measure inlet-outlet concentrations. However, limited attention was dedicated to understanding the contribution and integration of the removal processes within these systems. This uncertainty requires further investigation to facilitate using FTWs for water/wastewater purification.

1.6.2 *Design Parameters of FTWs*

The most important design and operational criteria of FTW performance are the plants used and their root development, coverage ratio, water depth and hydraulic performance and system buoyancy.

1.6.2.1 Plants Used and Root Development

A number of emergent water plant species have the potential to form floating islands in nature (Glen, 2005), and many of them can grow successfully as self-buoyant hydroponic root mat or on rafts with the aim to improve water quality. In order to select plant species for FTW application, Wang and Sample (2014) established the following criteria: (1) native and non-invasive species; (2) perennial plants; (3) terrestrial plant species; (4) wetland plants or plants with ability to thrive in a hydroponic environment; and (5) plants

with aerenchyma. The capability of these plants to establish a dense, submerged network is one of the most critical criteria for FTWs. Further, water quality and climate conditions are determining which plant species need to use for FTWs. As in soil-based CWs, various species of *Cana*, *Typha*, *Cyprus*, *Carex*, *Juncus*, and *Phragmites* are the most common species employed in FTWs (Kadlec and Wallace, 2009b). These species are characterized by greater biomass, longer and faster growing root systems, and large aerenchyma tissues in their roots and rhizomes, which improve their ability for buoyancy (Dushenkov et al., 1995). However, most studies on floating plantation have been made on herbaceous species with shorter root longevity (on average 40 days), and characterized by high transpiration and root oxygen loss rates (Lai et al., 2011). The fibrous roots of these plants are relatively thin (< 3 mm) and characterized by relatively high porosity (10-33%) which make them more efficient to remove total nitrogen (Li et al., 2013). Different factors can influence root development, e.g. plant species, plant age, nutrient concentration, redox conditions of the water, and the use of supporting mats or rafts (Chen et al., 2016). Despite root development is specially influenced by trophic status of the water, a high nutrient load can be harmful, in particular to young plants, when it leads to accumulation of sulphide generated under anaerobic conditions (Lamers et al., 2013).

Plant species selection is critical, not only to pollutant removal, but also to the local ecosystem integrity. Successful biomass establishment seems to linearly and predominantly correlate to nutrient removal rates (Bu and Xu, 2013).

1.6.2.2 Coverage Ratio

The coverage percent of the buoyant system, and therefore the shading area, strongly influence the dissolved oxygen and light penetration into the water column, and therefore controls FTW performance (Hubbard, 2010). Reaeration by atmospheric oxygen is substantially eliminated by vegetation cover (Zhou and Wang, 2010). Further, the growth of vegetation prevents light penetration in the water column, which leads to decline in the population of photosynthetic organisms, but improve microbial biofilm of non-photosynthetic bacteria attached onto root system under shaded condition (Lembi, 2001; Headley and Tanner, 2012). The coverage ratio is a parameter that varies greatly throughout the studies. Many studies have used 100% coverage ratio (Sun et al., 2009; Zhou and Wang, 2010; Xian et al., 2010), while others used 50% (Borne et al., 2013), or even less than 20% (Li et al., 2010). Most of these studied reported a positive relationship

between coverage percentage with removal efficiency of nutrients. For example, Van de Moortel et al. (2010) reported high reduction of total nitrogen from sewer overflow water with the increased raft coverage (50 and 100%) planted with *Carex acutiformis* compared with an unplanted control.

Removal of a contaminants, e.g. nitrogen, is typically associated with a specific microbial functional group, and the microbial community can be influenced by the presence of plants (Faulwetter et al., 2009). The plant-microbial interaction can be described as roots and attached biofilm interact with each other, where plant roots supply microbial population with carbon source essential to degrade contaminants, while associated microorganisms provides essential nutrients required for plant growth and development (Khan et al., 2013a). Differences in the composition of the heterotrophic microbial community originate largely in the spatial dynamics of oxygen and carbon availability throughout the plant root mat and can have effect on the removal processes for different contaminants (Chen et al., 2016). A significant source of organics matter in wetland systems comes from rhizodeposition products, which are especially important for microbial denitrification and sulphate reduction of low-organic-carbon loaded wastewater (van Oostrom, 1995). In addition to increase catabolic rates, a higher microbial taxonomic diversity in the root zone than in the water bulk has likely a greater catabolic range, and the microbial community might be more resilient and resistant towards disturbances (Tanaka et al., 2012). In eutrophic reservoir, ammonia oxidising bacteria (AOB) and archaea (AOA) were found around the roots of floating macrophytes where the relative abundance of these two microbial groups differed with various macrophytes (Zhang et al., 2015b). The ratio of AOA to AOB may be used as microbial indicator to determine the condition of oligotrophic wetlands, and their relative abundance is important for nitrification (Sims et al., 2013; Meng et al., 2014).

Therefore, floating mat coverage, including vegetation, of FTW can change the environmental condition (dissolved oxygen, light penetration, carbon releases etc.) and further influence the microbial community established in the root zone and subsequently leading to affect treatment processes of different contaminants.

1.6.2.3 Water Depth and hydraulic performance

The water level, system permeability and hydraulic characteristics are very important parameters when using FTWs. The choice of the water level in the FTW systems is vital

both for providing an adequate root cover over the water column, and for preventing the plants from anchoring, and thus, losing the privilege of water fluctuation resistance (Pavlineri et al., 2017b). Also, selection and maintenance of proper water depths depends on the treatment purpose, the inflow variations and the wastewater types (Chen et al., 2016). For example, in case of low water depth, which prevents the buoyancy of the root mats, the system can function like a filter that is more suitable for the removal of fine particles and dissolved pollutants ensuring a much more direct contact between plant roots, wastewater and microbial biofilm (Chen et al., 2012). However, when a high water depth is maintained, the plant root mats are floating and a free water zone is formed below the mat down to the bottom of the water body, the system can function similar to the treatment pond for remediating water with a high content of coarse suspended solids by sedimentation (Headley and Tanner, 2012). Although that some wetland plants are sensitive to water level fluctuations, species such as *Typha spp., Phragmites australis, Cyprus spp., Juncus spp., Phalaris arundinacea* show a high morphological adaptation to water-level variation (Bonilla-Warford and Zedler, 2002).

Water permeability of the FTWs can be influenced by design, e.g. geometrical dimensions (length, width and depth); the location of inlets and outlets; and their orientation regarding the dominant wind direction as well as the vegetation (Jenkins and Greenway, 2005). For instance, a good hydraulic performance could be obtained in shallow basins (0.1-0.15 m), where aerobic conditions are dominated (Chen et al., 2015). Further, the hydraulic characteristics of the system can be influenced by vegetation coverage. The hydraulic efficiency (ratio of the time of the peak outflow concentration to the nominal residence time) of the system becomes double with full plant coverage (Persson et al., 1999). The free zone between the floating plant roots and the bed of the system will allow more laminar flow conditions as well as avoiding re-suspension of settled particles to take place (Chen et al., 2016). However, the flow behaviour in the FTWs usually showed pronounced dispersion (i.e. less ideal flow) presumably because of the heterogeneity in root density and its depth distribution (Seeger et al., 2013). Eventually, the hydraulic performance in the FTWs was found to be depending on the position, size and placing arrangement of the floating mats and the inlet arrangement (Khan et al., 2013b; Lucke et al., 2019).

1.6.2.4 System Buoyancy

Natural floating wetlands are found worldwide in various climates and settings (Kadlec and Wallace, 2009b; Tsujino et al., 2010). Self-buoyance in natural systems is derived from the oxygen gas stored in the plant roots and other gasses associated with the decomposition of organic matter (Nichols et al., 2016). An important factor for self-buoyancy is the aerenchyma in many helophytes, which creates air spaces especially within the rhizomes (Hogg and Wein, 1988a). Entrapped gases under the root mats such as methane and nitrogen as final products of methanogenesis and denitrification are also important for floatation (Hogg and Wein, 1988b). A number of alternative technologies have been tested to ensure the buoyancy of the floating frame, and patented mats are commercially available. The buoyancy of the medium is achieved through various means such as foam injection, or the use of hollow, sealed components (Lucke et al., 2019). However, a cheap and effective alternative for frame construction includes naturally buoyant materials, e.g. bamboo and coconut coir bed (Pavlineri et al., 2017b). The use of peat as matrix, however, is not recommended because it settles, does not support plant growth and causes a high own oxygen demand which can affect the plants (Chen et al., 2016). Seo et al. (2013) indicated that buoyant or substratum material should be hydroponic, as such materials enhance rapid bacterial adhesion and absorb nutrients, while desorption is almost negligible.

1.6.3 *Nitrogen Removal Mechanisms in FTWs and Influencing Factors*

FTWs for N removal combines several different mechanisms including microbially-mediated transformations on the mat and the bed, and banks of the wetland, plant uptake, volatilization, sedimentation, algal/bacterial assimilation in the water column, and adsorption to settled material (Headley and Tanner, 2012; Wang and Sample, 2014).

1.6.3.1 Role of Microorganisms

Most wetland reactions are microbially mediated (Kadlec and Wallace, 2009b). Most microbes including nitrifiers, are often found in close-knit communities encased in an extracellular matrix and attached to a surface, forming what is known as a microbial biofilm (Karimi et al., 2015). Therefore, the microbial biomass tends to grow as fixed biofilm more than suspended flocs (Headley and Tanner, 2006). In an attached growth system, this process is also called a fixed film process in which the individual bacteria are immobilized (Chen et al., 2006). In this way, it is possible to obtain greater microbial

biomass in the system to undertake contaminant removal efficiently. Moreover, fixed biofilm can contain a higher concentration of active biomass in which the substrate gradients of the biofilm can result from the development of variant microbial populations (Safwat, 2018). Therefore, as the film builds up, diverse habitats are provided for different transformation processes of contaminants (Shahot et al., 2014). Biofilm kinetics can be complex. The substrate supply into the layer-like aggregation of the bacterial film is a diffusion-controlled process driven by the concentration gradient across the biofilm (Chen et al., 2006). The increase of biofilm surface area enables contaminant to pass over the media and increase the volume of the substrate that can be adsorbed from the influent (Maksimova, 2014; Shahot et al., 2014). This increases the removal efficiency of contaminants. Floating matrix is fundamental in FTW, as most biochemical processes take place within the mosaic structure of the aerobic and anaerobic zones (Tanner and Headley, 2011). The entire underwater surface of the floating mat serves as a potential habitat for different microbial populations (Tanner and Headley, 2011). Nitrogen removal is mainly accomplished by mineralization, nitrification, and denitrification (Rousseau et al., 2004). Wetlands systems are often highly buffered which keeps the pH in the neutral range (6-8) where microbial activity is optimal (Hammer, 1989). Metabolic pathways can also be affected by the prevalent redox potential within the biofilm, oxygen inputs, and the availability of the other electron acceptors (Coban et al., 2015b). For instance, plant roots can support settled microbial populations via various exudates, maintaining a mosaic of zones of aerobic and anaerobic processes (Faulwetter et al., 2009; Rajkumar et al., 2010).

1.6.3.2 Role of Vegetation

Plants can affect N removal by seasonally storing and releasing nitrogen in a “flywheel” effect (Kadlec and Wallace, 2009b). If biomass is removed, this can be regarded as a permanent sink. Vegetation growing in FTWs may be more effective in removing N directly from water column than those in bed sediment of traditional wetlands because they grow hydroponically (Wang and Sample, 2014). Root systems also provide mechanical support for microbial community attachment, enhancing biofilm development (Bissegger et al., 2014). Plant root systems also help regulate the microbial community structure and function within the surrounding rhizosphere (Bissegger et al., 2014). Plant roots can transfer oxygen from aerial tissues into the rhizosphere, and thus create conditions favorable for nitrification (Bastviken, 2006).

Up to 25% of total photosynthetic carbon can be secreted as root exudates, which are used by heterotrophic bacteria as a carbon source for denitrification (Sim, 2003).

Although the potential for macrophytes to reintroduce nitrogen back into the water column is a long-standing controversy in aquatic ecology (Benvenuti et al., 2018). Nevertheless, combinations of plant and biofilm mechanisms have been shown to remove the majority of nitrogen during wastewater treatment (Kadlec and Wallace, 2009b).

1.6.3.3 Physicochemical Processes

Abiotic responses in the nitrogen cycle include advection with running water, ammonia volatilization, sedimentation, and ammonium adsorption (Vymazal, 2010). Volatilization requires high pH so that a significant fraction of ammoniacal N is in the unionized form. This mechanism includes (i) diffusion of NH_3 to the air-water interface, (ii) release of NH_3 to the air at the interface, and (iii) diffusion of NH_3 from the air-water interface into the air above (Kadlec and Wallace, 2009b). Ammonia volatilization is thought to be a minor loss mechanism under non-alkaline waters and soils (Reddy and Patrick, 1984). This is because < 1% of total ammonia is present as NH_3 at pH values < 8, however, volatilization may affect > 1% because NH_4^+ will continuously convert to NH_3 to replace the NH_3 lost to volatilization (Poach et al., 2002). High density of hanging root systems may act as filters to capture suspended particles and encourage sediment settling by slowing water flows (Headley and Tanner, 2006). Elevated of litterfall coupled with low velocities promote deposition of N-containing materials, increasing burial and accretion processes (Bastviken, 2006; Coban et al., 2015b).

1.6.4 *Nitrogen Treatment Performance Assessment in FTWs*

Several studies have attempted to quantify the treatment performance of FTWs in removing nitrogen, using different removal pathways (Faulwetter et al., 2011; Ijaz et al., 2015; Vázquez-Burney et al., 2015; Nichols et al., 2016; Urakawa et al., 2017). However, there is still relatively little information on the specific mechanisms involved (i.e., CWs are often regarded as black boxes) (Rousseau et al., 2004). Better design to enhance the performance of FTW systems can only come from a better understanding of the processes operating. This section provides a review of approaches to describe FTW processes with the aim of providing further insights into their performance.

1.6.4.1 First Order Kinetic Models

Many individual wetland removal processes, can be described using first-order kinetics (Andersson, 2005; Kadlec and Wallace, 2009b).

$$J = k.C \qquad (1.9)$$

where J is the rate of nitrogen removal (g m^{-3} day^{-1}), k is the rate coefficient (day^{-1}), and C is the concentration of nitrogen (g m^{-3}). k can represent several different 1st order processes:

$$k = k_{vol} + k_{nit} + k_{up} \qquad (1.10)$$

where k_{vol} is rate constant of NH_3 loss via volatilization (day^{-1}), k_{nit} is rate constant of NH_4^+ removal via nitrification (day^{-1}), and k_{up} is rate constant of N removal via plant uptake (day^{-1}).

First order models can also be area dependent, where removal is estimated per unit wetland area (Kadlec, 2000).

$$J_A = \frac{k.C}{Z} \qquad (1.11)$$

where J_A is removal in g m^{-2} day^{-1} and Z is the average depth of the wetland (m).

The solution of the 1st order equation can be written (Gajewska and Skrzypiec, 2018):

$$C_{out}/C_{in} = exp^{(-kt)} \qquad (1.12)$$

Where C_{in} and C_{out} are the inlet and outlet concentration in a steady state wetland system, and t is the hydraulic retention time (day).

One drawback of the 1st order approach is that some nitrogen removal processes may not follow 1st order kinetics (e.g., some processes are known to follow zero-order or mixed order kinetics) (Bastviken, 2006).

It is clear that the individual components of any chemical mixture, e.g., TN, within multimedia environments may be degraded at different rates, and that there will be a corresponding difference in removal rate constants (Kadlec, 2003). Therefore, there is a need for models that can provide predictions complex processes in a multimedia environment (Wynn and Liehr, 2001). Modeling approaches based on 1st order kinetics to simulate multiple removal mechanisms include that of Wang and Sample (2013). Here, a variety of processes are represented including water body reaction, the reactions associated with mat, and settling of sorbed material during sediment deposition processes (Wynn and Liehr, 2001).

$$C_{iFTW} = C_0 e^{\left(-k_w + v_f\left(\frac{\gamma}{H}\right)\right)t} = e^{-t(k_w + k_m)} \quad (1.13)$$

where C is concentration at time t (mg N L^{-1}), C_0 is concentration at time 0, k_w is an overall 1st order rate constant for the water column including settling (day^{-1}), k_m is a 1st order rate constant for the mat (day^{-1}, i.e., $k_m = v_f.\ \gamma/H$), v_f is FTW apparent uptake velocity (m day^{-1}), γ is FTW coverage area (m^2), H is water depth (m). In this model, the function of the mat is separated from other processes going on within the water body.

1.6.4.2 System Dynamics Models

FTWs are complex multimedia environments in which a number of physical, chemical and biological processes interact. 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 framework for integrating the combined effects of several different interacting processes (Forrester, 1961; Sterman, 2001; Matinzadeh et al., 2017). Such models describe the interaction between different entities in a system, in an attempt to mimic the various removal processes operating in natural systems (Xuan et al., 2012b). They provide a framework for thinking for about system complexity and behavioral feedback (Wolstenholme, 2003). When there is an agreement between modeled predictions and observations, our existing understanding is reinforced. However, the poor agreement can challenge existing understanding and lead to new insights. Thus, system dynamics models facilitate the analysis of the complex system and provide more reliable outcomes. Such approaches have been employed to address a variety of biological, ecological sciences including agricultural management (Ouyang et al., 2010; Ouyang et al., 2012; Matinzadeh et al.,

2017) and stormwater management (Chang et al., 2014a; McAndrew and Ahn, 2017b). Mayo and Hanai (2014), for example, applied a system dynamics model to improve the knowledge of the nitrogen behavior in a free water surface stabilization pond receiving wastewater. The model was used to explore the contribution of transformation processes in removing N and showed that microbially mediated processes contribute to 69.1% of all losses, followed by volatilization (23.8%), and sedimentation (7.1%). However, few investigations have evaluated the performance of FTWs for wastewater treatment using such an approach.

1.6.5 *Disadvantages of FTWs*

As with any new treatment technology, there are number of limitations that should be assessed prior consideration of FTWs for use in full-scale applications. These limitations include some physical, chemical and biological limitations that should be take into account in order to optimise treatment performance and minimise any negative impacts as much as possible.

1.6.5.1 Physical Limitations

Floating infrastructure and buoyancy could be regarded the most physical limitations of FTWs. Like other buoyant objects, floating mat are very susceptible to strong waves, that could seriously damage the structure (Wang and Sample, 2014). Further, there is often a risk of biomass accumulation that may exceed the buoyancy provided by floating mats. Such limitation could be solved by reasonable prediction of the maximum biomass during design stage.

1.6.5.2 Chemical Limitations

Chemical properties of water can be influenced by the presence of the floating mats. Low dissolved oxygen (DO) was often observed within water column in the FTWs (Headley and Tanner, 2006; Tanner and Headley, 2011). Such phenomenon could be explained as a consequence of a lack of atmospheric diffusion of oxygen due to abstraction by floating mats as well as insufficient photosynthetic activities due to the shading effects of mats. However, some literatures reported high DO levels in FTWs, particularly with high vegetation density, where high root oxygen releases could occur compared to diffusion from air side (Nduwimana et al., 2007; Van de Moortel et al., 2010).

1.6.5.3 Biological Limitations

Water purification efficiency may be limited by the performance of vegetation and microorganisms. The presence of different contaminants at high levels within the water body of FTW could influence treatment performance. For example, oil present in the polluted urban run-off and wastewater discharges may pose great challenges to biological treatment systems. It is reported that different plant species (e.g. *Typha orientalis*, *Angelonia goynazensis* and *Celosia argentea*) were severely damaged by oil spills from domestic wastewater and some recreational activities, where the root systems was coated with oil and therefore results in high mortality rates of these species (Mitsch et al., 2008).

The choice of plant species for FTW and other practices is not only critical for the contaminant removal but also for system integrity. If invasive species were introduced, these plants could form monotypes and significantly affect biodiversity, ecosystem functionality and human uses of the affected environments (Zedler and Kercher, 2004). Although some invaders can enhance FTW nutrient removal, through high uptake rate and rapid growth (Sheley et al., 2006), the negative impacts on the ecosystem or the costs of habitat restoration may be more significant.

1.7 Summary and Current Research Gaps

Nitrogen is a fundamental component of biosphere and the most abundant form at the atmoshere. Though the eco-benificial role of nitrogen, the negative consequences of accelerated fluxes of this element to the environment are substaintial and manifold, ranging from eutrification, ecosystem acidification, and water quality deterioration. Because of the coexistence of benificial and deterimental impacts, a betttere understanding of N cycle processes is required to develop efficient mitigation stratigies sustaining water quality. In the recent decades, constructed wetlands (CWs) are being increasingly used for water quality improvement during wastewater treatment (Matamoros and Salvado, 2012). This is, in part, a result of increasingly strict water quality standards which mean that conventional secondary treatment may not be sufficient to comply with targets in receiving waters. CWs are engineered ecosystems that exploit different natural remediation mechanisms from biotic and abiotic components for water quality improvement (Hammer, 1989; Vymazal, 2010). These systems have been created with different designs including free water surface constructed wetlands (FWS CWs), and subsurface flow constructed wetlands (SSF CWs) (Hammer, 1989; Kadlec

and Wallace, 2009a). FWS CWs, the most common design, have been used worldwide as a tertiary polishing stage in wastewater treatment, and their performance in removing nitrogen were evaluated (Stone et al., 2002; Andersson et al., 2005; García-Lledó et al., 2011; Wu et al., 2015a). One advantage of FWS CWs is that they are inexpensive to operate and maintain. However, inadequate surface area for microbial growth for a given volume and the lack of direct contact between water column and plant roots are deemed critical limitations in their performance for removing nitrogen.

To tackle these limitations, one possible solution is the floating wetland technology. Floating treatment wetland (FTW) is an eco-engineered system, that integrate the functions of the FWS CW and floating treatment island for water quality improvement (Headley and Tanner, 2012). Increasing decontamination surface areas and direct contact with contaminants as well as harnessing range of biogeochemical processes such as microbial transformations, plant uptake, and physical filteration to eliminate contaminants are the central mechanisms of applying FTWs (Tanner and Headley, 2011; Headley and Tanner, 2012; Keizer-Vlek et al., 2014). FTW has, therefore, been developed as an lternative and effective wetland design for watre-wastewater quality improvement.

Although, there are some studies have been dedicated to evaluate FTWs performance in removing nitrogen from different sources, however information about the functionality of these systems in domestics wastewater treatment is limited. The relative contribution of different processes to overall losses of nitrogen is poorly documented. Further, the stat-of-the-art of FTWs performance in removing ammonia, as integral part of N cycle, still consists of black-box approaches, whereby the inherent complexity of these systems is entirely neglected. Specifically, the kinetics of ammonia removal under different design characteristics are not well understood. There is still lack of understanding of the extent to which different treatment proccesses (e.g. microbial transforamtions, plant related processes, algal uptake, and physicochemical processes) influence the ammonia removal, and therefore the optimal design and operating criteria have not been established yet. Therefore, it is critical to understand ammonia removal processes under different design criteria in order to determine critical design that influence ammonia removal and thus optimise system performance.

1.8 Research Strategy

The intent and focus of this research is to investigate research gaps regarding performance of FTWs as tertiary treatment for domestic wastewater. The work reported in this thesis integrates three scaled up studies within the domain of FTW technology. These include (a) microcosm experiments which focuses on the effect of biofilm surface area associated with floating matrix as a key design factor on ammonia removal and nitrification kinetics. Well controlled bench-top treatments with different mat surface areas, ammonia concentrations, and aerations were constructed to examine process controls. (b) A mesocosm experiment which focuses on the effect of some design criteria on the relative contribution of different ammonia removal processes. Mesoscale treatments under open field conditions with different surface area of mat material, plant density, and water depth were constructed to understand the magnitude of different processes and to identify the most critical design for ammonia removal. (c) A pilot-scale study which focuses on evaluating the effectiveness of a critical FTW design in removing ammonia from domestic wastewater under different operational conditions. A field system of treatment chambers with different operational volumes were employed for polishing final effluent from small sewage treatment plant. The research strategy of this work is based on the integration of scaled-up methodology, where the findings from a simple experimental design are used as a platform for designing more complex systems with the aim of optimising treatment performance of ammonia. A critical FTW design form well-controlled lab experiments is scaled up to less controlled field studies to optimise system performance in removing ammonia. widening the application of elected design to larger scales may help to establish optimal design and operation criteria of FTWs. In addition, upscaling the knowledge of ammonia behaviour from different experimental systems has the potential to improve understanding of ammonia fate in FTW systems. Also, this can be a useful approach to confirm the kinetics of ammonia removal derived from systems examined. Kinetic parameters of ammonia removal can be chosen based on through process analysis in the experimental systems. Removal rate constants of nitrification (k_{nit}), plant uptake (k_{up}), and volatilization (k_{vol}) are chosen as kinetic parameters to compare treatment efficiency of ammonia under different design criteria. Since FTW is a complex multimedia environment where various biogeochemical processes are interacted, the contribution of different ammonia removal mechanisms can be assessed most effectively via application of numerical models.

A model-based analysis can provide an interpretive framework for integrating the combined effects of different interacting processes. In this research, an attempt to quantify the contribution of different ammonia removal processes and complex interactions using combined efforts of both experimental-based process analyses and model-based analyses rather than classical approach that rely only on experimental process analyses. For this, this project employed system dynamics approach as a framework for robust understanding of the treatment mechanisms and system performance in removing ammonia. A reliable mechanistic understanding of the treatment mechanisms of ammonia is fundamental to improve design and operation criteria in FTW systems.

1.9 Research Aim, Objectives and Hypotheses

The overall aim of this research is to develop a mechanistic understanding of ammonia removal in FTW systems to improve design and operation.

This will be achieved by meeting the following objectives:

1) To investigate kinetics of ammonia removal in FTWs under different design criteria using scale-up experimental approach.
2) To determine most critical FTW design for ammonia removal.
3) To evaluate treatment performance of a critical FTW design in removing ammonia from domestic wastewater under different operational conditions.
4) To improve understanding of the treatment processes and system performance in treating ammonia using system dynamics modelling.
5) To use the measured kinetics parameters from small scale FTWs to confirm kinetic parameters derived from field scale.

The hypotheses tested are:

1) Nitrification rate is directly and linearly proportional to the submerged mat surface area (which is assumed to function as a habitat for nitrifying bacteria).
2) Higher ammonia removal is associated with higher plant density (which will control uptake rates and support microbial community in the rhizosphere with carbon and oxygen releases).
3) Reaction rate constants of ammonia removal are inversely proportional to water depth (i.e., nitrification rate is assumed to be higher by reducing water depth as redox potential tend to be higher in shallower systems because the ratio of the air-water interface area to volume is higher).
4) A critical FTW design can enhance ammonia removal in a pilot-scale system receiving domestic wastewater discharge under field conditions
5) Supplementing a well-controlled laboratory study with field study could confirm kinetics parameters derived from laboratory data.

1.10 Thesis Structure

This thesis is comprised of five further chapters, summarised as follows:

Chapter 2 (Materials and Methods): General methods that are used in all the results chapters are described here. This including, sampling strategies, analytical methods, calculations of performance and removal kinetics, general model description and statistical analyses. Specific details of the individual experiment's designs are described in the corresponding Results chapters.

Chapter 3 (1st Results Chapter): This chapter devoted to process analysis in order to understand ammonia dynamics under controlled conditions. Experimental data from laboratory experiments conducted under different design characteristics were used to quantify the potential of microbial transformation in ammonia removal. The effect of different of mat surface area, aeration and ammonia concentration were examined. Alongside with experimental data, A model-based analysis was employed to improve mechanistic understanding of ammonia removal. Data were used to support Objectives 1, 2 and 4.

Chapter 4 (2nd Results Chapter): In this chapter, a mesocosm experiment was conducted outdoors. The focus was to quantify the magnitude of different removal mechanisms of ammonia under the effect of different design parameters and to determine the most critical design for treatment performance. The role of some design factors such as water depth, floating mat area, and plant density in altering ammonia removal were examined. All mesocosms were operated under approximately steady state conditions using raw tap water amended with ammonia concentration. Process-based modeling was used to describe N dynamics in each experimental treatment. Data are used to address research objectives 1, 2 and 4.

Chapter 5 (3rd Results Chapter): Experimental data were obtained from pilot-scale FTWs used to evaluate the performance of a critical FTW design, scaled up from the mesocosm study, in treating ammonia from domestic wastewater from a small sewage treatment plant serving a hotel complex. The kinetics of the ammonia removal with two different operational volumes with and without floating islands were also assessed. The treatment chambers were operated under batch-mode (i.e., static) conditions. Again, the behavior N species was analyzed using the modeling approach. This work addresses research objectives 3 and 4.

Chapter 6 (Discussion): In the final chapter, the data from the Results chapters is compared and discussed. Some general conclusions are presented along with some suggestions for future research.

Chapter Two - Materials and Methods

2.1 A note on nomenclature

Ammonia nitrogen (NH_x) is used here to denote the nitrogen present as sum of the ammonium (NH_4^+) and free (unionized) ammonia (NH_3). The ratio of the two forms is pH and temperature-dependent. The fraction of NH_x which is unionized increases with increasing pH (Poach et al., 2002). Oxidized nitrogen (NO_x) is used here to denote the sum of the nitrite (NO_2^-) and nitrate (NO_3^-). Total inorganic nitrogen (TIN) is the sum of NH_x and NO_x. Total nitrogen (TN) represents the sum of the total Kjeldahl-N (TKN-N) and NO_x.

2.2 Sampling Strategy

2.2.1 Water Samples

The design of the sampling regimes, preservation, and handling of samples was carried out in accordance with EPA and APHA standards for water quality sampling (EPA, 1993; APHA, 2005b). Aqueous samples were collected using an auto-pipette equipped with disposable plastic tips. Triplicate composite samples were made up using 15- and 50-mL conical centrifuge tubes (Thermo Fisher scientific Inc., USA) (Figure 2.1). All samples were filtered using a syringe filter with a pore size of 0.45µm before analyses (Cheeseman et al., 1989). Depending on the sample volume, samples for ON, NH_x, and NO_x were immediately preserved by adding 0.025 or 0.1 mL of concentrated sulfuric acid (98 %) (Table 2.1a). All samples were refrigerated at 4 °C. Since preservation for NO_2^- is not possible, samples for NO_2^- were frozen at -20 °C prior to analysis (Cheeseman et al., 1989). During transportation, samples form field systems were stored in a cool box with ice packs to maintain the temperature.

2.2.2 Biomass Samples

Nitrogen uptake during the study period was quantified by biomass measurements and tissue analysis for total-N content. Plant biomass was collected for each of the vegetated mats (n = 3 for each species) at the start and end of experimental time. Sampling was performed with three replicates of *Juncus effusus* and *Phragmites australis* using dark packs to prevent any photosynthesis during transportation and storage.

The root system was differentiated from shoots and rinsed carefully with deionized water to determine initial and final biomass and TN content (Figure 2.1b). All plant samples were frozen at -20 °C before analysis.

For microbial analysis, water, mat material, and plant root samples were collected to determine the change of microbial abundance. Water samples (50 mL) were taken at 15 cm from the surface using an auto-pipette equipped with disposable plastic tips. Solid samples were handled gently to avoid distributing attached biofilm and other material prior to placement of mat material and root specimens into sterile dilution water (Figure 2.1c). Sampling was always performed in triplicate using 50 mL conical centrifuge tubes (Thermo Fisher Scientific Inc., USA). All samples were transported and stored at 4 °C. During transportation, sample containers were covered with aluminum foil to eliminate any possibility of algal growth and stored in a cool box with ice packs.

Figure 2. 1 Sampling strategy: (a) Water, (b) plant biomass and (c) biofilm samples.

2.3 Analytical Methods

2.3.1 Water analysis

The analytical data presented in this thesis rely considerably upon the use of an automated multi-chemistry discrete analyzer (AQ2: SEAL Analytical, UK, Figure 2.2) to assess N concentrations in the samples (Figure 8). It is, therefore, appropriate to explain the scientific basis of this method.

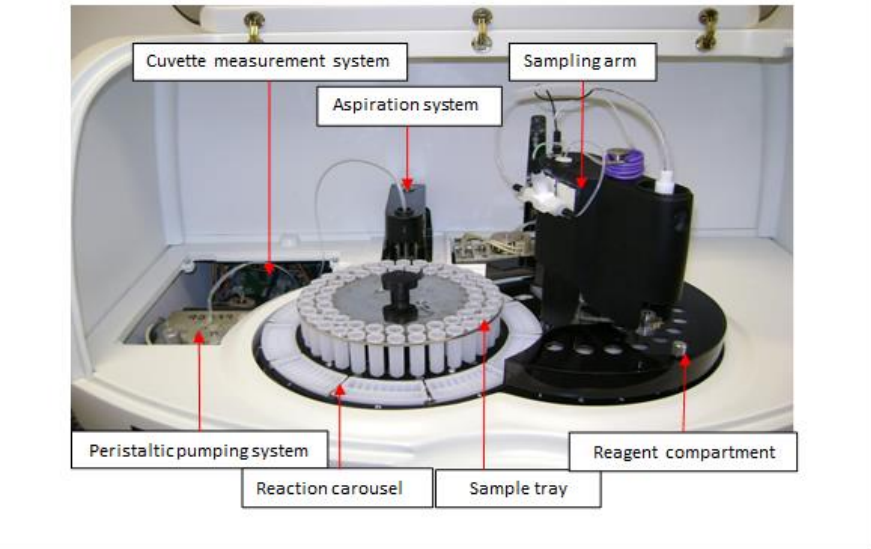

Figure 2. 2 AQ2 Main System Components.

The AQ2 analyzer is an automated colorimetric system. The main components of this instrument are reagent and sampling stations, reactional carousel system, aspirator, and photometer. Sample and reagent are picked up and mixed in a reaction well (which is heated at 37 ºC to ensure stable reaction conditions irrespective of fluctuating ambient conditions) for a chemical reaction using a diluter system. A wash station washes the robotic probe after every liquid contact to prevent cross-contamination. Once the reaction time is complete, the reaction mixture is aspirated into the cuvette using a peristaltic pump, where the appropriate filter is selected, and absorbance readings are taken. When applicable, auto-dilution is performed to ensure the accuracy of the detection within the calibration range. The cuvette is comprehensively washed between aspirations to minimize carryover. The absorbance data is then used to calculate concentrations in the samples based on a calibration curve. Specialized software controls the testing process (SEAL Analytical, 2014).

Details of the methods employed are shown in Table 2.1. All methods comply with Standard Methods for Examination of Water and Wastewater (APHA, 2005b) and US EPA Methods (EPA, 1993). Standard quality control procedures for sampling, sample preservation, and handling were adopted to safeguard the accuracy of the results. All analyses were carried out in triplicate. For Quality Assurance/Quality Control (QA/QC), laboratory blanks and quality control solutions of known concentration were included in each batch of analyses.

Table 2. 1 Summary of wastewater quality parameter collection and analysis.

Constituents	Preservation	Laboratory testing methods
Ammonia-N	H_2SO_4 (< 2 pH), <4°C	AQ2 method No: EPA-153-A Rev. 2.0 (SEAL Analytical, 2015)
Nitrite-N	Freezing 20°C	AQ2 method No: EUR-608-A, Rev.1.0 (SEAL Analytical, 2013a)
Nitrate-N	H_2SO_4 (<2 pH), <4°C	Ultraviolet spectrophotometric screen method (APHA, 2005b)
Nitrate+Nitrite	H_2SO_4 (< 2 pH), <4°C	AQ2 method No: EUR-616-A (SEAL Analytical, 2011)
Organic-N	N/A	Calculated as TKN-ammonia (Kadlec and Wallace, 2008)
Total Kjeldahl-N	H_2SO_4 (< 2 pH), <4°C	AQ2 method No: 111-A Rev. 5.0 (SEAL Analytical, 2013b)
Total inorganic-N	N/A	Calculated as ammonia+Nitrate+Nitrite (Kadlec and Wallace, 2008)
Total-N	N/A	Calculated as TKN+ Nitrate+Nitrite (Kadlec and Wallace, 2008)

A brief description of the reaction principal for each colorimetric analysis is described in the following subsections.

2.3.1.1 Determination of total ammonia-N (NH_4^+-N+NH_3-N)

The alkaline salicylate method with hypochlorite and sodium nitroprusside (MDL: 0.02 mg N L^{-1} (range: 0.2 – 10 mg N L^{-1}) was followed to determine total ammonia-N in the aqueous samples (SEAL Analytical, 2015). At alkaline pH, NH_4^+ and NH_3 in the aqueous sample react with hypochlorite ($HClO^-$), previously liberated from dichloroisocyanurate (Table 2.2). The chloramine formed then reacts with salicylate, at a pH of at least 12.6, in the presence of nitroferricyanide. During static incubation at 40ºC, a blue-green indophenol dye forms, which is measured photometrically at 660 nm. This method is equivalent to USEPA method 350.1 Rev.2.0 (EPA, 1993).

2.3.1.2 Determination of Nitrite-N (NO_2^--N)

The sulphanilamide reaction in the presence of N-(1-naphthylethylenediamine) dihydrochloride method (MDL: 0.0015 mg N L^{-1} (range: 0.01 – 0.15 mg N L^{-1}) was followed to determine nitrite-N in the aqueous samples (SEAL Analytical, 2013a). Nitrite reacts with Sulphanilamide (Table 2.2) to form a diazonium compound which, in dilute phosphoric acid, couples with N-(1-naphthylethylenediamine) dihydrochloride to form a reddish-purple azo dye. This species is measured photochemically at 546 nm or 520 nm. This method is equivalent to the ISO 13395-E standard method (International Water Quality Standard, 1996).

2.3.1.3 Determination of Nitrate-N (NO_3^-)

An ultraviolet spectrophotometric screen method 4500-NO_3^- was followed to determine nitrate-N in the samples (APHA, 2005b). Measurement of UV absorption at 220 nm enables rapid determination of NO_3^- for filtered and acidified (1 N HCl) water samples. Empirical correction of the NO_3^- absorbance values against associated dissolved organic matter was used by making a second measurement at 275 nm. Using corrected absorbance, NO_3^- concentrations were obtained directly from a standard curve using KNO_3 standards.

2.3.1.4 Determination of total oxidised-N (NO_2^--N+NO_3^--N)

Nitrate is chemically reduced to nitrite by alkaline hydrazine sulphate, in the presence of copper (II) (MDL: 0.03 mg N L^{-1} (range: 0.2 – 5 mg N L^{-1}) (SEAL Analytical, 2011). Aqueous samples are an incubated in an alkaline solution of hydrazine sulfate, with Cu (II) present for catalysis, to reduce nitrate to nitrite (Table 2.2). Nitrite originally present, plus nitrite produced by chemical reduction, react with sulphanilamide to form a diazonium species. This precursor, in dilute phosphoric acid, couples with N-(1-naphthyl)-ethylenediamine dihydrochloride to form a reddish-purple azo dye. This compound is measured photometrically at 546 nm or 520 nm. This method is equivalent to standard methods 4500-NO_3 H (21^{th} Ed.) (APHA, 2005b).

2.3.1.5 Determination of Total Kjeldahl Nitrogen-N (TKN)

Kjeldahl digests (copper catalyst) are reacted with salicylate in the presence of hypochlorite and sodium nitroprusside (MDL: 0.07 mg N L^{-1} (range: 0.2 – 4.0 mg N L^{-1}) (SEAL Analytical, 2013b).

The total Kjeldahl nitrogen (TKN) method demands sample digestion prior to testing with the discrete analyzer (EPA, 1993). Here, an AIM600 digester block was used for digestion of TKN samples. The main units of the instrument are a digestion block, a programmable controller, a set of digestion tubes, tube rack, and cooling stand. The digestion block contains 50 wells for placing 100 mL glass sample tubes. The programmable controller is used to control digestion temperature and the time needed for the specified method.

In the digestion process for TKN, 25 mL samples and 5 mL of digestion reagent were poured into 100 mL Kjeldahl flask with boiling stones. Samples were heated at 160 ºC for 1 hour and 380 ºC for 30 min (SEAL Analytical, 2013b). The residue from digestion is briefly cooled, diluted to 25 mL using ammonia-free water and placed in the discrete analyzer for ammonia determination. The analyzer mixes finished digest with complexing pH buffer to achieve alkaline pH without precipitation of calcium, magnesium or heavy metal species (Table 2.2). The released ammonia reacts with hypochlorite to form chloramine. Chloramine then reacts with salicylate, at a pH of at least 12.6 in the presence of nitroferricyanide to intensify the color reaction. During static incubation at 40 ºC, a blue-green analog of indophenol blue forms. The absorbance is measured photometrically at 670 nm. This method is equivalent to USEPA method 351.2, version 2.0 (EPA, 1993).

Table 2. 2 Specific reagents and standards for analyzed N parameters.

Constituents	Reagents and standards	Reagent-grade Chemicals
Ammonia-N	Sample matrix	
	Salicylate	Trisodium citrate dihydrate, Sodium salicylate, Sodium nitroferricyanide, Deionized water.
	Dichloroisocyanurate (DCI)	Sodium hydroxide, Sodium dichloroisocyanorate dihydrate, Deionized water.
	Ammonia top standard	Ammonium chloride anhydrous, Deionized water.
Nitrite-N	Sulphanilamide (NEDD)	Concentrated phosphoric acid, Sulphanilamide, N-(1-naphthylethylenediamine) dihydrochloride, Sodium nitrite, Deionized water.
	Nitrite top standard (1.0 mg NL^{-1})	
Nitrate-N+ Nitrite-N	Buffer, alkaline	Sodium hydroxide, Sodium phosphate dibasic, heptahydrate, Deionized water.
	Working hydrazine Sulfanilamide (NEDD)	Hydrazine sulfate stock, copper (II) sulfate pentahydrate stock, zinc (II) sulfate heptahydrate concentrated phosphoric acid, Sodium hydroxide, Sulphanilamide, N-(1-naphthylethylenediamine) dihydrochloride, Deionized water.
	Nitrate top standard (5-mg NL^{-1})	Sodium nitrate, Sulfuric acid, Deionized water.
TKN-N	Digestion solution	Potassium sulfate, Sulfuric acid, Copper (II) Sulfate pentahydrate, copper (II) sulfate
	TKN buffer	sodium phosphate, dibasic heptahydrate, Sodium hydroxide, Sodium potassium tartrate, Deionized
	Salicylate/nitoferri-TKN hypochlorite	Sodium salicylate, Sodium nitroferricyanide, Sodium hypochlorite, Deionized water.
	Ammonia top standard	Ammonium chloride, anhydrous, Sulfuric acid, Deionized water.

2.3.2 Biomass and Tissue Analysis

Root and shoot samples of *Juncus effusus* and *Phragmites australis* were dried (24 hr) to a constant weight using freeze drier (SciQuip Ltd, UK) to obtain the initial and final dry weights for biomass analysis. Nitrogen contents in both initial and final plant tissues were

analyzed. Dried samples of roots (including root hairs) and shoots were ground to pass through a 0.45 mm screen. Approximately, 3 mg of tissue was weighed in standard tin-capsules alongside reference samples (wheat flower standard-OAS-SERCON Ltd.) using a four-digit balance (Sartorius.co Ltd.). Total nitrogen concentration then was determined using an elemental analyzer: SERCON ANCA GSL according to an established protocol (Fry, 2006). All analyses were carried out in triplicate.

Samples of the microbial biomass of fixed biofilm from mat, roots, rhizomes as well as free suspended bacteria were analyzed via the total viable count method (TVC) (Yao et al., 2000; Truu et al., 2009). Samples were serially diluted in a sterile 1 mM phosphate buffer solution (PBS) before plating.

Floating matrix and plant root samples (1 g) were each mixed with 9 mL of PBS (4 mL of 0.2M K_2HPO_4 + 1 mL of 0.2M KH_2PO_4 per deionized water) and homogenized with a sterile glass rod. The mixture was vigorously shaken via vortex shaker for 2 minutes at 120 rpm. After that, the samples were sonified in an ultrasonic bath at 40 kHz for 20 sec. to detach microbial biofilm from the adsorbed surface (Kyambadde et al., 2006). Likewise, a diluent of 10^{-1} of water samples was performed using sterile PBS solution. 20 µL of diluted solutions was transferred to180 mL of PBS to make a 10-fold dilution. Dilution series for each sample up to 10^{-3} were made using 96 sterile micro-well plates. An inoculum of 50 µL was transferred to three replicate Petri-dishes containing autoclaved (121 °C for 15 min) nutrient agar. The plates were incubated at 37 °C for 24hr in a dark incubator (HERA CELL/Heraeus-UK). Plate counts are given as colony forming units (CFU) per gram for solid samples and aqueous samples as CFU/mL water (Yao et al., 2000; Hallberg and Johnson, 2005). All collected data were log transformed and expressed and presented as Log base 10 values of CFU.

2.3.3 Physicochemical measurements

At each sampling event, analyses of water quality variables (pH, DO, EC, and water temperature) were measured at 15 cm from the surface using portable pH/EC/DO/˚C meters (Hanna Instrument Inc., USA; Yellow Springs International Inc. Ohio, USA; Hach Co., India). All sensors were pre-calibrated using standard buffer solutions, and APHA (2005b) test methods were used.

Evapotranspiration was estimated using the Penman-Monteith approach (Allen et al., 1998) which was executed via the Wasim-ET Model-1.8 software utilizing daily

meteorological data collected at the University of Leicester-AWS-UoL (air temperature, humidity, sunshine hours, solar radiation and wind speed). Suitable season-dependent crop coefficients (K_c) were employed to estimate ET per each wetland cell (Allen et al., 1998):

$$ET_{cell} = \left[(1 - f) \times K_{c-open\ water} \times ET_{\circ}\right] + \left[f \times K_{c-Cattails} \times 0.7 \times ET_{\circ}\right] \qquad (2.1)$$

where ET_{cell} = evapotranspiration per wetland cell (L m^{-2} day^{-1}), f = fraction floating mat coverage area, $K_{c\text{-open water}}$ = crop coefficient (open water < 2 m depth = 1.05) (Allen et al., 1998), ET_O = reference evapotranspiration (L day^{-1}), $K_{c\text{-}cattails}$ = crop coefficient for wetland cattails and bulrushes (1.2) (Allen et al., 1998), $K_{c\text{-}cattails}$ = 0 in the case of Control, Multiplier for K_c reduction = 0.7.

2.4 Calculations of wetland performance and removal kinetics

2.4.1 Water and Mass Balance

In order to calculate an accurate mass balance of nitrogen entering and leaving examined systems an assessment of the change in water balance during the experimental period was needed. This was based on operating a water budget combined with local meteorological parameters (rainfall, evaporation and transpiration rates). Since the experimental systems were run under different operational conditions: continuous-flow in the case of the mesocosm experiment (chapter 4) and static batches in case of the pilot-scale study (chapter 5), a more extensive explanation on the water and mass balance methods is described in the relevant chapters.

2.4.2 Kinetics and Performance Evaluation

Ammonia removal in all three experiments was assumed to follow first order kinetics.

In first order kinetics (Lin et al., 2002; Finnegan et al., 2009):

$$C_t = C_i\, e^{-k.t} \qquad (2.2)$$

where C_t = concentration at time t (mg N L^{-1}); C_i = initial concentration (mg N L^{-1}); k = removal rate constant (k_{amo}, k_{nit} and k_{denit}, day^{-1}); t = reaction time (day).

In the mesoscale experiment, a continuously-stirred tank reactor approach (CSTRs) was used to represent mass changes between input and output under steady-state conditions. In the bench top experiment (chapter 3) and the pilot-system (chapter 5), static batches were studied over time. More details on the kinetics and performance evaluation methods used in both systems are presented in the following sub-sections.

2.4.2.1 Microcosm experiments

Removal rate constants for ammonia (k_{nit}) for different treatments were calculated as:

$$k = \frac{ln(C_{fin}/C_{init})}{t} \qquad (2.3)$$

where k = removal rate constant for NH_x (day^{-1}); $C_{init/fin}$ = initial or final concentration (mg N L^{-1}); t = reaction time (day). The reaction time for these experiments was simply the time from initiation to sampling.

The half-life (day) of the N-forms dynamic in the system were calculated as (Finnegan et al., 2009):

$$T_{1/2}\frac{\ln(2)}{k} \qquad (2.4)$$

where $T_{1/2}$ = half-life (day); k = removal rate constant for NH_x (day^{-1}).

2.4.2.2 Mesocosm system

In the mesocosm experimental system, removal rate constants (k) could be estimated by plotting $Q.(C_{in}\text{-}C_{out})$ versus $HRT.C_{out}$ based on equations (2.5-2.9). The removal rate of NH_x can be expressed as:

$$-\frac{dM}{dt} = Q_{in}.C_{in} - Q_{out}.C_{out} - k.C_{out}.V \qquad (2.5)$$

where Q_{in} and Q_{out} are the inflow and outflow discharge (L s^{-1}).

This assumes each mesocosm behaves as a CSTRs. At steady state, ammonia change within each mesocosm over time (*dM/dt*) is negligible. Equation (2.6) can, therefore, be rearranged as:

$$V.k.C_{out} = Q.(C_{in} - C_{out}) \qquad (2.6)$$

Assuming $Q = Q_{in} = Q_{out}$. This yields:

$$k = \frac{Q(C_{in} - C_{out})}{V\ .\ C_{out}} \qquad (2.7)$$

If the nominal hydraulic retention (*HRT*) time is defined as:

$$HRT = \frac{V}{Q} \qquad (2.8)$$

This then yields:

$$k = \frac{(C_{in} - C_{out})}{HRT\ .\ C_{out}} \qquad (2.9)$$

where k is removal rate constant for NH_x (day^{-1}), C_{in} is the influent concentration (mg L^{-1}), C_{out} is the effluent concentration (mg L^{-1}), *HRT* is the nominal residence time (day), Q is flow rate (L day^{-1}), and V is operational volume (L). This method has been widely adopted to simulate nutrient behavior in constructed wetlands and ponds (Gao et al., 2017).

The degradation half-life can also be calculated from k_{nit} using Equation 2.4.

The removal efficiency (*RE %*) of NH_x in each treatment was calculated based on (Wang and Sample, 2014):

$$RE = \frac{(C_{in} - C_{out})}{C_{in}} \times 100\% \qquad (2.10)$$

where $C_{in/out}$ (mg L^{-1}) is the inflow or outflow concentration of a constituent in each mesocosm tank.

2.4.2.3 Pilot-scale system

In the pilot scale system, calculated rate constants for ON, NH_x, and NO_x were estimated using a linear best fit (Kadlec and Wallace, 2009b). Optimize the fit between the model output and the observed data was by minimizing the root square error between observed and predicted N concentration. Half-lives (day) were calculated using equation (2.4).

2.5 Calculation of Biomass, Plant Uptake, and volatilization

All biomass data in chapters 4 and 5 are reported as dry weight (g). The above and below mat biomass was determined for each vegetated treatment at the start and end of the experiment. Total vegetation biomass was determined by multiplying measured biomass by the total number of plants. The average growth rate of plant biomass (g day^{-1}) for the entire study period was calculated for the two selected species by subtracting the initial plant biomass (g) for the 3 test replicates from the final biomass and then dividing by the number of days for the period of measurements (Keizer-Vlek et al., 2014). In order to determine the net growth of plant biomass during the experimental phase of the study, biomass at the start of experimental phase in each treatment was estimated by adding the calculated growth rates (g day^{-1}) of each treatment to the initial biomass value over the study period.

Dried tissue samples of above and below mat biomass taken at the start and end of the study were used to determine the total nitrogen content in selected species. Assimilated TN in the plant tissues throughout the study was determined based on (Tanner and Headley, 2011):

$$F = \left(\frac{N_r}{100} \times R_m\right) + \left(\frac{N_s}{100} S_m\right) \qquad (2.11)$$

where F = assimilated N mass (mg N); $N_{r/s}$ = N in root/shoot (%); R_m = root dry mass (mg N); S_m = shoot dry mass (mg N).

Accordingly, plant uptake rate (J_{up}) during the study are expressed:

$$J_{up} = \frac{F_{fin} - F_{init}}{t} \qquad (2.12)$$

Where J_{up} = plant uptake rate (mg N day^{-1}); $F_{init/fin}$ = initial and final TN content in plant tissue (mg N); t = time between sampling (days).

In order to determine the mass of N assimilated by plants during experimental phase, N-content at the start of experimental phase in plant tissues for each vegetated treatment was estimated by adding the J_{up} of each treatment to the initial tissue N-content over the study period.

Total established microbial biomass per gram of floating mat (matrix and root) was estimated as follows:

$$B = B_r + B_m \qquad (2.13)$$

where B = total microbial biomass per g of floating island (CFU g^{-1}); B_r = epiphyte microbial biomass (CFU g^{-1}); B_m = epimatrix biomass (CFU g^{-1}). Free bacteria within the water column were calculated directly using the culturable colonies on agar (CFU mL^{-1}). For simplification, all collected data for microbial biomass were log transformed and presented as Log base 10 values of CFU.

The mean change in of microbial biomass in each floating island was calculated by:

$$\mu_{max} = \frac{B_{fin} - B_{init}}{t} \qquad (2.14)$$

where μ_{max} = maximum growth rate of microorganisms per floating island (CFU g^{-1} day^{-1}); $B_{init/fin}$ = initial and final plant and microbial biomass per FTW (g); t = time between sampling (day).

The losses of ammonia-N via volatilization of unionized ammonia was estimated, assuming full and instantaneous thermodynamic equilibrium between NH_3 and NH_4^+ (Whelan et al., 2010):

$$C_{NH_3} = C_{NH_x} . f_{FREE} \qquad (2.15)$$

where C_{NHx} is the concentration of total ammonia N (mg L^{-1}), f_{FREE} is the fraction of NH_3 which is:

$$f_{FREE} = \left(\frac{1}{1 + 10^{(pKa - pH)}}\right) \qquad (2.16)$$

In which *pKa* is the temperature-dependent dissociation constant which was set at 9.24 (for system temperature of 25 °C) (Finnegan et al., 2009).

A combined mass transfer coefficient-k_T (m h^{-1}) can be calculated using the two-film resistance model (Kadlec and Wallace, 2009b):

$$\frac{1}{k_T} = \frac{1}{k_W} + \frac{1}{k_A . k_{AW}} \qquad (2.17)$$

In which k_A and k_W are partial mass transfer coefficients for the air side (m h^{-1}) and water side of the air-water interface (m h^{-1}), k_{AW} is the air: water partition coefficient for NH_3 (dimensionless Henry's law constant: 0.0007). Values for k_A and k_W were set at 1 and 0.01 m h^{-1}, respectively (Mackay, 2001).

The volatilization rate coefficient (k_{vol}, day^{-1}) was estimated by dividing k_T by water depth. The rate constant for the loss of ammonia N by volatilization of NH_3 is, therefore (Whelan et al., 2010):

$$k_{vol} = \frac{k_T}{Z} . f_{\frac{NH_3}{NH_4}} \qquad (2.18)$$

2.6 System Dynamics Modelling

System thinking concept is widely used to address and manage complex feedbacks systems (Forrester, 1961; Sterman, 2001). Based on system thinking, System Dynamics modeling is a well-established methodology designed to better understand complex management and dynamic behaviour of the systems via feedback process during the time (Xuan et al., 2012a). It uses software to map processes and policies at a strategic level, populates the map with data, and simulate the evolution of the processes under the

transparent assumption and scenarios (Wolstenholme, 2003). Every dynamic system is determined by interdependency, interaction and information feedbacks (Matinzadeh et al., 2017). The system dynamics approach provides a set of thinking skills (e.g., dynamic thinking, operational thinking, and quantitative thinking) required to understand system complexity, and a set of modeling tools used to integrate policies across system compartments where behavioural feedback is essential, and analyse variation (Wolstenholme, 2003). Therefore, processes in system dynmics approach are viewed in terms of unique causal loop diagrams and stock and flow diagrams to form a system dynamics model for applications (Forrester, 1968; Xuan et al., 2010). In system dynamics approach, modeling process consists identifying a problem and developing a dynamic hypothesis explaining the cause of the problem in model formulation, which then will be used to test a computational simulation model under alternative policies in the problem (Marimon et al., 2013). Dynamic hypothesis, a type of thinking skill, aims to explain system behaviour through hypothesizing feedback relationships and the causation of the seen behaviour using conceptual models (Richmond, 2004). System dynamic analysis method is based on the hypothesis of feedback processes obtained from exist observations, which are used to anticipate new behaviours in the future (Matinzadeh et al., 2017). In SD approach, feedback loops represent a sequence of the causes and effects in which change in one variable leads to the change in the loop, with a similar effect on the other variables (Saysel and Barlas, 2001). A system dynamics diagram can entail N-cycle and relevant processes with feedbacks, in which plus signs represent positive feedback and minus signs represent negative feedback in the system (Marimon et al., 2013). The causal loop diagrams (CLDs), as system modeling tools, are a combination of variables that are connected with each other by arrows, representing the causal effects between variables**.**

2.6.1 Model Description

Structural Thinking and Learning Laboratory with Animation-STELLA (ISEE systems, New Hampshire, USA) is a dynamic modeling software which is commonly used to simulate the behavior of dynamic systems. STELLA is essentially a programming environment that is specially designed to create and test system dynamics models with a proprietary "visual programming language" (McAndrew and Ahn, 2017b). The program uses inputs to construct a set of differential equations that are integrated over time using standard numerical techniques to describe the time behavior of chemical decay or enrichment in different pools (Bice, 2006). Rate coefficients were estimated by fitting (calibrating) the model to experimental data for a limited part of the data (e.g., one treatment). The key variables used in this study are state variables (Stocks): i.e., N-mass within a pool which is build up or depleted over time as input and output rates into them change; (Flows): i.e., exchange rates, which describe exchanges of N between pools. Flows contain differential equation controlling exchanges of N-masses between state variables using constants contained on converters. Convertors: building blocks holds values for constants. Connectors: a join among modeling features to transmit information and actions (Wolstenholme, 2003; Ouyang et al., 2010).

Conceptually, changes in state variables are controlled by changes in the flows. A conceptual diagram of the models used here to represent mineral N dynamics in the experimental systems is shown in Figure 2.3.

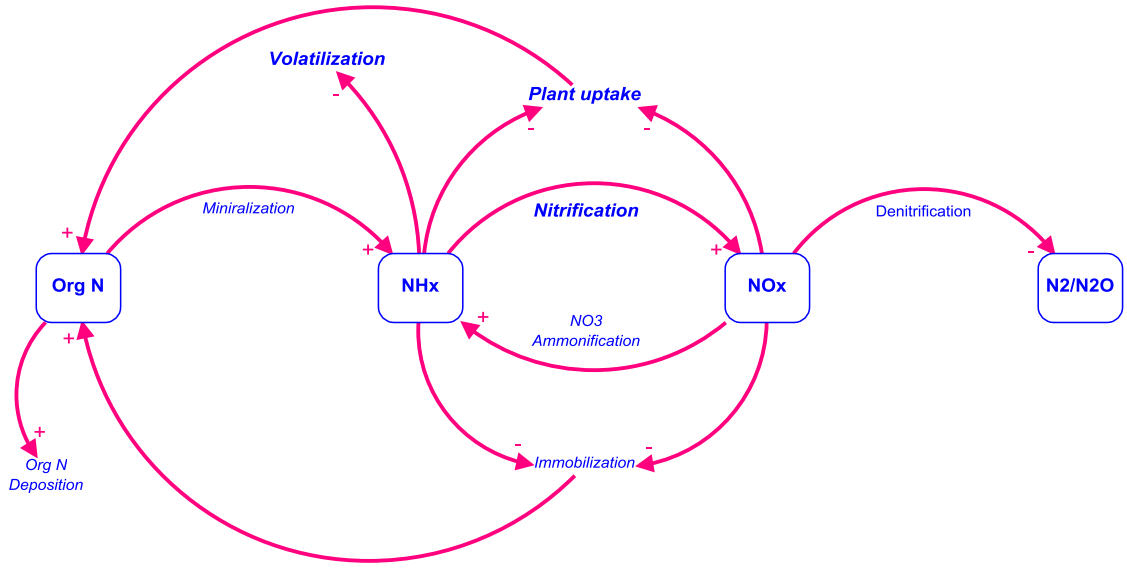

Figure 2. 3 Causal-loop diagram for the nitrogen cycle in the experimental systems under different operational conditions.

To understand how experimental systems behave over time, in terms N-cycle, two organic and inorganic pools are considered. The organic pool comprised an organic-N form, and the inorganic pool consisted ammoniacal-N and oxidized-N forms as well as gaseous N products. As nitrogen load was applied to the systems, the mineralization reaction of Organic-N could be taking place, increasing the NH_x formation in the water column, which, in turn, may promote higher immobilization rates to reform Organic-N in the system and the formation first positive loop. In the second positive loop, with the increase in the amount of immobilized Organic-N levels in the water table, the deposition of particulate organic matters to the bottom of the of the system is increased. The mineralized NH_x concentration in the system lead to increasing in nitrification reactions, and depleted ammonium form the system. Accelerated nitrification rates cause an increase in NO_x concentration in the water column; this, in turn still possible, leads to additional nitrate ammonification in the system bed to reduce NO_3^- to NH_4^+, and forming the third positive loop. With the access of NH_x concentrations into the system, the amount of the constituent taken up by the settled plants is elevated, causing to a reduction in the ammonium concentration in the water and the formation of the fourth negative loop. In the fifth loop, volatilization process might reduce NH_x accessed to the system; resulting in decreasing in free ammonia and the formation of the negative loop. In the sixth loop, by increasing NO_x concentration due to the elevated nitrification rates, further denitrification, which ultimately leads to a reduce NO_3^- to N_2, thereby forming a negative loop. In the seventh negative loop, by increasing of NO_3 levels in the water table, the plant uptake of nitrate is increased; thus, results in a reduction in the dissolved NO_3^- concentrations.

Figure 2.4 shows the modeling scheme for the three different experimental systems examined in this research (microcosm, mesocosm, and pilot-scale studies).

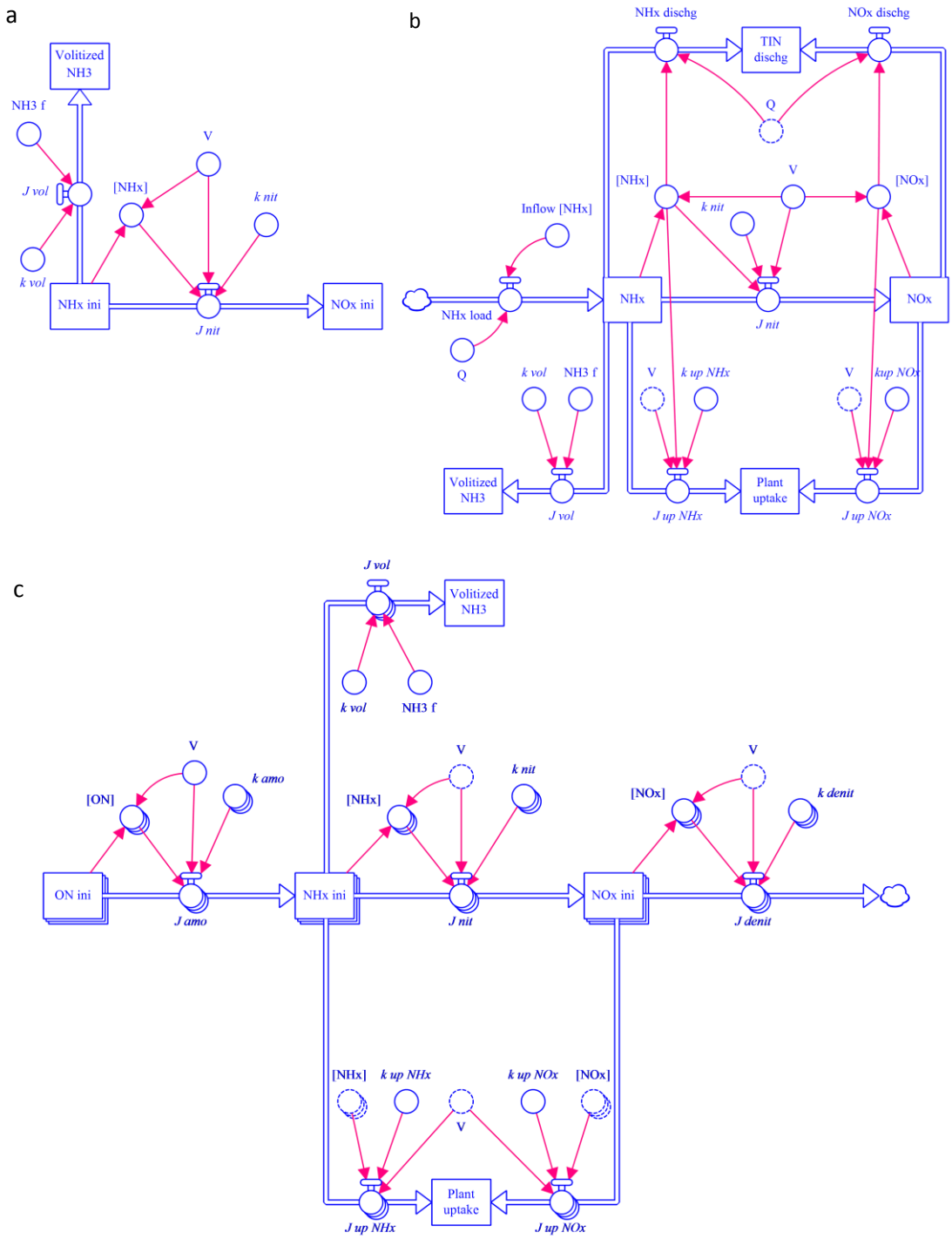

Figure 2. 4 The formulation of STELLA models for (a) the microcosm experiment (b) the mesocosms and (c) the pilot-scale study.

(mg N); Plant $_{uptake}$ = assimilated N in plant (mg N); TIN_{dischg} = inorganic N discharge (mg N); k_{amo} = ON rate constant (day^{-1}) ; k_{nit} = NH_x rate constant (day^{-1}), k_{denit} = NO_x rate constant (day^{-1}); k_{up} NH_x = uptake rate constant of NH_x (day^{-1}), k_{up} NO_x = uptake rate constant of NO_x (day^{-1}); k_{vol} = volatilization rate constant (day^{-1}); J_{amo} = ammonification rate (mg N day^{-1}); J_{nit} = nitrification rate (mg N day^{-1}); J_{denit} = denitrification rate (mg N day^{-1}); $J_{up\ NHx}$ = uptake rate of NH_x (mg N day^{-1}); $J_{up\ NOx}$ = uptake rate of NO_x(mg N day^{-1}); J_{vol} = NH_3 volatilization rate (mg N day^{-1}); $NH_{x\ dischg}$ = ammonia discharge (mg N day^{-1}); $NO_{x\ dischg}$ = oxidised N discharge; NH_x load= ammonia N flux (mg N day^{-1}); Inflow $_{[NHx]}$ = ammonia concentration in the influent (mg N L^{-1}); Q = flow rate (L day^{-1}); V = volume (L).

Initial values for stocks, and parameters in the microcosm, mesocosm, and pilot-scale models, along with the optimization procedures during calibration, are presented in chapter 3, 4 and 5, respectively.

2.6.2 Sensitivity analysis

A classical one-at-a-time sensitivity analysis was carried out to investigate model sensitivity to selected parameters (k_{nit}, $k_{up\ NHx}$ and k_{vol}). Parameters were varied systematically one at a time over a range, with other factors held at their base (best estimate) values (Whelan, 2013; Whelan et al., 2015). This allowed the identification of which parameters have the greatest impact on the model predictions and could help to understand the relative magnitude of the main processes operating (nitrification, plant uptake and volatilization). Keeping all other parameters the same as used in model calibration, different k_{nit}, k_{upNHx} and k_{vol} (day^{-1}) were varied systematically one at a time in the model interface in increments of 20%. Changes were then observed in the predicted time series of NH_x concentration.

2.6.3 Models Performance

A statistical comparison between observed and simulated data-sets of the concentration of different N species in each experiment was made to assess the accuracy of the model. Linear regression was used to determine the significance of the relationship between simulated and measured concentrations. The coefficient of determination (R^2), Slope of the linear regression and root mean squared error (RMSE) were employed to compare actual and modeled data-sets (Abadi et al., 2015).

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(O_i - S_i)^2}{n}} \qquad (2.21)$$

Where O_i and S_i are the observed and simulated values of the variable under consideration for a time step, and n is the number of observations.

2.7 Statistical Analysis

Analysis of variance (ANOVA) (two-way) and linear regressions were employed (Field et al., 2012). Three replicates were always used for each variable and the data presented represent averages unless otherwise stated. Error bars represent standard deviations. The Shapiro-Wilk test for homogeneity of variances and normality was performed to test the distribution of the data. In cases of significant differences in ANOVA, *Tukey's post hoc* test was used to analyze any significance between samples means (Rohatgi and Saleh, 2015). The level of significance was set at $p < 0.05$. Correlations between variables were assessed using Pearson correlation analysis. All statistical analysis was performed using Microsoft Excel and R (R-Studio v. 099.489).

Chapter Three – The Role of Microbial Biofilm in Removing Ammonia in Floating Treatment Wetlands

3.1 Rational

In this chapter, Laboratory experiments were conducted under controlled conditions in order to quantify the potential of microbial transformation associated with floating matrix in ammonia removal and nitrification kinetics. The effect of different design parameters on ammonia removal from synthetic media amended with ammonia concentrations was investigated in order to optimize system performance. The aspects investigated include: (a) the effect of different biofilm surface areas; (b) the effect of ammonia concentration (c) the effect of aeration. A simple model of mineral nitrogen transformation was used as a framework for interpreting the experimental results.

We hypothesise that nitrification rate is directly and linearly proportional to the mat surface area (i.e., that nitrifiers predominantly inhabit fixed biofilms attached to the mat). We also hypothesise that nitrification rate will be inhibited at higher free ammonia concentrations and that nitrification will be enhanced by aeration (i.e., that oxygen will limit nitrification in unaerated systems. Better mechanistic understanding of the fundamental processes operating in FTWs should provide the basis for improving FTW design and efficacy.

3.2 Methods

3.2.1 Experimental

Prior to the experimental phase, floating mat material consisted of extruded plastic (obtained from Frog Environmental, UK) was pre-cut into pieces approximately 2 cm^2 and weighting 0.570g±0.054. The cut mat materials were incubated for four weeks in 5000 mL conical glass flasks containing 4000 mL of mineral medium and approximately 200g of sediment collected from a local pond. Substrate media contained 0.272 mM $MgSO_4$, 0.6 mM $CaCl_2$, 0.24 µM $FeSO_4$, 0.174 µM EDTA, 3 mM K_2HPO_4, 1.4 µM Phenol red prepared in deionised water and was used (Chapman et al., 2006). The sediment was used as a mixed culture of micro-organisms, in order to allow biofilms to

development (Figure 3.1). The flasks were covered with foil to maintain dark conditions for microbial growth and to prevent algal growth. Stock cultures were fed on a daily basis by adding 4 mL of 1 M NH_4HCO_3 to encourage nitrifier growth and biofilm establishment. Cultures pH were readjusted daily to pH 7.4, depending on the phenol red colour change, using 0.1 M Na_2CO_3. All flasks were capped with a rubber bung fitted with a tube for aeration via an air stone attached to an aquarium pump. Gas venting of the headspace was achieved via a water trap (brewers' trap) containing a disinfectant. The temperature was maintained at 29 °C using thermostats. pH, temperature and dissolved oxygen were monitored daily using a Hanna HI 8711E pH meter (Hanna Instruments Inc.) and YSI Professional Plus multi-probe meter (Yellow Springs International Inc. Ohio, USA).

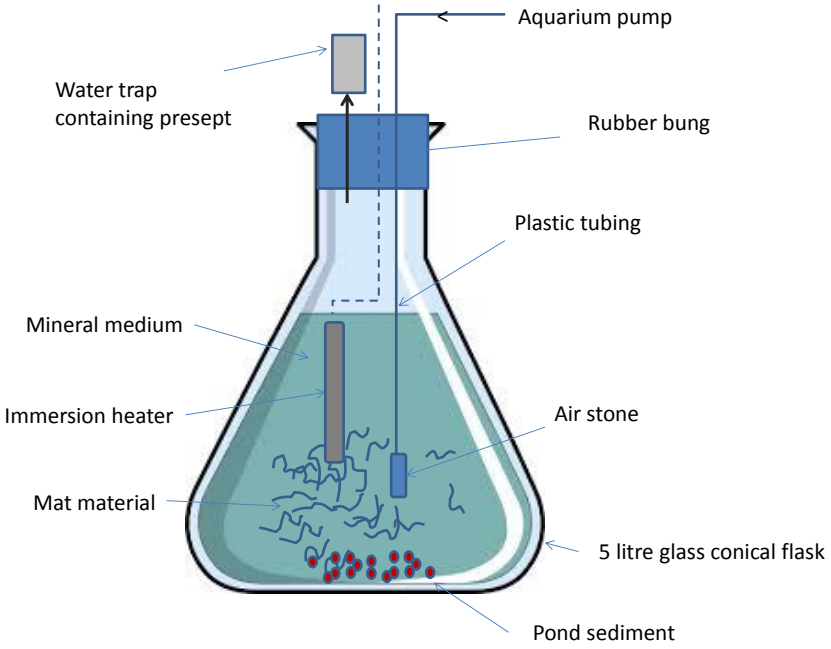

Figure 3. 1 Illustration of the inoculation phase set up.

Two microcosm experiments were established. Experiments 1 investigated the effect of mat area on ammonia removal. 1000 mL amber Duran glass bottles were used at room temperature (20 – 21 °C) (Figure 3.2). The same volume of synthetic mineral media spiked with ammoniacal nitrogen were used in all treatments. pH was maintained (between 7.3 and 7.4) by adding 0.1 M Na_2CO_3. All treatments were established in triplicate. The treatments were as follows: T_1 (4 cm^2 mat material); T_2 (8 cm^2 mat material); T_3 (12 cm^2 mat material); T_4 with (16 cm^2 mat material); (T_5) (20 cm^2 mat

material) and control (no mat material). The initial concentration of ammonia-N in each treatment was 72±2 mg N L^{-1}. The bottles were gently shaken daily.

Experiment 2 investigated the influence of different concentrations of ammonia under aerated and non-aerated conditions on nitrification kinetics. The treatments were as follows: T_1 (15 mg N L^{-1} without aeration), T_2 (15 mg N L^{-1} with aeration), T_3 (30 mg N L^{-1} without aeration), T_4 (30 mg N L^{-1} with aeration), T_5 (60 mg N L^{-1} without aeration), and T_6 (60 mg N L^{-1} with aeration). All treatments used a mat area of 20 cm^2. All treatments were aerated using aquarium air pumps (50 L hr^{-1}). Airflow rate in each reactor was monitored using a bubble flow meter.

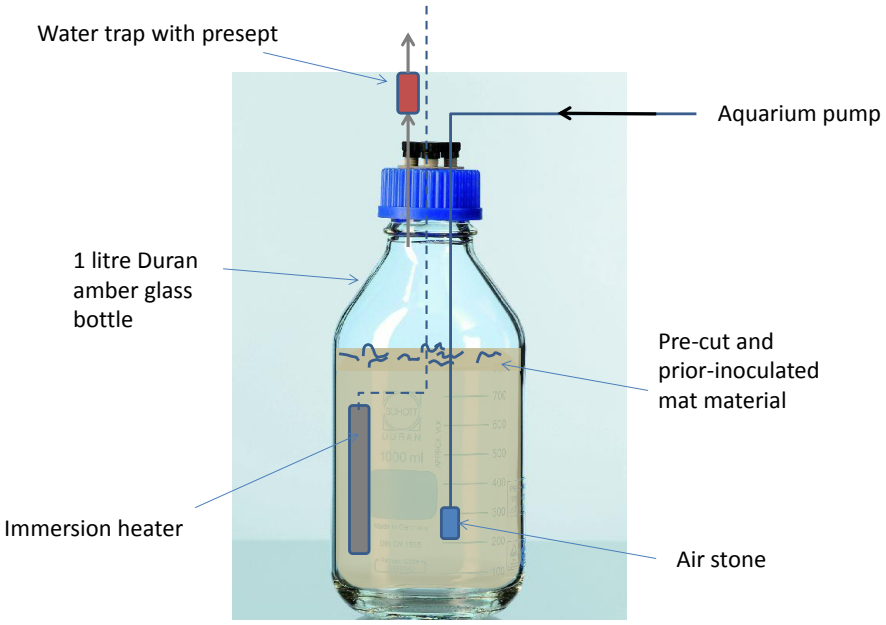

Figure 3. 2 Experimental set up for the nitrification experimental phase.

In each experiment, aqueous samples (15 mL) were collected from each bottle for nine sampling intervals over a two week period. Samples for ammoniacal N and total oxidized N analysis were immediately preserved by adding 0.025 mL concentrated sulfuric acid (98 %) and cooling to 4 °C (EPA, 1993). Samples for nitrite analysis were either analysed directly or were frozen at -20 °C until analysis (Cheeseman et al., 1989). Concentrations of NH_x, NO_2^- and NO_x were analyzed according to established protocols (SEAL Analytical, 2011; SEAL Analytical, 2013a; SEAL Analytical, 2015) using an automated discrete colorimetric instrument (AQ2: SEAL Analytical, UK). Unionised NH_3 concentration was calculated assuming full and instantaneous thermodynamic equilibrium between NH_3 and NH_4^+ (section 2.5). DO, pH, EC and temperature were

measured at the time of each sampling. using portable probes (see inoculation phase). For a more extensive description of the analytical methods, the reader is referred to chapter 2, sections 2.3 and 2.4.

3.2.2 Model

A simple numerical model was developed to describe NH_x and NO_2^- behavior in each experiment. A detailed description of the model in Chapter 2. Experimental data obtained from one treatment (T_5) in experiment 1 were used for model calibration (Table 3.1). The model fit to the measured NH_x concentration data was optimized by minimizing the root mean square error (RMSE) between the simulated and measured concentrations in a trial and error optimization of the parameter k_{nit} (Rousseau et al., 2004). The model was run over 14 days and employed Euler's method of integration with a time step of 0.25 day.

Table 3. 1 Description of symbols used in the microcosm model. T_5 is treatment number 5 (containing 20 cm^2 mat material).

STELLA symbol	Description	Initial value/Units	Source
State variables			
$NH_{x\ ini}$	NH_3+NH_4^+ mass	72.25 mg N	
$NO_2^-{}_{ini}$	NO_2^- mass	mg N	
Volatized NH_3	Free ammonia volatilization	mg N	
Flow variables			
J_{nit}	Nitrification rate	mg N day^{-1}	Calculated using data in T_5
J_{vol}	Volatilization rate	mg N day^{-1}	(Whelan et al., 2010)
Parameters/coefficients			
$[NH_x]$	NH_x concentration	mg N L^{-1}	
k_{nit}	Nitrification rate constant	0.033 day^{-1}	Calculated using data in T_5
k_{vol}	Mass transfer coefficient of NH_3	0.08 day^{-1}	Estimated using data in T_5
$NH_{3\ f}$	Concentration of NH_3 gas	0.015 mg N	Estimated using data in T_5
V	volume	L	

The mass balance equation for the total ammonia N is expressed as follows:

$$V.\frac{d[NH_x]}{dt} = k_{vol}.f_{FREE}.[NH_x].V - k_{nit}.[NH_x].V \qquad (3.2)$$

where k_{vol} is the mass transfer coefficient of free ammonia across the air-liquid interface by volatilization (day^{-1}), f_{FREE} is the fraction of total ammonia which is present as free. NH_3 (dependent on pH and temperature), k_{nit} is a first order rate constant for nitrification (day^{-1}), $[NH_x]$ is the total ammonia N concentration in the treatment (mg N L^{-1}), and V is the vessel volume (L).

The calibrated value for k_{nit} then adjusted in proportion to the mat area for other treatments, after subtracting the background control value for k (*k control*). This represents the background nitrification rate due to biofilm on the surfaces of the vessel. A specific rate constant (k_{spec}) per unit mat area (A) was estimated for T_5 by divided k_{nit} by the total surface area of the mats. k_{spec} of T_5 (0.0015), mat surface area (A), and *k control* were used to calculate predicted overall rate constants (k_{inde}) for other treatments.

$$kindep = kcontrol + (kspecT5 * A) \qquad (3.1)$$

Since adjustments to the rate constants for the other treatments was made only using the surface area, these predictions can be considered to be an independent validation.

The calibrated value of k_{nit} derived from experiment 1 was also applied to other treatments from experiment 2 to compare ammonia loss in both experiments and to illustrate the extent of the inhibition effect of free NH_3 on nitrification. Then, the calibrated k_{nit} value was optimized for each treatment to get best fits between simulated and measured concentrations. Optimized k_{nit} were compared with calibrated k_{nit} from experiment 1. The ratio of optimized k_{nit} to calibrated k_{nit} was regarded as a quantitative index of the extent of nitrifier inhibition by free NH_3.

3.3 Results and Discussion

3.3.1 Experimental data

3.3.1.1 Effect of mat area (Experiment 1)

Changes in the concentration of ammonia, nitrite and total oxidized N over time for each treatment are shown in Figure 3.3. NH_x reduction was observed in all treatments soon after the introduction of the mat material which continued over the 14-day experimental period. NH_x removal differed significantly between treatments (ANOVA, $F_{5, 54} = 9.276$; $P < 0.05$).

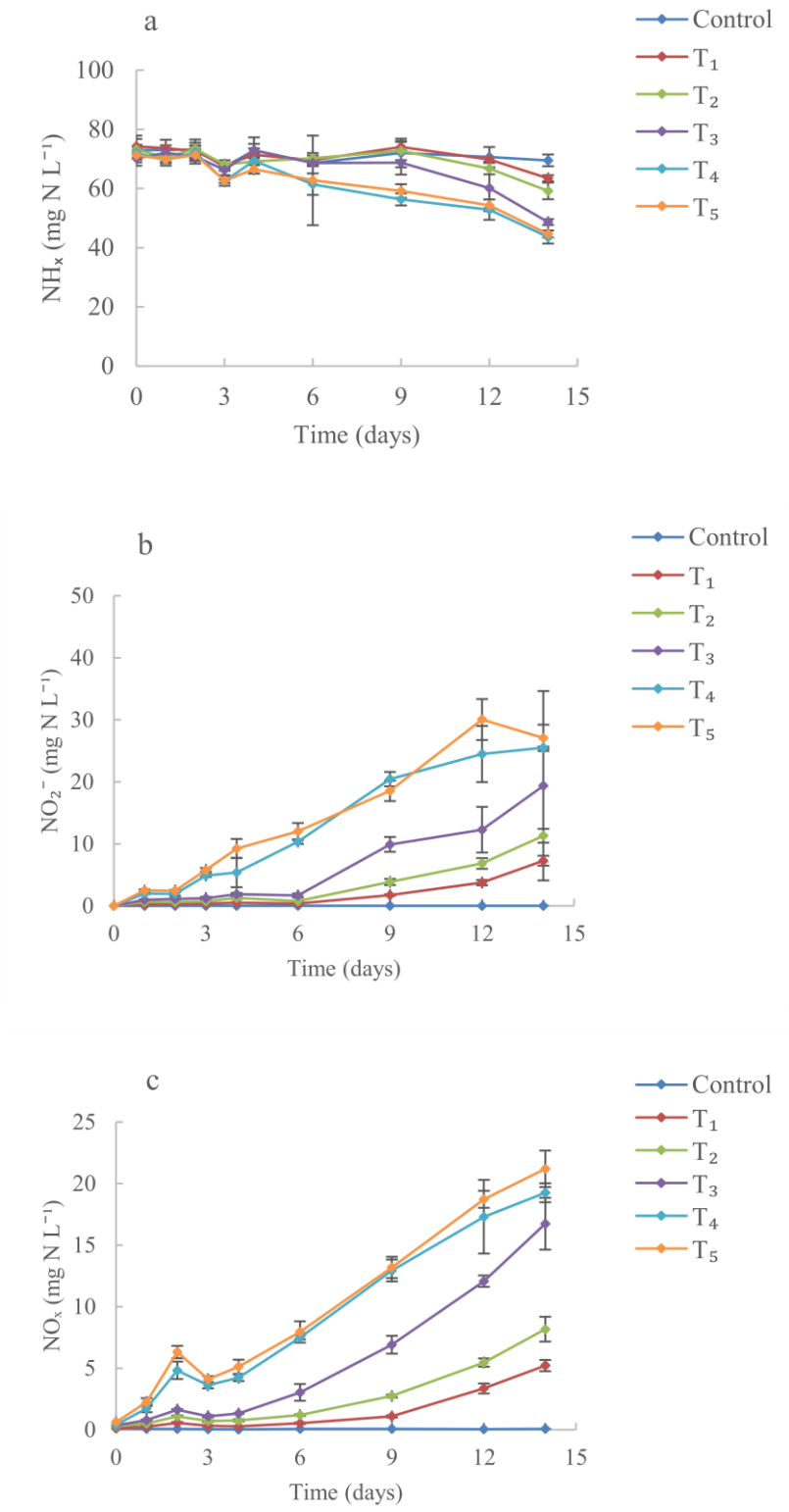

Figure 3. 3 Changes in the concentration of (a) NH_x, (b) NO_2^-, (c) NO_x over time (mean ± standard deviation). Control (without mat); T_1 (4 cm^2 mat material); T_2 (8 cm^2 mat material); T_3 (12 cm^2 mat material); T_4 (16 cm^2 mat material); T_5 (20 cm^2 mat material).

biofilm activity than with those with low surface area. Consistently, higher rate constants were observed in T_4 and T_5 treatments compared to T_1, T_2 and T_3, respectively. In contrast, Removal half-life for NH_x concentrations within T_4 and T_5 treatments were shorter than T_1, T_2, and T_3, respectively.

Table 3. 2 Net removal (mg N L^{-1}), rate constant (k, day^{-1}), and half-lives ($T_{1/2}$, day) for the NH_x concentrations in the treatments.

	Net removal (mg N L^{-1})	***k*** (day^{-1})	***$T_{1/2}$*** (day)
Control	3.47	0.003	198.73
T_1	10.78	0.011	61.85
T_2	14.03	0.015	45.59
T_3	21.57	0.026	26.43
T_4	28.46	0.035	19.31
T_5	26.63	0.033	20.73

The observation of an increased rate of nitrification with an increase in the mat area is consistent with the expectation that microbial processes are closely associated with solid surfaces (Kadlec and Wallace, 2009b). The rate constants for NH_x are plotted against mat surface area in Figure 3.4.

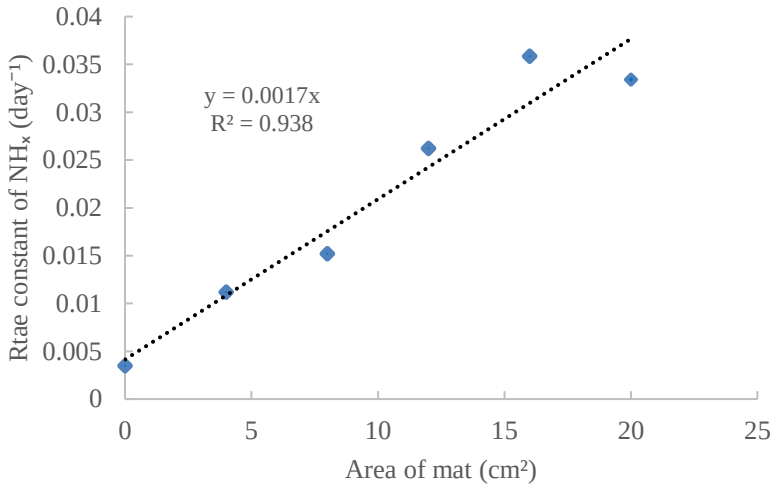

Figure 3. 4 Removal rate constants of NH_x at different area of floating mats.

Rate constant was positively correlated with the increase of mat area ($r^2 = 0.96$; $p = 0.05$). These results are in good agreement with those obtained by Wang et al. (2015) who

reported higher removal efficiency of NH_x (89–91 %) when the surface area of floating mat was increased by adding a biological grid which allows for larger bacterial populations.

In parallel, nitrite concentrations increased over time. The rate of NO_2^- production was initially low and then increased after 3-6 days. The rate of NO_2^- production was consistent with the rate of NH_x loss and was highest in T_4 and T_5 which had the highest mat surface area. (Figure 3.3b). Initial delay of nitrification activity in the treatments at the start of the experiment (lag phase) could be attributed to acclimatization of the microbial population to the high concentration of ammonia in this system. The most prolonged lag phase was observed in the treatments with smaller mat area (T_1, T_2, and T_3). A possible explanation is that higher microbial biofilm is metabolically capable of tolerating and nitrifying high concentration of NH_x faster than those with a smaller amount of biofilm (Costerton et al., 1994). Treatments with higher mat area had higher accumulated concentrations of NO_2^- (ANOVA, $F_{5, 54} = 10.088$; $p < 0.05$). The net production of NO_2^- concentration were 7.27, 11.3, 19.36, 25.5, and 27.1 mg N L^{-1} in T_1, T_2, T_3, T_4, and T_5, respectively. NO_2^- concentration in the control treatment remained very low (0.001 mg N L^{-1}).

Total oxidized N concentrations in all treatments were similar to those of nitrite suggesting that there was the very little production of nitrate (Figure 3.3c). This indicates that the growth and activity of *Nitrobacter spp.* responsible for the conversion of NO_2^- to NO_3^- was inhibited in this experimental system. The most likely explanation for this is that the concentration of NH_3 was high. There is a general agreement that *Nitrosomonas* are less sensitive to high NH_3 concentrations than *Nitrobacter* (Park et al., 2010). Inhibition of *Nitrosomonas* is usually reputed in the range of 10-150 mg N L^{-1}, whereas for *Nitrobacter* it can start in the concentration range 0.1-1.0 mg N L^{-1} (Anthonisen et al., 1976). The dynamics of NH_3 alongside with average concentrations in the treatments throughout the experiment are presented in the Figure 3.5 and Table 3.3. The range of NH_3 concentrations observed (0.9-1.1 mg NH_3 L^{-1}) are consistent with those reported above, which might explain the inhibition effect of free ammonia on *Nitrobacter sp*, therefore depress the conversion of NO_2^- to NO_3^-.

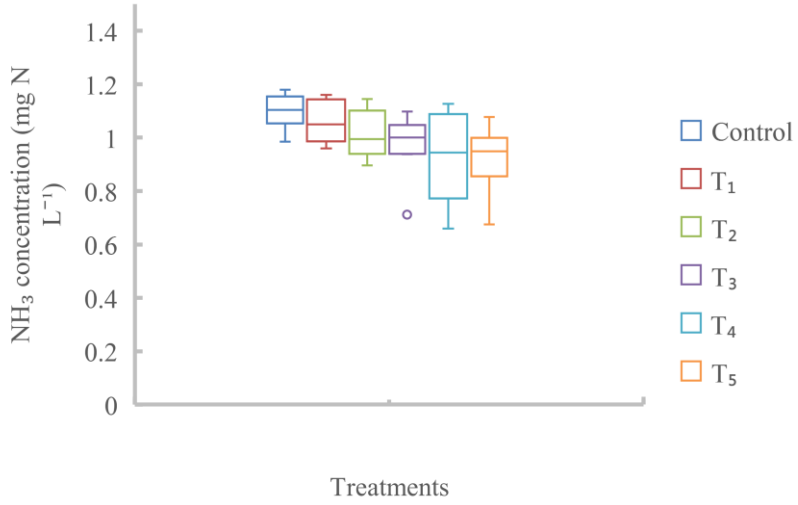

Figure 3. 5 Concentration of NH_3 in treatments under different areas of mat material

Table 3. 3 Average concentrations of NH_3 in the treatments during the experiment

	Treatments					
	Control	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
NH_3 (mg N L^{-1})	1.1±0.06	1.06±0.08	1.01±0.09	0.96±0.11	0.92±0.16	0.92±0.11

Dissolved oxygen (DO) concentrations was differed between treatments (ANOVA, $F_{5, 54} = 18.864$; $P < 0.05$). There was an exponential decrease in DO concentrations in T_4 and T_5 from time zero to an asymptote at almost 9 days. Decreases in DO concentrations were also seen in the other treatments containing mat material, but the rate of change here was initially slow and increased progressively in T_1 and T_2 over the course of the experiment (Figure 3.6a). In T_3 DO concentration also reached an asymptote at 9 days. The rate of change in DO concentration appeared to be proportional to the nitrification rate. In control, there was very little DO depression. Nitrifying bacteria are obligate aerobes and require oxygen to derive energy from reduced ammonia (Hargreaves, 1998).

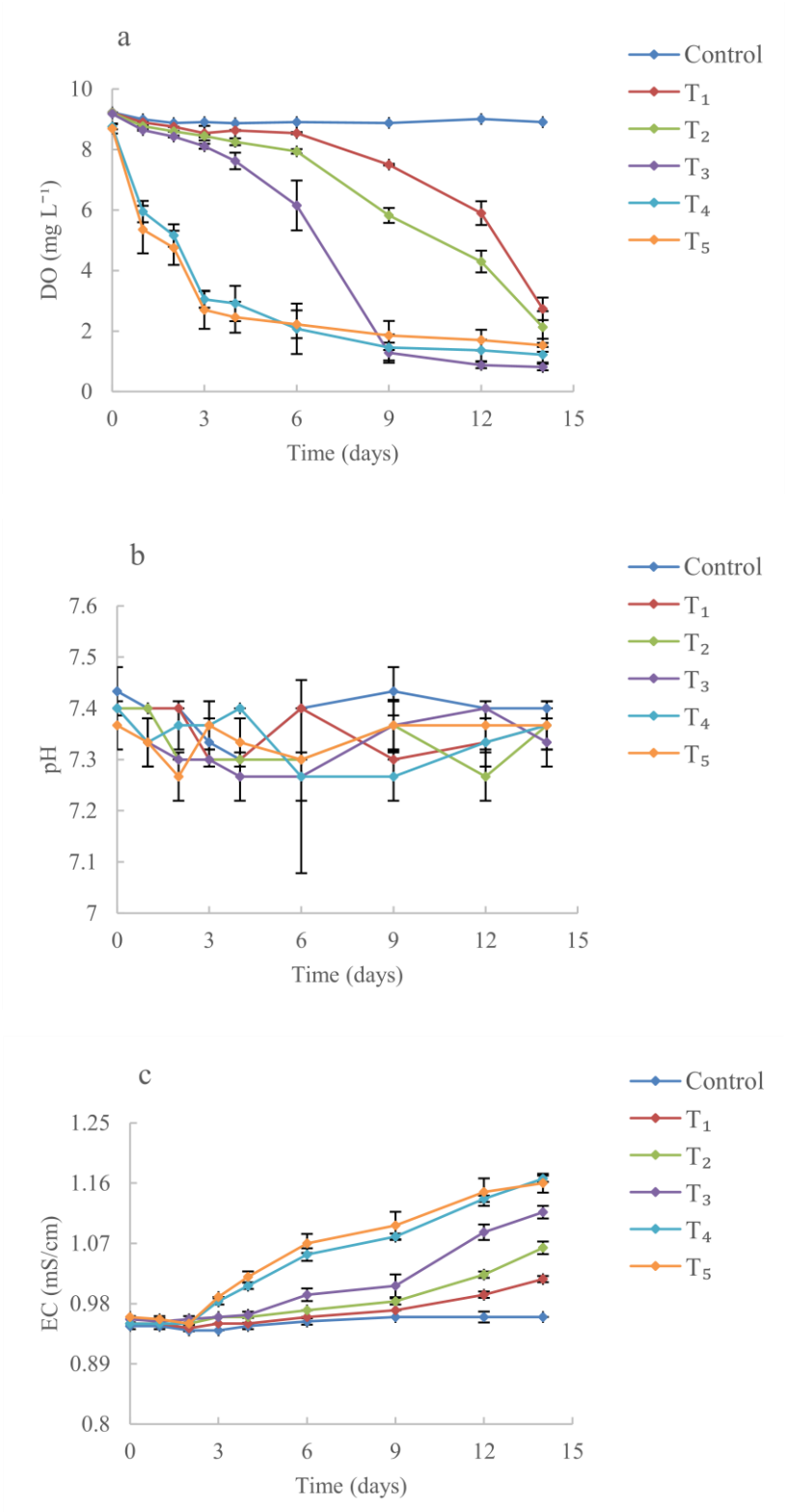

Figure 3. 6 Changes in the (a) DO; (b) pH and (c) EC over time (mean ± standard deviation). Control (without mat); T_1 (4 cm^2 mat material); T_2 (8 cm^2 mat material); T_3 (12 cm^2 mat material); T_4 (16 cm^2 mat material); T_5 (20 cm^2 mat material).

The observation of an increased rate of nitrification with the decreased DO concentration is consistent with the expectation that ammonia oxidation is correlated with DO and can be regarded as the primary sink of the dissolved oxygen. The rate constants for NH_x are plotted against DO concentration in Figure 3.7. A negative correlation was observed between removal rate constant of ammonia and DO concentration ($r^2 = 0.99$; $p = 0.05$).

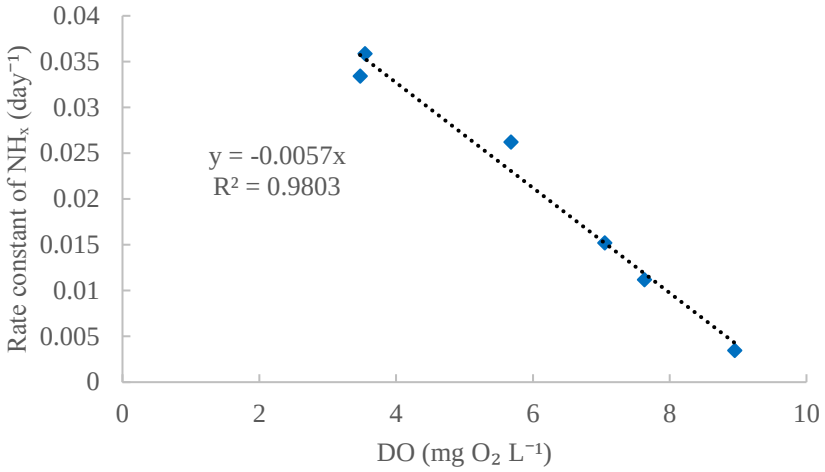

Figure 3. 7 Removal rate constants of NH_x versus DO concentrations in the treatments.

Although, there was a clear reduction in the DO concentration in the treatments with mat materials over the course of the experiment, however measured O_2 levels in the media were not limiting to the microbial activity (e.g. > 2 mg O_2 L^{-1}). Since media pH during the experiment was maintained between 7.3 and 7.4 (Figure 3.6b), volatilization was calculated to be low in all treatments. The loss of ammonia via volatilization of NH_3 was found to be 0.001 day^{-1}. In all treatments, there was a progressive increase in electrical conductivity over the experiment (Figure 3.6c) This was probably due to the increase of NO_2^- concentration in all the treatments.

3.3.1.2 Model performance (Experiment 1)

Observed and modeled (calibrated) concentrations of NH_x and NO_2^- for the T_5 treatment are shown in Figure 3.8. Optimized RMSE values were 4.06 and 0.98 mg N L^{-1} for NH_x and NO_2^-, respectively. The good fit demonstrates that the model was able to reproduce the concentrations of N species well and that the rate of nitrite accumulation was consistent with the rate of ammonia loss (i.e., nitrification was the principal process).

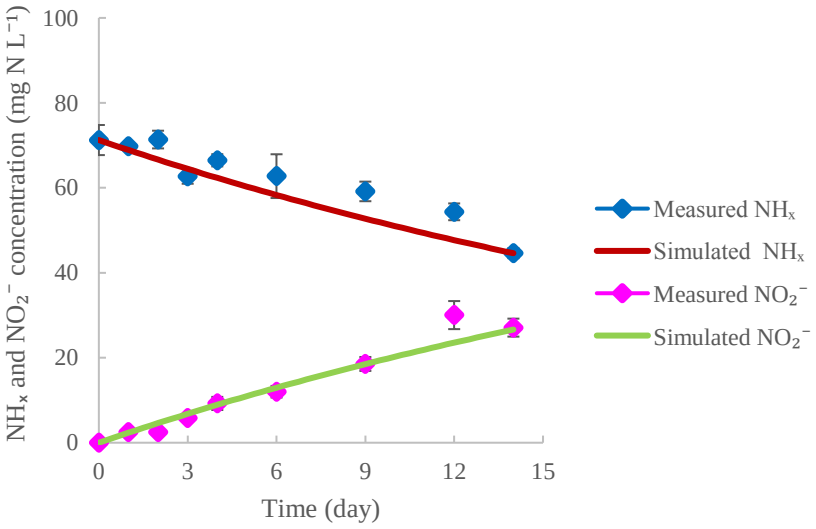

Figure 3. 8 Measured time series of mean NH_x and NO_2^- concentrations in the T_5 treatment (symbols) and simulated values (lines) produced using the calibrated parameters. Error bars shows standard deviations.

Calibrated value of k_{nit} for T_5 alongside with the calculated values for independent treatments k_{inde} (T_1, T_2, T_3, and T_4) are presented in the Table 3.4.

Table 3. 4 Calibrated k_{nit} for T_5 and calculated k_{inde} for independent treatments.

k_{nit} (day^{-1})	k_{inde} (day^{-1})				
T_5	**Control**	**T_1**	**T_2**	**T_3**	**T_4**
0.0333	0.0035	0.0095	0.0155	0.0215	0.0274

Measured and simulated time series for NH_x and NO_2^- in the other treatments are shown in Figure 3.9. R^2, slope regression line and RMSE values between the mean observed and predicted concentrations are shown in Table 3.5.

Overall, there is a good agreement between model prediction and measured data for most treatment. This suggests that the mat area provides an adequate scaling factor for the experimental system and support the hypothesis that nitrifier biomass and activity are proportional to mat surface area.

Table 3. 5 Statistical analysis for model performance.

	NH_x			NO_2^-		
	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})
Control	0.39	0.69	1.99	0.45	0.0006	1.84
T1	0.63	0.68	2.78	0.88	0.65	2.67
T2	0.71	0.62	3.98	0.91	0.69	3.72
T3	0.81	0.96	5.88	0.93	0.98	3.28
T4	0.95	1.13	3.07	0.98	1.22	2.46

Although the trends of NH_x and NO_x concentrations were generally captured well, the model performed poorly in some points in some treatments. For example, the initial rate of increase in NO_2^-concentrations was overpredicted for T_1, T_2, and T_3. This is due to the apparent lag phase in the observed data for these treatments, which was not taken into account in the model.

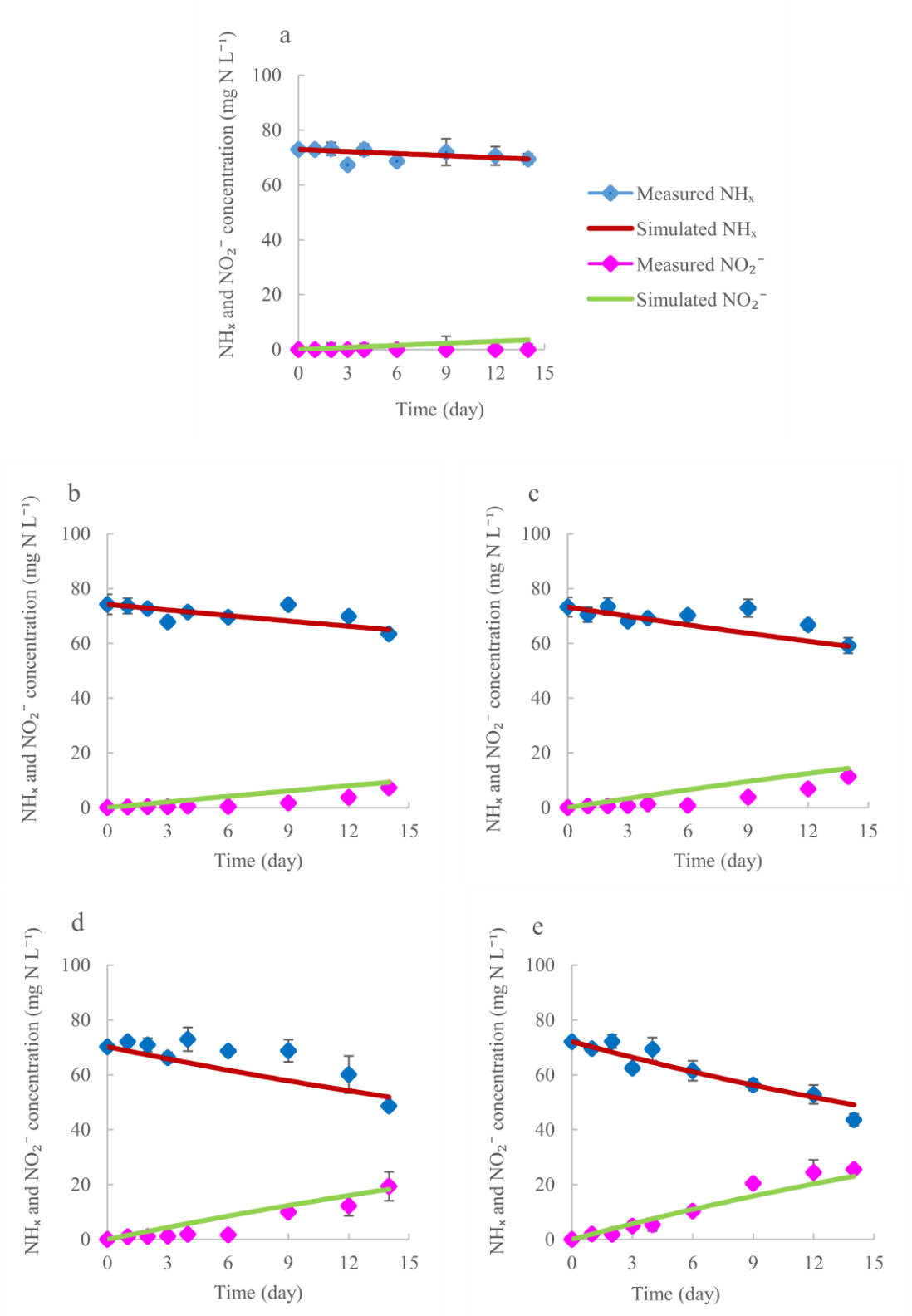

Figure 3. 9 Changes in average measured and predicted values of NH_x and NO_2^- in the validation exercise: (a) control (without mat); (b) T_1 (4 cm^2 mat material), (c) T_2 (8 cm^2 mat material), (d) T_3 (12 cm^2 mat material) and (e) T_4 (16 cm^2 mat material). Error bars show standard deviations for measured concentrations.

The results suggest that the submerged surface area provided by operational FTWs will support the growth of microbes, and could significantly increase N removal compared with conventional free surface constructed wetlands (Stewart et al., 2008). The results support the findings reported by Zhang et al. (2016), of significant promotion of nitrification (82%) in the presence of floating islands in an experimental system containing synthetic wastewater. The final measured concentrations are plotted against modeled values in Figure 3.10. Overall, there is a good agreement in final concentrations for both NH_x and NO_2^- (R^2 = 0.88 and 93; slopes = 1.14 and 0.75 mg N L^{-1}, respectively).

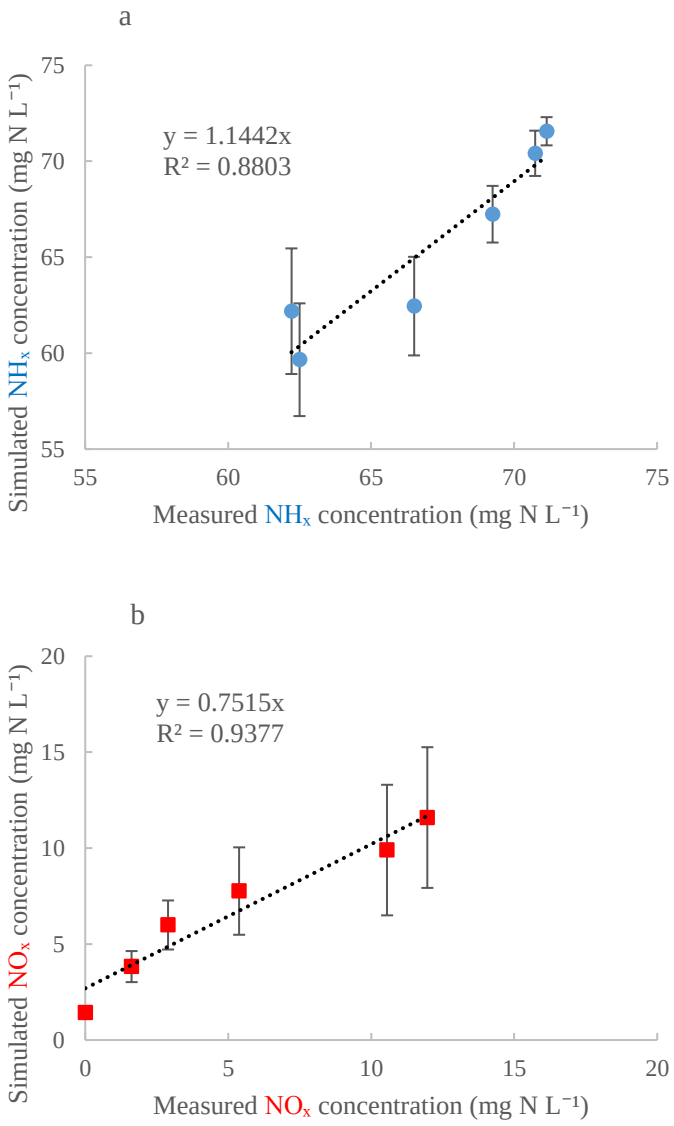

Figure 3. 10 Final average measured and simulated concentrations of (a) NH_x and (b) NO_2^- in the validation treatments. Error bars show standard deviation for measured concentration.

Overall, the good agreement between the modeled and measured data of NH_x and NO_2^- concentrations suggests that the model well described different treatment investigated under different mat surface areas. Keeping other factors controlled and no limits to the microbial activity (e.g. O_2 limitation), these results support our hypothesis that the mat area can be used as a design parameter to optimize system performance in removing ammonia. Ammonia removal is directly proportional to the increase in mat area and therefore established microbial biofilm.

3.3.1.3 Effect of ammonia concentrations and aeration (Experiment 2)

The similarity between the total oxidized N and nitrite concentrations observed in experiment 1 suggested that the second stage of nitrification was inhibited (i.e., that *Nitrobacter* type organisms were unable to grow and or operate efficiently). This could be due to high NH_3 concentrations which are known to affect *Nitrobacter* more. We also hypothesized that aeration could enhance nitrification rates through increase DO concentration available for nitrifiers biosynthesis and activity. To test these ideas, the performance of the experimental system was examined under different total ammonia concentrations with and without aeration. Changes NH_x, $NO_2^-$and NO_x concentrations over time for the different treatments are shown in Figure 3.11. NH_x loss differed between treatments. There was an apparent lag phase in NH_x loss in all treatments up to day 5, but the lag appeared to be more pronounced in the treatments with the highest initial NH_x concentrations.

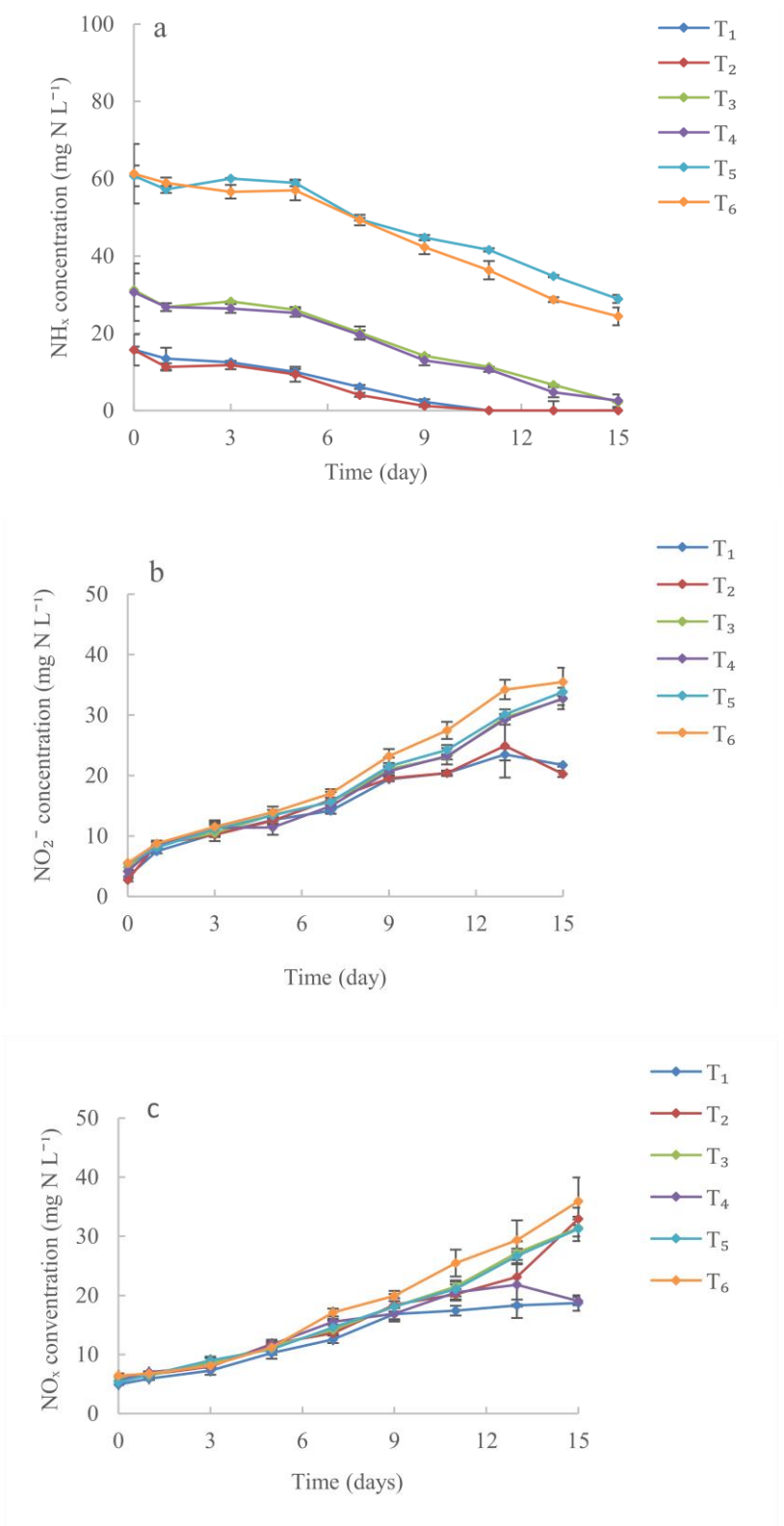

Figure 3. 11 Changes in the concentration of (a) NH_x, (b) NO_2^-, (c) NO_x in the treatments during experimental time (mean ± standard deviation). T_1 (15 mg N L^{-1}); T_2 (15 mg N L^{-1}, aeration); T_3 (30 mg N L^{-1}); T_4 (30 mg N L^{-1}, aeration); T_5 (60 mg N L^{-1}); T_6 (60 mg N L^{-1}, aeration).

First order rate constants fitted to the NH_x data show that the overall loss was higher in the treatments with the lowest initial NH_x concentrations and lowest in those treatments with high initial NH_x concentrations (Table 3.6) Consistently, higher net removal was observed in the treatments with low NH_x concentrations than with high. However, $T_{1/2}$ values were low in treatments treated with low NH_x concentration (T_1 and T_2) compared to those treated with high concentrations (T_3, T_4, T_5, and T_6). No significant differences were observed in the NH_x between treatments with and without aeration ($p < 0.05$) despite the marked differences in DO concentrations (showing later in Figure 3.12a).

Table 3. 6 Net removal (mg N L^{-1}), rate constant (k, day^{-1}), and half-lives ($T_{1/2}$, day) for the NH_x concentrations in the treatments.

	Net removal (mg N L^{-1})	***k*** (day^{-1})	***$T_{1/2}$*** (day)
T_1	15.74	0.53	1.29
T_2	15.67	0.57	1.21
T_3	29.03	0.17	4.12
T_4	28.05	0.17	4.07
T_5	31.85	0.05	13.33
T_6	36.90	0.07	10.34

Lower rate constants in treatments with high initial ammonia-N concentration suggest that the nitrifiers may have been inhibited by high NH_3 concentrations in the treatments. It has been well documented that NH_3 have toxic impacts on nitrification (Ruiz et al., 2003; Vadivelu et al., 2007; Daalkhaijav and Nemati, 2014). Partially for NO_2^-, AOBs are known to be more tolerant to NH_3 than NOB in activated sludge systems (Qiao et al., 2010).

Nitrite concentrations gradually increase in all treatments over the study period (Figure 3.9b). Rates of NO_2^- production were consistent with rates of NH_x loss. significant differences in NO_2^- production between treatments were observed (ANOVA, $F_{5, 54} = 6.576$; $P < 0.05$). The net production of NO_2^- were by 18.48, 17.53, 27.82, 28.59, 28.41, and 29.95 mg N L^{-1} in T_1, T_2, T_3, T_4, and T_5, and T_6 treatments, respectively. NO_2^- concentrations stop increasing in T_1 and T_2 (lowest NH_x concentration) at 15 days coincident with the exhaustion of NH_x supply in their treatments.

Like the findings of experiment 1, obtained data showed that the total oxidized N was characterized by NO_2^- concentrations, but no analytical evidence of NO_3^- generation (Figure 3.11c).

Free ammonia generation alongside with their average concentrations in the treatments over the course of the experiment are presented in Figure 3.12 and Table 3.7. Higher concentration of NH_3 was observed in the treatments with high concentration of total ammonia (T_5 and T_6) compared t those exposed to low NH_x levels (T_1, T_2, T_3, T_4).

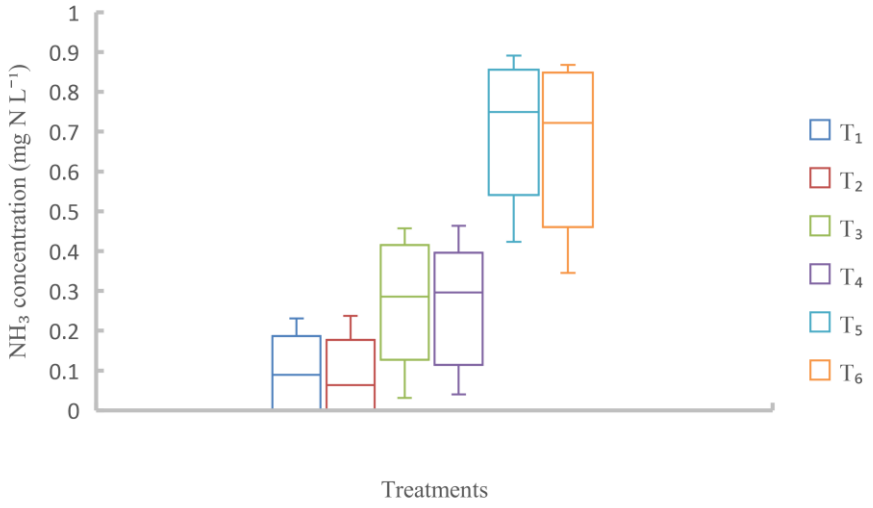

Figure 3. 12 Concentration of NH_3 in treatments under different ammonia N concentrations with and without aeration.

Table 3. 7 Average concentrations of NH_3 in the treatments throughout the experiment.

	Treatments					
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
NH_3 (mg N L^{-1})	0.1±0.09	0.09±0.09	0.27±0.15	0.26±0.15	0.7±0.17	0.66±0.20

The differences of the metabolic responses to the toxic effects of NH_3 on the nitrifying population have been proposed as the primary cause resulting in NOB elimination (Sun et al., 2015). For instance, Vadivelu et al. (2007) found that the growth of *Nitrobacter* was decreased by 12 % when NH_x concentration increased from 0 to 9 mg N L^{-1}, indicating inhibitory effects on the respiratory capacity of the genus. Also, Philips et al. (2002) reported that the NH_3 inhibition threshold for *Nitrobacter* in the range of 0.02-0.82 mg NH_3-N L^{-1}. This could explain the higher accumulated rates of NO_2^- in the treatments as the NH_3 concentration is increased in the present study.

Treatments which were aerated had DO concentration close to the equilibrium level for the system temperature (8.5 mg L^{-1}). However, in treatments which were not aerated DO concentrations declined progressively due to oxygen consumption in nitrification (Figure 3.13a). However, even the lowest oxygen level observed in the nonaerated treatments (an average of 3.96±0.76 mg DO L^{-1}), nitrification was observed to be proceeded (i.e., DO not limit). A notable recovery of DO concentration in T_1 treatment at the end of the experiment was probably due to the oxygen concentration in this treatment was able to return to saturation levels once this sink was switched off. NH_x depletion in this treatment and a consequent decline in nitrification (the main sink for dissolved O_2).

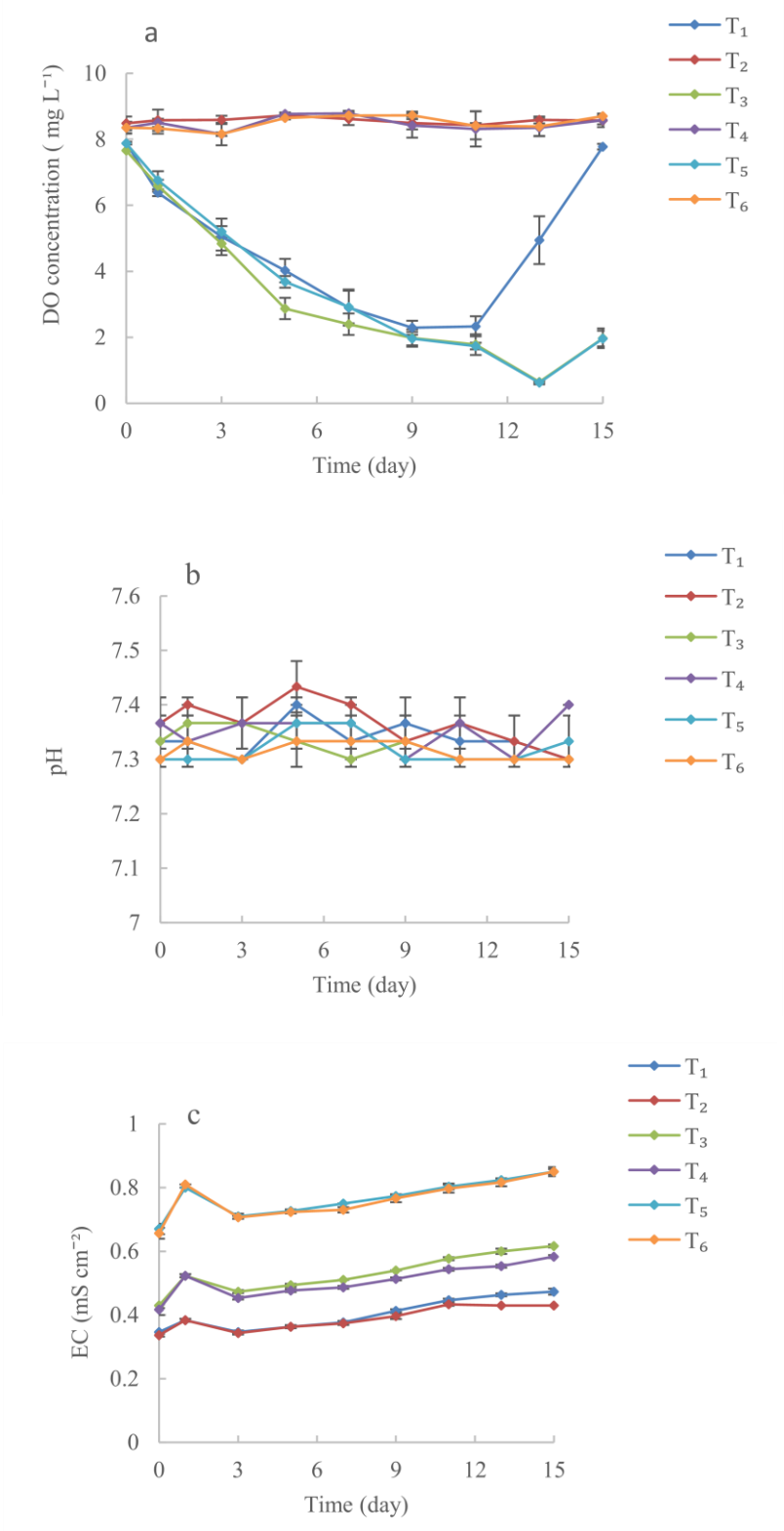

Figure 3. 13 Changes of (a) DO; (b) pH and (c) EC in the treatments during experimental time (mean ± standard deviation). T_1 (15 mg N L^{-1}); T_2 (15 mg N L^{-1}, aeration); T_3 (30 mg N L^{-1}); T_4 (30 mg N L^{-1}, aeration); T_5 (60 mg N L^{-1}); T_6 (60 mg N L^{-1}, aeration).

Although blowing air in the system could encourage volatilization through the stripping process, however, the first order NH_3 volatilization rate constant at pH ranged between 7.3-7.4 was calculated to be 0.001 day^{-1}. Generally, all treatments showed a gradual increase in EC over the course of the experiment. (Figure 3.9f) Such increase was consistent with the extent of NO_2^- concentrations produced in the treatments during the experiment.

3.3.1.4 Model Performance (Experiment 2)

Modeled and measured concentrations of NH_x and NO_2^- for T_5 model from experiment 1 against treatments from experiment 2 are shown in Figure 3.14. RMSE values between the measured and predicted concentrations are shown in Table 3.8.

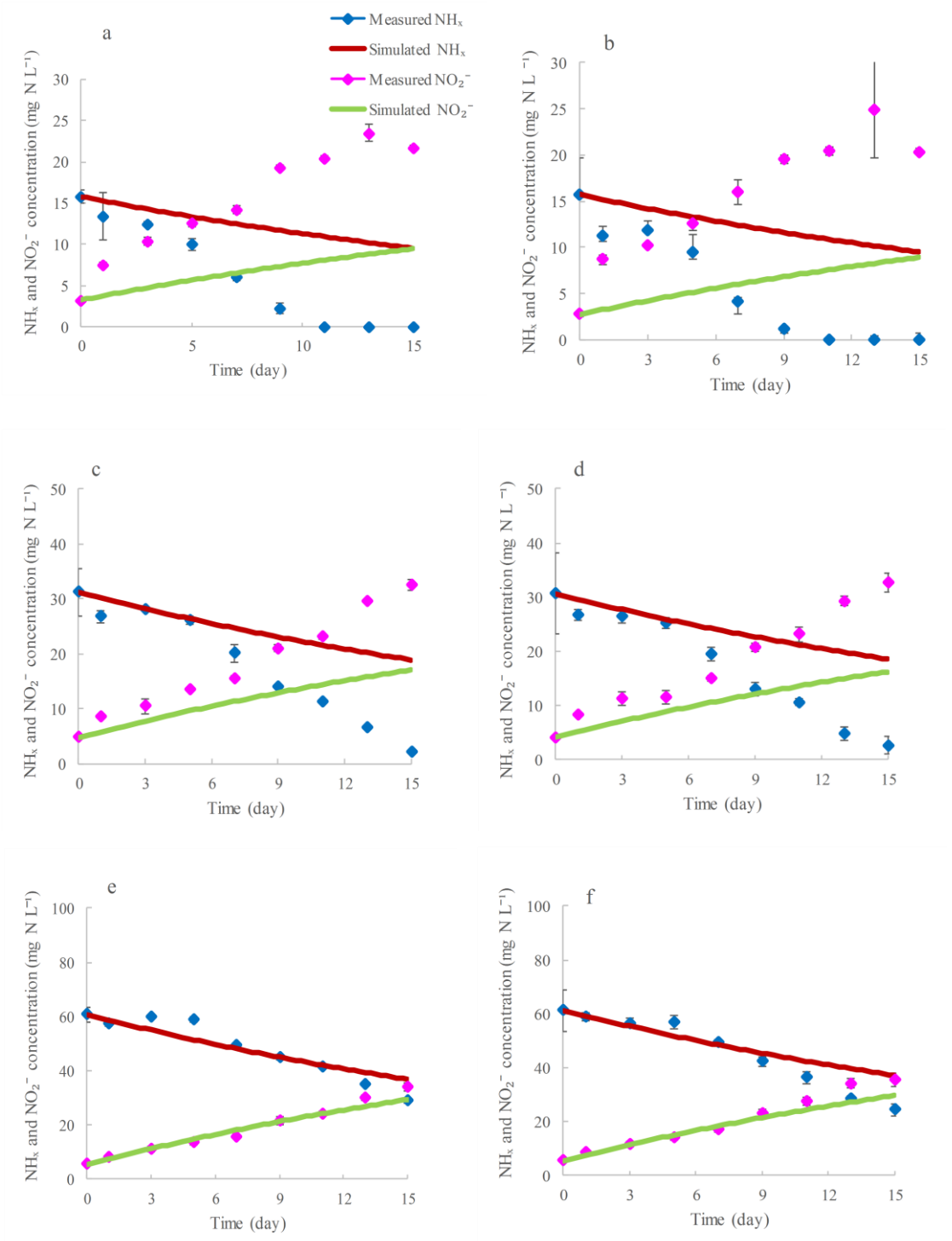

Figure 3. 14 Changes in average measured and predicted values of NH_x and NO_2^- in the validation exercise: (a) T_1 (15 mg N L^{-1}); (b) T_2 (15 mg N L^{-1}, aeration); (c) T_3 (30 mg N L^{-1}); (d) T_4 (30 mg N L^{-1}, aeration); (e) T_5 (60 mg N L^{-1}) and T_6 (60 mg N L^{-1}, aeration). Error bars show standard deviations for measured concentrations.

Table 3. 8. Statistical analysis for model performance.

	NH_x			NO_2^-		
	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})
T_1	0.95	2.81	7.14	0.95	3.08	9.51
T_2	0.92	2.67	7.63	0.90	3.04	10.27
T_3	0.94	2.29	8.70	0.96	2.14	8.29
T_4	0.95	2.34	8.95	0.95	2.21	8.81
T_5	0.89	1.30	4.34	0.96	1.13	2.07
T_6	0.93	1.54	6.30	0.96	1.25	3.34

Although measured and observed time series for NH_x and NO_2^- concentrations in the T_6 and T_5 were generally captured well, the model performed poorly in other treatments. This is due to the apparent inhibition effect of free NH_3 on the nitrifiers at a high level of ammonia N concentrations.

Table 3.9 compares best fits from all treatments. The ratio of optimized k_{nit} to calibrated T_5 model (T_5-k_{nit}) was used as a quantitative index of the extent of nitrifiers inhibition by NH_3.

Table 3. 9. Optimized k_{nit} for treatments operated under different NH_x concentrations with and without aeration and the ratio of nitrifiers inhibition by NH_3.

Treatment	Optimised k_{nit} (day^{-1})	Optimised k_{nit} /T_5 k_{nit}
T_1	0.16	4.8
T_2	0.17	5.3
T_3	0.09	2.8
T_4	0.08	2.5
T_5	0.04	1.2
T_6	0.05	1.5

These results suggest different extents of inhibition effects of free NH_3 on nitrification as a consequence of nitrifiers activity depression. High ratio of the optimized k_{nit} to calibrated k_{nit} were observed in the treatments treated with low NH_x concentrations, while low ratio was associated with treatments with high concentration of NH_x. High index indicates low inhibition effect of NH_3 on nitrification at low concentration of ammonia, but low index indicates higher inhibition with higher concentration.

3.4 Conclusions

The findings presented in this chapter suggest that the changes in the surface area of floating mat matrix can affect ammonia removal via nitrification. There was an approximately linear relationship between the removal rate constant and mat surface area. The higher ammonia removal efficiency was caused by a larger surface area which could support the growth of more microbes. There was also a clear inhibitory effect on second stage nitrification in experiment 1 manifested as low production of nitrate. This is probably due to very high ammonia concentrations. This was further investigated in experiment 2 in which ammonia concentration was varied. Here, there appeared to be an effect of increased NH_x concentration on nitrification (with a higher rate of nitrification in treatments with lower initial NH_x concentrations) which confirm the free ammonia toxicity hypothesis. However, NO_3^- production was still not observed. There was no major effect of oxygen saturation on NH_x removal using aerated and non-aerated conditions.

Understanding of mineral N dynamics in the experiments described here was facilitated using a simple dynamic model. In experiment 1, the model was calibrated using experimental data from one treatment (T_5) and validated on the other treatments after simple linear adjustments reflecting a *priori* hypotheses about the contribution of mat surface area. Considering the relatively simple nature of process representation, the model did a fairly good job in predicting N concentration dynamics and removal efficiencies. The findings also support the hypothesis underpinning the conversion of rate constants obtained via calibration in treatment T_5.

Overall the findings show that increasing surface area of the floating mat improves the efficiency of ammonia removal, and that high free ammonia concentrations can depress nitrification. The work demonstrates the use of a simple dynamic model as a framework to improve a mechanistic understanding of NH_x removal under different experimental conditions. These findings will be used as a platform for designing the mesoscale and pilot-scale studies described in chapters 4 and 5, respectively. Upscaling the knowledge of ammonia behavior obtained from well-controlled lab-experiment to less controlled field studies has the potential to improve understanding of the ammonia behavior at different experimental conditions.

Chapter Four – The Contribution of Different Processes in Removing Ammonia in Floating Treatment Wetlands

4.1 Rational

In this chapter, mesocosm experiment was conducted in order to quantify the relative contribution of different processes in removing ammonia under different design criteria and to demonstrate the most critical FTW design for ammonia removal. The magnitude of the nitrification, plant uptake, and volatilization in removing ammonia was investigated using FTW system operating under steady-state flow conditions with different design parameters (two water depths and three levels of mat coverage, including control and two different plant destinies). The study answers several scientific questions: (1) How might different water depths impact ammonia removal capability with and without floating mats? (2) Is the overall performance in terms of ammonia removal directly proportional to the mat coverage area? (3) Does the plant density influence ammonia removal? Each experimental treatment was described using a simple model in order to disentangle the relative contribution of different processes to overall performance.

The first hypothesis of this study is that shallower systems promote higher ammonia removal rates than deeper systems. The second hypothesis is that ammonia removal rates are higher with the system fully covered by mat than when semi-covered. The third hypothesis is that higher removal efficiency of ammonia is associated with higher vegetative density than lower, or indeed without.

4.2 Methods

4.2.1 Experimental

A mesoscale FTW system was operated under real weather conditions in Brookfield campus at the University of Leicester site location (52.3814° N, 1.0754° W), Leicestershire, UK. The experimental design was aim to simulate environmental conditions in an actual domestic wastewater treatment system under different design criteria. The experimental setup included ten treatments with three replications following a completely randomized block design (Figure 4.1).

Thirty experimental mesocosms were established. Each mesocosm consisted of an 80 L polyethylene tank (length 58 × width 38 × depth 48.5 cm) which was subjected to the following treatments: half the mesocosms used shallow water depths (0.2 m, volume 36 L) and half were deep (0.4 m, volume 72 L). In each half (shallow or deep) there was a control treatment which contained no floating mat (C_1 and C_2, respectively, for shallow and deep). All remaining treatments contained floating mats at either full coverage of the water surface (100%) or half (50%) coverage. The floating mats were constructed from a loose matrix of extruded plastic injected with polystyrene foam to increase buoyancy and were obtained from Frog Environmental, UK. The mats were either vegetated or unvegetated, using rushes (*Juncus effusus*, order: Poales, family: Juncaceae) (McCorry and Renou, 2003) with a density of two plants per mat for 50% coverage or four plants per mat for 100% coverage. Vegetated mats were drilled with 7 cm diameter holes to accommodate pots containing seedlings of plants and used a bed of sawdust to support the plant settlement. Plant roots were washed carefully to remove all attached soil before insertion. All macrophytes had the same growth history, maturity and originated from the same batch at a local nursery. The different treatments are summarized in Table 4.1.

Table 4. 1 Summary of experimental treatment characteristics and treatment codes.

Treatment	**C_1**	**M_1**	**M_2**	**V_1**	**V_2**	**C_2**	**M_3**	**M_4**	**V_3**	**V_4**
Water depth(m)	0.2	0.2	0.4	0.2	0.4	0.4	0.2	0.4	0.2	0.4
Mat cover (%)	0	50	50	50	50	0	100	100	100	100
Plants per mat	0	0	0	2	2	0	0	0	4	4

I

II

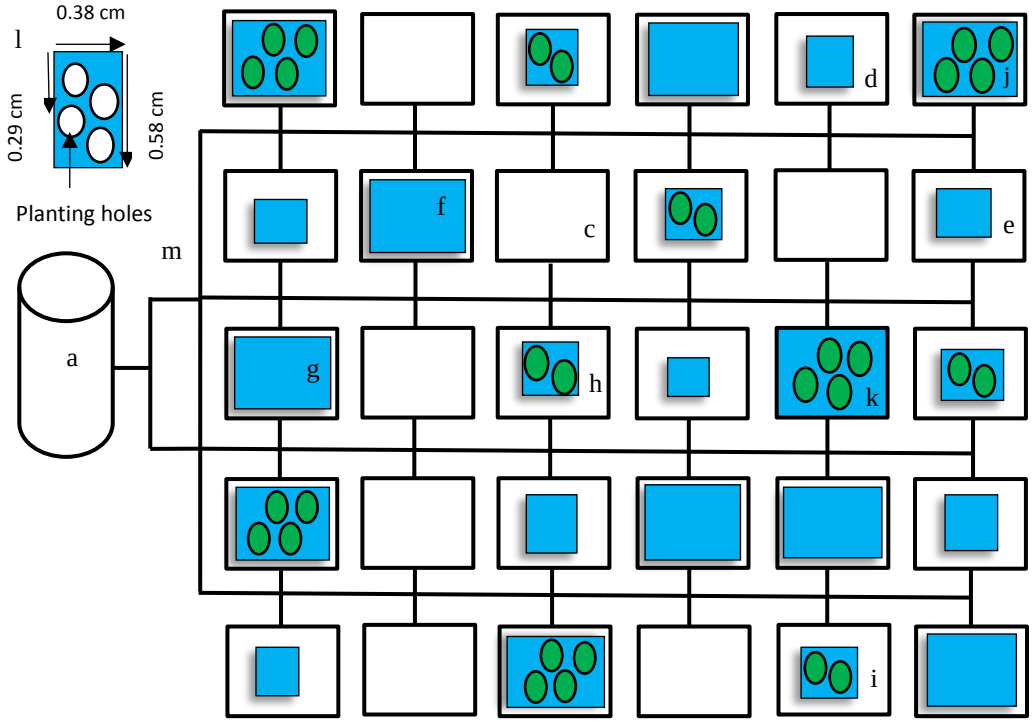

Figure 4. 1 Experimental set-up: (I) Photo of the treatments in the mesocosm experiment, (II) a-top view of the wetland series and mesocosm floating system. (a) central tank; (b) C_1; (c) C_2; (d) M_1; (e) M_2; (f) M_3; (g) M_4; (h) V_1; (i) V_2; (j) V_3; (k) V_4; (l) floating mat and (m) pumping system.

All treatments were subjected to a steady-state continuous flow regime in which an influent containing a relatively high concentration of ammonia was pumped into each tank at a flow rate of 5.1±0.2 L day^{-1} for the shallow treatments and 10.3±0.5 L day^{-1} for the deep treatments. Each tank was allowed to overflow via an outlet tube which could be sampled periodically. Since the concentration of the influent water was always the same (9.4 mg N L^{-1}) for each treatment, the mass loading rate of ammonia was 48.2±0.5 mg N day^{-1} for the shallow treatments and 96.4±1.1 mg N day^{-1} for the deep treatments.

Prior to the experimental phase (6 weeks), each tank was operated under steady-state conditions for eight weeks 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, the retention pond was empty and influent water was therefore obtained from the domestic supply and dechlorinated using an activated carbon dichlorination filter prior to pumping into a central holding tank (~210 L) where it was spiked with an ammonium stock solution (6.2 g NH_4Cl L^{-1}) on a daily basis to create a constant initial concentration of approximately 9.4 mg N L^{-1}. In addition to simulate ammonia concentration in diluted domestic wastewater (≤ 12 mg N L^{-1}), the choice of applied this concentration was to avoid any possibility of nitrification inhibition due NH_3 toxicity at higher ammonia concentrations (≥ 15 mg N L^{-1}) based on outcomes obtained from the lab experiments. This was passed via three intermediate reservoirs to each tank using a system of eight dosing pumps which were calibrated to achieve the required flow rates. Water samples (50 mL) from the outflow of each experimental tank were collected over the experimental phase.

4.2.1.1 Water and mass balance

The hydraulic profile of the mesocosm tanks during the study was evaluated using influent and effluent flow rates combined with local meteorological data.

Since the inlet flow was controlled by setting the pump rate, outflow rates were calculated based on the water balance equation:

$$Q_{out} = Q_{in} - ET_{tank} \qquad (4.1)$$

where Q_{out} is the effluent flow rate (L day^{-1}), Q_{in} is the influent flow rate (L day^{-1}), and ET_{tank} is evapotranspiration of the mesocosm tank (L m^{-2} day^{-1}). Outflow was estimated for each mesocosm tank on a daily basis.

The following equation was used to estimate flux rates (J) for NH_x in the influent and effluent of each mesocosm (Alley et al., 2013):

$$J = C \times Q \qquad (4.2)$$

where J is the flux rate of NH_x (mg N day^{-1}), C is the concentration of the NH_x in the inflow or outflow water (mg N L^{-1}) and Q is the daily inflow or outflow rate (L day^{-1}).

Mass removal of NH_x was determined as:

$$M_r = J_{in} - J_{out} \qquad (4.3)$$

where M_r is the net NH_x mass removal rate (mg N day^{-1}), J_{in} is the inflow of NH_x (mg N day^{-1}), and J_{out} is the outflow of NH_x (mg N day^{-1}).

The removal efficiency of NH_x during the study was estimated as follows:

$$M_E = \frac{M_r}{J_{in}} \times 100\% \qquad (4.4)$$

where M_E is the mass removal efficiency of NH_x (%).

Assuming ammonia loss via NH_3 volatilization is negligible, the net rate and contribution of the nitrification process to the overall removal can be estimated as follows (Gao et al., 2017):

$$J_{nit} = M_r - J_{up} \qquad (4.5)$$

where: J_{nit} = nitrification rate (mg N day^{-1}); M_r = overall mass removal rate (mg N day^{-1}); J_{up} = plant uptake rate (mg N day^{-1}) and J_{vol} = volatilization rate (mg N day^{-1}).

$$C_E = \frac{J_{nit}}{M_r} \times 100\% \qquad (4.6)$$

where: C_E = nitrification or plant uptake efficiency (%); R = nitrification rate or plant uptake (mg N day^{-1}); M_r = total N mass removal (mg N day^{-1}).

4.2.2 Model

A system dynamics model was constructed to represent mineral N dynamics in the experimental system. Extensive details of the model's construction are presented in Chapter 2. To implement the model for the continuous flow system described above, each mesocosm was assumed to behave as a continuously stirred tank reactor (CSTR) with loss processes occurring via first-order kinetics (Marimon et al., 2013). The influx rate of NH_x (mg N day^{-1}) in the influent was assumed to be the product of the inflow rate (Q_{in}: L day^{-1}) and the influent NH_x concentration (mg N L^{-1}).

In the model, ET was assumed to have a negligible effect on the net water balance, therefore Q_{out} was assumed to be equal to the inflow rate (Q_{in}). Ammonia volatilization is generally insignificant below a pH of 7.3, but can account for nearly 10% of the total ammonia in aquatic systems at pH of 8.3 (Poach et al., 2002). The rate constant for volatilization (k_{vol}) was estimated as the combined mass transfer coefficient for volatilization as derived using two-film resistance theory (Kadlec and Wallace, 2009b) divided by the water depth (Whelan et al., 2010). Since the average water pH throughout the experimental phase was 6.4, volatilization was found to be negligible in any case (i.e., value of mass transfer coefficient-k_{vol} were estimated to be 0.003 h^{-1} (0.08 day^{-1}) and 0.001 h^{-1} (0.04 day^{-1}) for the shallow and deep systems, respectively.

The model was calibrated using data from the V_4 treatment (deep water with full mat cover and plants, Table 4.2). This was achieved by minimizing the root mean square error (RMSE) between the simulated and measured steady-state concentrations in a trial and error optimization of the parameter k_{nit} (Rousseau et al., 2004). The model was run over 40 days and employed Euler's method of integration with a time step of 0.25 day.

Table 4. 2 Description of parameters used in the V_4 model.

STELLA symbol	Description	Value/Units	Source
State variables			
NH_x	NH_3+NH_4^+ mass	mg N	Calculated, V_4 cell
NO_x	NO_2^-+NO_3^- mass	mg N	Calculated, V_4 cell
Plant $_{uptake}$	Total-N content in plant	mg N	Measured, V_4 cell
Volatized NH_3	Free ammonia volatilization	mg N	Estimated, V_4 cell
TIN $_{dischg}$	Total-inorganic discharge	mg N	Calculated, V_4 cell
Flow variables			
NH_x load	NH_3+NH_4^+ loading rate	mg N day^{-1}	Measured, V_4 cell
J_{nit}	Nitrification rate	mg N day^{-1}	Calculated, V_4 cell
J_{vol}	Volatilization rate	mg N day^{-1}	Whelan et al. (2010)
$J_{up\ NHx}$	Plant uptake rate of NH_x	mg N day^{-1}	Estimated, V_4 cell
$J_{up\ NOx}$	Plant uptake rate of NO_x	mg N day^{-1}	Estimated, V_4 cell
NH_x $_{dischg}$	NH_3+NH_4 surface discharge	mg N day^{-1}	Measured
NO_x $_{dischg}$	NO_2+NO_3 surface discharge	mg N day^{-1}	Measured
Parameters/coefficients			
Inflow $_{[NHx]}$	Influent NH_3+NH_4 concentration	9.38 mg N L^{-1}	Measured
$[NH_x]$	NH_3+NH_4 concentration	mg N L^{-1}	
$[NO_x]$	NO_2+NO_3 concentration	mg N L^{-1}	
k_{nit}	nitrification rate constant	1.71 day^{-1}	Calculated, V_4 cell
$k_{up\ NHx}$	Uptake rate constant for NH_x	0.012 day^{-1}	Estimated, V_4 cell
$k_{up\ NOx}$	Uptake rate constant for NO_x	0.012 day^{-1}	Estimated, V_4 cell
k_{vol}	Mass transfer coefficient of NH_3	0.04 day^{-1}	Estimated, V_4 cell
NH_3 f	Concentration of NH_3 gas	0.006 mg N	Estimated, V_4 cell
Q	Flow rate	10.27 L day^{-1}	Measured, V_4 cell
V	Operational volume	72 L	Measured, V_4 cell

The mass balance for NH_x can be written as:

$$V.\frac{d[NH_x]}{dt} = Q_{in}.[NH_{x\,in}] - k_{vol}.f_{FREE}.[NH_x].V - k_{nit}.[NH_x].V - k_{up\,NH_X}.[NH_x].V - Q_{out}.[NH_{x\,out}] \quad (4.7)$$

where $[NH_{x\,in}]$ is the ammonia concentration in the influent (mg N L^{-1}), $[NH_{x\,out}]$ is the ammonia concentration in the effluent (mg N L^{-1}), k_{vol} is the mass transfer coefficient of free ammonia across the air-liquid interface by volatilization (day^{-1}), f_{FREE} is the fraction of ammonia which is present as free NH_3 (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}), $k_{up\ NHx}$ is a rate constant for ammonia uptake by plants (day^{-1}), and Q_{out} is the discharge in the outflow (L day^{-1}).

The mass balance for oxidized N can be written as:

$$V.\frac{d[NO_x]}{dt} = V.k_{nit}.[NH_x] - k_{upNO_x}.[NO_x].V - Q_{out}.[NO_{x\,out}] \quad (4.8)$$

where [NO_x] is the total oxidized-N concentration (mg N L^{-1}), [$NO_{x\ out}$] is the total oxidized concentration in the effluent (mg N L^{-1}), and $k_{up\ NOx}$ is a rate constant for oxidized-N uptake by plants (day^{-1}). Plant uptake is assumed to occur for both NH_x and NO_x. Note that, here, there is no distinction between first and second stage nitrification and that denitrification is assumed to be negligible.

The measured average TN uptake was calculated to be 0.025 g N day^{-1}. With between two and four plants per square meter, this is within the range of plant uptake rates reported in the literature of between 0.002-2.8 g m^{-2} day^{-1} (Wang et al., 2014). Despite there being no analytical indication of the preference of the plants for either NH_4^+ or NO_3^-, ammonium uptake is presumed to be more favoured than nitrate uptake in this study. NH_4^+ is known to be readily assimilated into plant tissues, as aquatic macrophytes demand utilization of enzymes (nitrate reductase and nitrite reductase) to convert oxidized N to usable forms (Kadlec and Wallace, 2009b); moreover, the production of these enzymes decreases when ammonium is presented in high concentrations (Hammer, 1989). This is consistent with the present study, where a relatively high ammonia concentration was continuously applied to the system.

The V_4-calibrated parameters were adjusted for the other treatments as follows: k_{nit} was assumed to be inversely proportional to water depth, i.e., 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); 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); k_{up} was assumed to be proportional to the number of plants in the system (thus, uptake rates for systems containing four plants would be twice those in systems containing two). For controls, as systems with no mats, the effect of the depth parameter was considered, but the effects of mat area and plant density parameters have been neglected.

However, unexpected autotrophic growth can create a heterogenous environment where NH_x concentration is gradually decreased, leading to variation in typical k_{nit} values. Therefore, via trial and error, k_{nit} for the controls has been adjusted to allow the best fit between measured and simulated data. Following these simple adjustments, the model predictions were compared with the observed concentrations in independent treatments. This can be regarded as a validation of the model since no further (optimizing) parameter adjustments were made.

4.3 Results and Discussion

4.3.1 Experimental data

Water balance, N forms dynamics, plant/microbial performance, mass balance and physicochemical responses of the treatments with and without FTWs under 0.2 and 0.4 m depths, 50 and 100% coverage area and low and high plant densities were compared and presented in the following sections.

4.3.1.1 Water Balance

Average changes in the water budget of mesocosm cells during the study are shown in Figure 4.2 which shows daily water loss and gain via evapotranspiration and rainfall and outflow of each cell.

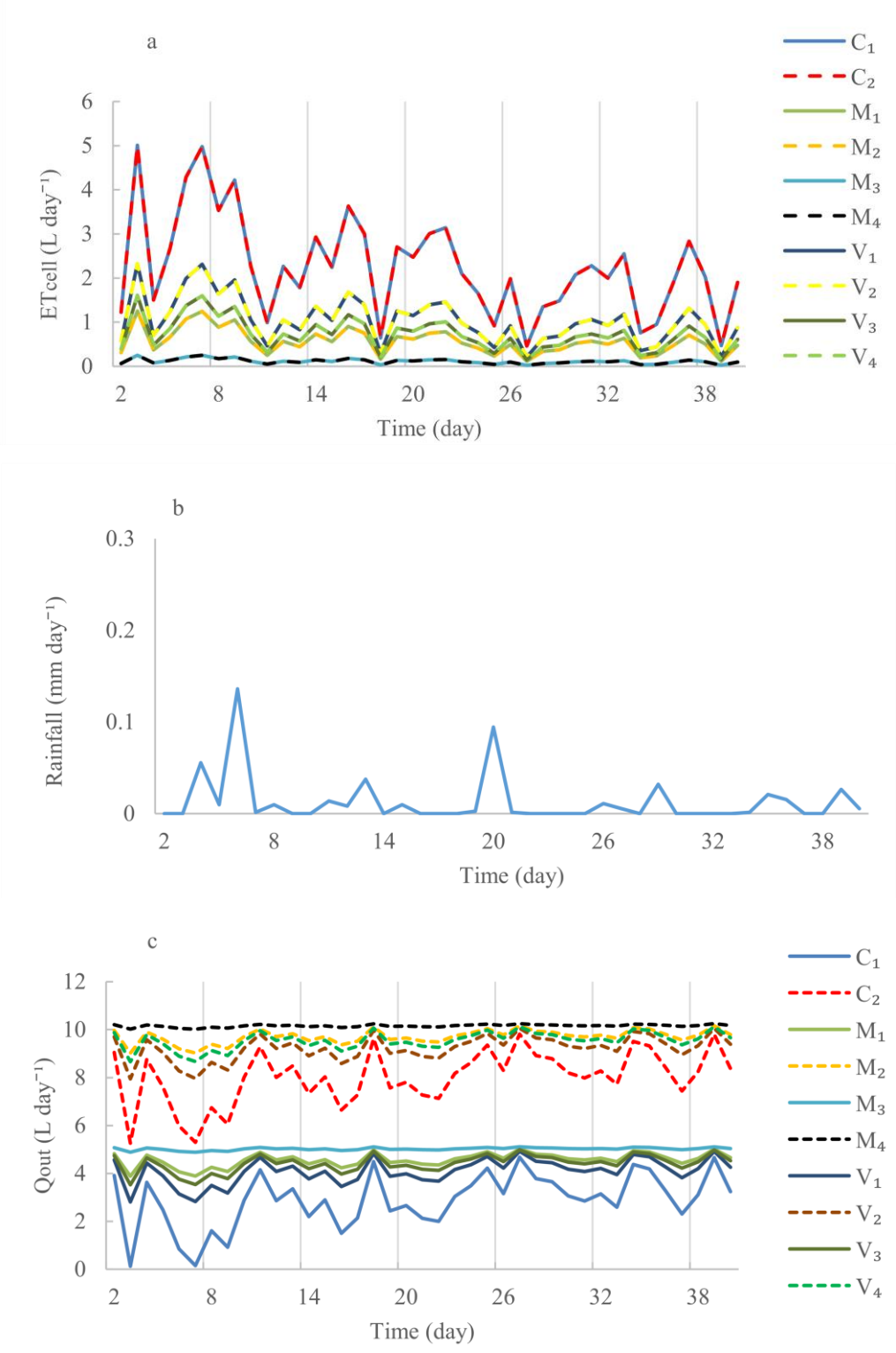

Figure 4. 2 (a) Evapotranspiration, (b) rainfall and (c) outflow of mesocosms during the study.

A nominal 7 days hydraulic retention time (HRT) was achieved by calibrating water inflow rates to the system. The operational mass loading rates (*J*) for treatments were calculated based on the inflow rates entering the shallow and deep systems. Through incorporating ET_{cell} coefficients in the daily water budget, the mean flows leaving the wetland series were relatively smaller than the mean flows entering the systems (Table 4.3). Water loss (WL) values for both FTW treatments were less than those of the controls. Treatments with plants had higher WL than those without plants during the study.

Table 4. 3 Average (±SD) inflow rate (Q_{in}), outflow rate (Q_{out}), evapotranspiration (ET_{cell}), water loss (WL, %) and mass loading rate (*J*) for wetland series at operational water depths (*Ƶ*) and volumes (*V*). *J* = mean (range).

Ƶ (m)	**V (L)**	**Q_{in} (L day^{-1})**	**Q_{out} (ET_{cell}) (L day^{-1}) (WL %)**					**J (mg N day^{-1})**
			C_1	**M_1**	**M_3**	**V_1**	**V_3**	
0.2	36	5.1±0.2	2.7±0.2 (2.4±0.2) (46%)	4.5±0.1 (0.6±0.1) (11%)	5.0±0.01 (0.1±0.01) (2%)	4.0±0.1 (1.1±0.1) (16%)	4.3±0.1 (0.7±0.1) (13%)	48.2±0.5 (45-52)
			C_2	**M_2**	**M_4**	**V_2**	**V_4**	
0.4	72	10.2±0.5	7.8±0.2 (2.4±0.2) (23%)	9.6±0.1 (0.6±0.1) (5%)	10.1±0.01 (0.1±0.01) (1%)	9.1±0.1 (1.1±0.1) (9%)	9.4±0.1 (0.7±0.1) (6%)	96.4±1.1 (91-104)

Based on the results obtained, water loss in M treatments was between 1-11%, while it was between 6-16 % in V treatments. The differences in the water budget between the planted and unplanted treatments could be due to the evapotranspiration, although the coverage effect of the floating mat likely restricts water loss in both FTW systems compared to the controls. Higher ET rates in V treatments could be attributed to the higher plant growth and the larger amount of biomass observed, and thus increase biological activity for evapotranspiration through mass transfer from the root zone to the atmosphere. Allen et al. (1998) stated that the plant transpiration rates may increase by an average of 10-30% when a specific surface area covered by a plastic cover (e.g. sheets of polyethylene) compared to uncovered area under specific cultivation practices. This increase is caused by the transfer of both sensible and radiative heat from the surface of the plastic cover to adjacent vegetation. However, a reduction by 50-80% of evaporation rates from the wetted surface was reported when using full coverage of these materials.

When the plastic material does not entirely cover surface area, then the reduction in evaporation rate will be less. This is consistent with the obtained results when the treatments with full coverage of floating materials achieved lowest water loss compared to those with semi-covered. The water budget in the C cells reduced by 23% and 46% in the deep and shallow systems, respectively. The greater WL for C_1 treatment was possibly due to the high ratio of the air-water interface area per unit volume and therefore more contact with atmospheric conditions compared to C_2 treatment, which may facilitate higher rates of evaporation. In any circumstances, none of the wetland series showed a negative WL as a response to the rainfall during the study. The daily average of rainfall (0.013 ± 0.01 mm day^{-1}) was low during the experimental time, which have not indicated a substantial impact on the water budget of the mesocosms.

4.3.1.2 Ammonia Removal Performance

Ammonia removal in the shallow wetland series was greater than for deeper systems throughout the study (ANOVA, $F_{9,120} = 41.018$; $P < 0.05$). For the wetland series (C_1, M_1, V_1, M_3 and V_3), average effluent NH_x were lower than for the C_2, M_2, V_2, M_4 and V_4 series, respectively (Figure 4.3). Removal rate coefficients for shallow systems (C_1, M_1, V_1, M_3 and V_3) were higher than those for deep systems (C_2, M_2, V_2, M_4 and V_4). Consistently, shorter degradation half-lives for NH_x concentrations ($T_{1/2}$) were observed in the shallow series compared to the deep (Table 4.4). Removal rate coefficients for the NH_x and increased water depths were negatively correlated ($r^2 = 0.60$; $p = 0.05$), whereas a insignificant correlation for removal half-life and water depth was observed during the study ($r^2 = 0.26$; $p = 0.05$). As a general concept, an inverse relationship between the first-order rate coefficient (day^{-1}) and water depth was proposed by Kadlec and Wallace (2009b) for the design of free surface wetland performance. Therefore, a directly proportional relationship between the degradation half-life of the contaminants and water depth was expected to be observed. Overall removal efficiencies were higher in the shallow treatments, but lower in deep ones. One reason for this is that the shallower systems have a greater bed surface area and, where present, of mat material per unit volume. This means that nitrifiers living in fixed biofilms have greater access to NH_x in the water column. The redox potential may also be higher in shallower systems because the ratio of the air-water interface area to volume is higher (García et al., 2005; Matamoros and Bayona, 2006; Holland et al., 2004).

Oxygen bioavailability is one of the main limiting factors dictating the removal rates of nitrogenous compounds in CWs (Nivala et al., 2013). These findings were consistent with the previous investigation conducted by Kotti et al. (2010), who observed higher removal performance for ammonia (18.26 mg N L^{-1}, equivalent to 53.9%) in five pilot-scale free surface constructed wetlands operating under shallow depth (0.10 m), a range of flows (18–58 L day^{-1}) and 14 day HRT. Further, Sanchez-Ramos et al. (2017) found that the shallower pilot-scale horizontal subsurface flow constructed wetland (0.27 m), as operated under flow rates between 20-45 mm day^{-1} and 2.4-5.4 day HRT, revealed higher removal efficiencies for NH_4^+ (73.8%) than deeper systems (0.5 m) operated under 4.4-10 day HRT, during a 3-year monitoring period.

For control series, periphyton growth on the experiment tank surface improved the NH_x removal efficiency considerably. Such unexpected autotrophic microorganism growth (e.g. algae) created a heterogeneous environment between the control and FTW wetland groups. Therefore, the C-group is not suggested as a control for retrofitted systems during the experimental period. Even though C-series showed a gradual decrease in NH_x concentration, retrofitted treatments reflected a significant improvement over the former. Regarding the effect of mat surface area and the associated microbial biofilm, NH_x removal in M-wetlands was significantly improved over the C-group (Figure 4.3b). A positive correlation was observed between mat area and removal rate ($r^2 = 0.60$; $p = 0.05$), but a negative correlation was observed with the half-life of NH_x concentrations ($r^2 = 0.37$; $p = 0.05$). Removal efficiencies were higher when mat area was increased, i.e., the overall NH_x removal efficiencies observed increased from 93 and 77% in M_1 and M_2 to 93 and 85% in M_3 and M_4, respectively. This indicates accelerated nitrification rates by increasing attachment surface area for the microbial biofilm that develops within such systems (Table 4.4). These results are consistent with the findings obtained from the lab experiments when removal rates of NH_x became higher with the increase of surface area available for microbial growth. These findings are also in good agreement with (Zhi and Ji, 2014; Zhang et al., 2016), who documented that the enhancement of ammonia reduction, which is associated with higher NO_x production due to treatment with mat material (90% coverage area) that relied mainly on the biofilm activity. However, the current results showed a low removal efficiency of NH_x compared to other, previous studies (Wang and Sample, 2014; Gao et al., 2017), who used a full coverage of floating mat and bio-carriers to remove N from natural and simulated influent, respectively.

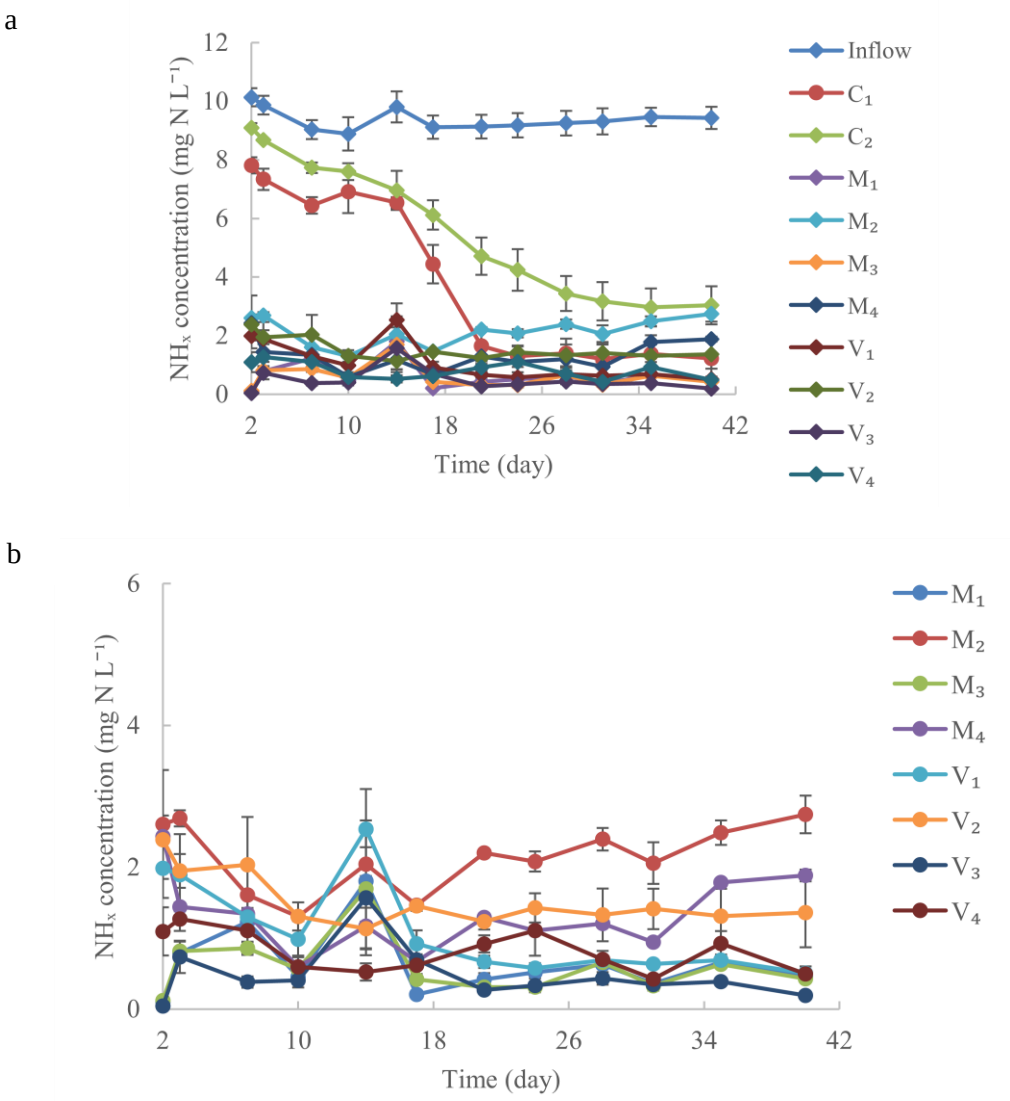

Figure 4. 3 NH_x concentration variations in (a) all treatments and (b) M and V series during experimental time (mean ± standard deviation).

For the planted treatments, the V-series showed an enhanced removal of NH_x than the corresponding M and C groups based on the effects of established vegetation (Figure 4.3b). Higher rate constants were observed in the V-series compared to the M- and C-series (Table 4.4). Removal $T_{1/2}$ for NH_x concentrations within the V series were shorter compared to M and C series, respectively. The correlation coefficient for the removal rate constants and increased plant density was positive ($r^2 = 0.67$; $p = 0.05$). Conversely, the half-lives of NH_x concentrations were negatively correlated with the abundance of the plants ($r^2 = 0.89$; $p = 0.05$).

The greater NH_x removal was observed for the V-series compared to the M- and C-series in the current study was consistent with the findings of (Wang and Sample, 2014; Zhang et al., 2016). These authors reported that the N removal in the vegetated treatments was mainly dependent on the combined impacts of plant uptake and microbial activity.

Table 4. 4 Average effluent fluxes (mg N L^{-1}), rate coefficients (*k*, day^{-1}), half-lives ($T_{1/2}$, day), and efficiencies (*RE*, %) for the N forms in the wetland cell effluents for the mesocosm tanks (mean ± standard error of the mean.

	NH_x				NO_2^-				NO_3^-
	Effluent flux (mg N L^{-1})	***k*** (day^{-1})	$T_{1/2}$ (day)	**%**	**Effluent flux** (mg N L^{-1})	***k*** (day^{-1})	$T_{1/2}$ (day)	**%**	**Effluent flux** (mg N L^{-1})
C_1	3.9 ± 0.8^{a}	0.4 ± 0.1^{ae}	7.1 ± 2.2^{d}	57	2.3 ± 0.6^{abc}	5.7 ± 3.6^{abc}	2.3 ± 0.7^{abc}	74	2.7 ± 0.4^{a}
C_2	5.6 ± 0.6^{b}	0.1 ± 0.1^{a}	22 ± 9.9^{a}	39	2.2 ± 0.5^{abc}	7.3 ± 3.6^{abc}	2.0 ± 0.5^{abc}	75	2.1 ± 0.2^{a}
M_1	0.6 ± 0.1^{cd}	3.4 ± 0.9^{bc}	0.3 ± 0.1^{bc}	93	1.8 ± 0.7^{abd}	4.2 ± 3.0^{a}	2.3 ± 1.1^{abd}	80	6.3 ± 0.6^{b}
M_2	2.1 ± 0.1^{ae}	0.5 ± 0.1^{ade}	1.4 ± 0.1^{b}	77	4.4 ± 0.4^{ce}	0.2 ± 0.1^{b}	5.5 ± 1.2^{c}	52	3.9 ± 0.2^{c}
M_3	0.5 ± 0.1^{cd}	3.2 ± 0.8^{bc}	0.3 ± 0.1^{bc}	93	1.6 ± 0.4^{abd}	5.2 ± 1.8^{abc}	1.2 ± 0.4^{abd}	83	6.6 ± 0.4^{b}
M_4	1.3 ± 0.1^{efg}	1.0 ± 0.1^{def}	0.8 ± 0.1^{bc}	85	3.5 ± 0.6^{bc}	0.8 ± 0.3^{bc}	3.9 ± 0.8^{bc}	62	4.2 ± 0.4^{c}
V_1	1.1 ± 0.1^{cfg}	1.4 ± 0.2^{df}	0.7 ± 0.1^{bc}	87	0.4 ± 0.1^{ad}	$7\pm1.3.0^{a}$	0.2 ± 0.1^{ad}	95	4.7 ± 0.2^{c}
V_2	1.5 ± 0.1^{ef}	0.7 ± 0.1^{adef}	0.9 ± 0.1^{bc}	83	1.2 ± 0.4^{ad}	5.2 ± 1.4^{ac}	0.9 ± 0.3^{ad}	86	4.5 ± 0.3^{c}
V_3	0.4 ± 0.1^{d}	5.6 ± 2.4^{b}	0.2 ± 0.1^{c}	94	0.05 ± 0.1^{d}	35 ± 5.7^{d}	0.02 ± 0.1^{d}	99	1.0 ± 0.1^{d}
V_4	0.8 ± 0.1^{cdg}	1.7 ± 0.2^{cf}	0.4 ± 0.1^{bc}	91	0.5 ± 0.1^{ad}	6.3 ± 1.3^{a}	0.3 ± 0.1^{ad}	94	4.4 ± 0.3^{c}

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

Obtained results have demonstrated a range of combined effects of the examined factors, which have revealed different potential roles in ammonium removal. The synergistic effects of (1) shallow depth and full mat area; (2) shallow depth and higher vegetation density; (3) full coverage mat area and higher plant abundance have improved NH_x removal compared to the other combinations. The additive effects of the shallow depth and full mat area with higher plant density within V_3 treatment were identical to achieve the highest removal efficiency of NH_x during the study.

The average rate of ammonia loss as NH_3 by volatilization in the experimental system was calculated to be negligible in all treatments, as average water pH was about 6.4 throughout the experimental phase. Estimated average rate constants for NH_3 loss were found to be 6×10^{-4} and 3×10^{-4} day^{-1} in the shallow and deep systems, respectively.

The dynamics of NH_3 alongside with estimated average concentrations of NH_3 in the shallow and the deep systems throughout the study are presented in the Figure 4.4 and Table 4.5.

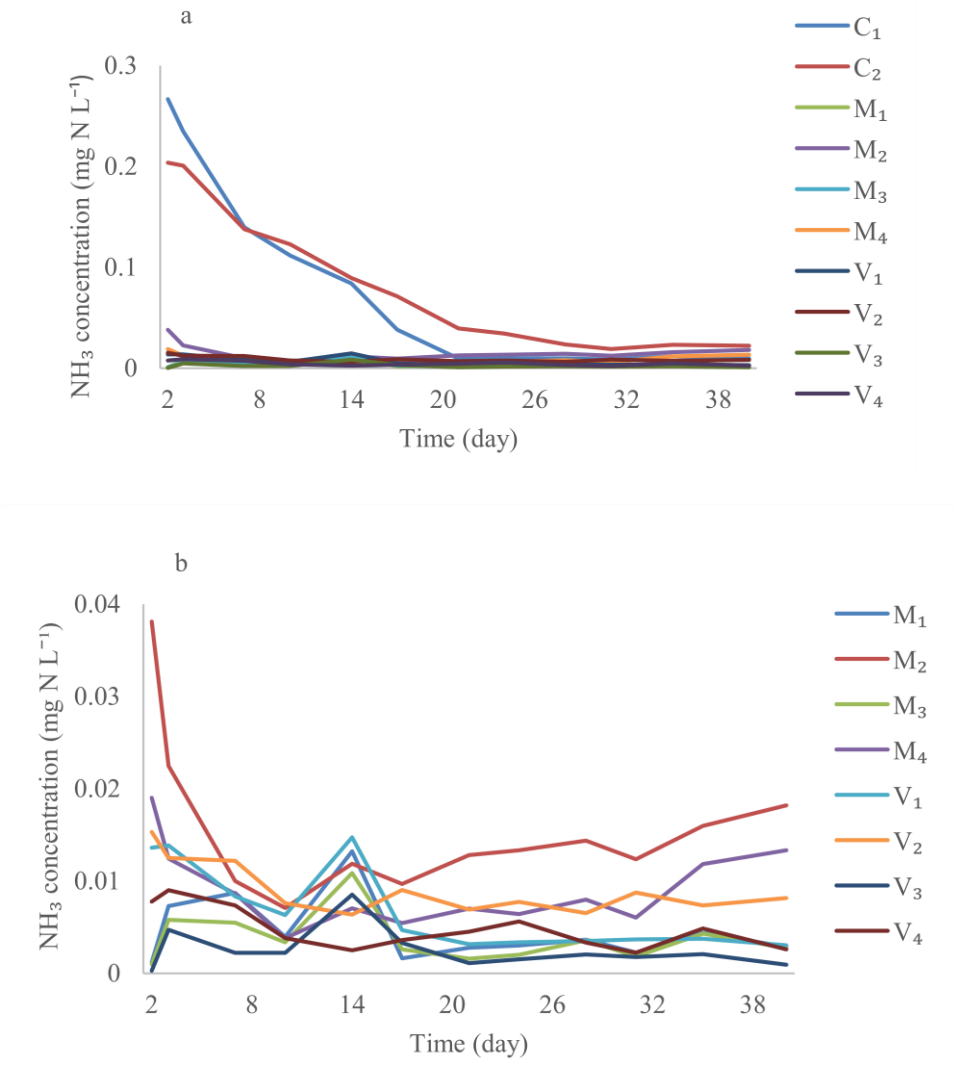

Figure 4. 4 Estimated concentrations of NH_3 in the mesocosm treatments

Table 4. 5 Average concentrations of NH_3 (mg N L^{-1}) in the mesocosms throughout the experiment.

Treatments									
C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
0.08 (±0.09)	0.08 (±0.06)	0.005 (±0.003)	0.01 (±0.01)	0.004 (±0.003)	0.01 (±0.004)	0.01 (±0.005)	0.01 (±0.003)	0.003 (±0.002)	0.005 (±0.002)

4.3.1.3 Oxidized Nitrogen Removal Performance

Figure 4.5 shows the changes of total oxidized nitrogen (NO_2^-+NO_3^-) concentrations in the treatments over the course of the experiment (ANOVA, $F_{10,132} = 42.790$, $P < 0.05$). The concentration of NO_x was high in most treatments except for the V_3 cell, which exhibited a lower effluent concentration. For the controls, a gradual increase in NO_x concentration was followed by tail-off to a constant concentration by the end of the experiment was observed.

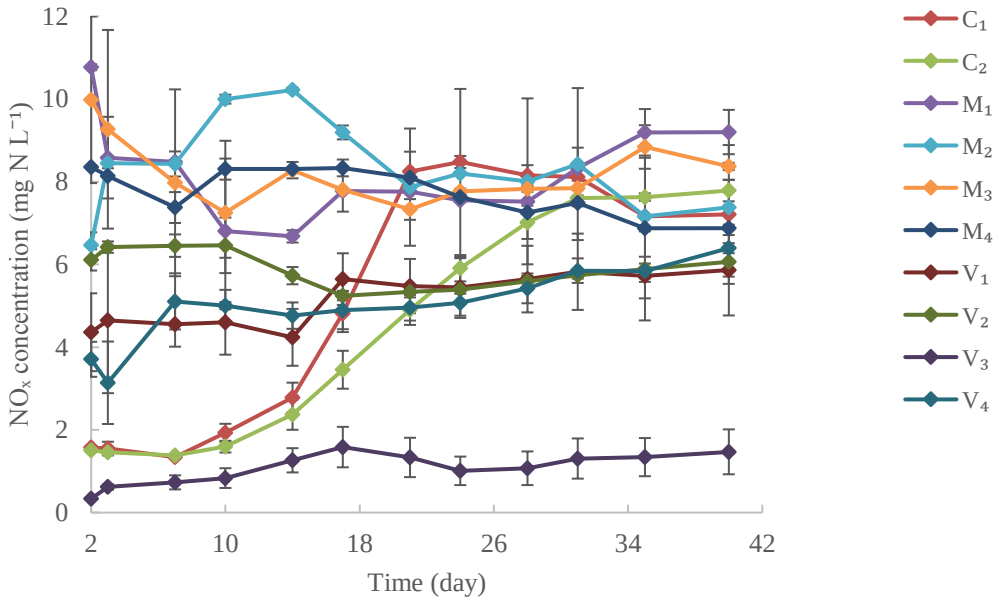

Figure 4. 5 Time series changes of effluent NO_x concentrations (NO_2^-+NO_3^-) in wetland series during the experiment (mean ± standard deviation).

In general, the dynamics of NO_2^- concentrations in the treatments during the study consisted of NH_4^+ reduction one the one hand, and NO_3^- production on the other. For FTWs with a 0.2 m depth**,** the extents of the removal and removal rate constants of NO_2^- were greater than for the wetland series with a 0.4 m depth (ANOVA, $F_{9,120} = 9.24$; $P < 0.05$). Consistently, shorter $T_{1/2}$ values for the NO_2^- concentrations in M_1, V_1, M_3 and V_3 were observed compared to M_2, V_2, M_4 and V_4 (Table 4.4). The larger surface areas available for microbial growth in the shallow systems could lead to an increased NO_2^- removal compared to the deeper systems (Figure 4.6).

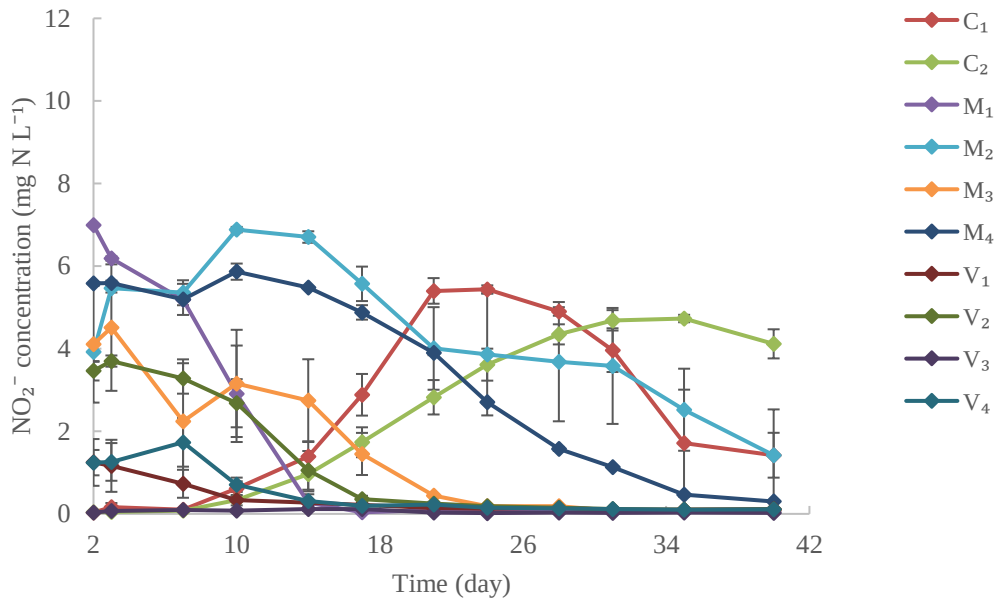

Figure 4. 6 Time series changes of effluent NO_2^- concentration in wetland series during the experimental time (mean ± standard deviation).

Removal rate constants for NO_2^- were found to decrease as depth increased ($r^2 = 0.39$; $p = 0.05$). Therefore, the $T_{1/2}$ for NO_2^- concentrations were found to be increased as depth increase ($r^2 = 0.39$; $p = 0.05$). Unexpectedly, NO_2^- concentrations within the controls exhibited a gradual increase followed by a reduction during the course of the experiment due to proliferating autotrophic biomass. For M-wetland series with 100% mat areas, the extents of removal of NO_2^- were greater than for the wetland series under 50% coverage. Average rate constants for the M series were as follows: $M_3 > M_1$, and $M_4 > M_2$. Accordingly, $T_{1/2}$ were shorter in the case of M_3 and M_4 than for M_1 and M_2 treatments, respectively (Table 4.4). Removal rate constants of nitrite correlated positively with the mat area ($r^2 = 0.19$; $p = 0.05$), but the correlation was negative between degradation half-life and mat area ($r^2 = 0.41$; $p = 0.05$). These results indicated that the nitrite oxidizers in the FTWs are more effective in converting NO_2^- to NO_3^- due to the increased surface area available for microbial growth. The production of NO_3^- may confirm the plausibility of this indication as will discuss later. Further, microbial analysis may support such explanation as will be discussed in the next section. Statistical analysis has indicated a positive correlation between rate constants of NO_2^- and the existing vegetation ($r^2 = 0.58$; $p = 0.05$), while it was negative between $T_{1/2}$ and vegetation ($r^2 = 0.59$; $p = 0.05$).

A portion of the nitrifying bacteria that attached to the flourishing roots, in addition to that which established on the floating mat and the internal surface area of the tanks may have contributed to the greater conversion rates of NO_2^-. Overall, the removal efficiency of effluent NO_2^- for the V-series was greater than for the M- and C-series (Table 4.4). The maximum removal efficiency of NO_2 concentrations was observed in the V_3 treatment.

For this study, the dynamics of NO_3^- concentrations were found to show a gradual increase during the course of the study (ANOVA, $F_{9,120} = 35.48$; $p < 0.05$) (Figure 4.6). NO_3^- production in the shallow series was greater than for the deeper systems ($r^2 = 0.13$; $p = 0.70$). Also, the extents of NO_3^- production for the M-series with 100% mat coverage were greater than for series under 50% coverage.

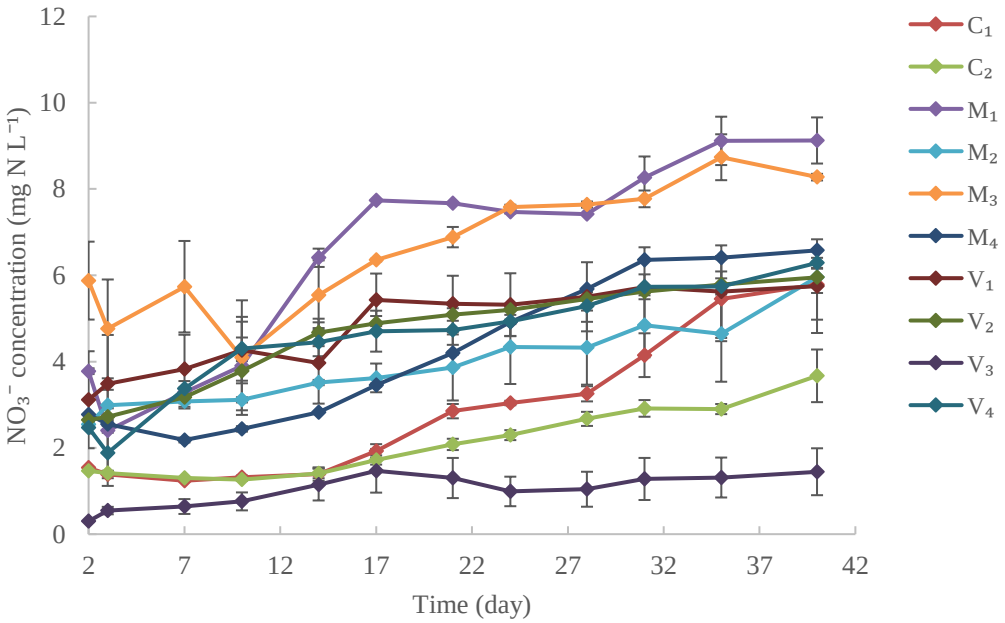

Figure 4. 7 NO_3^- concentration variations of the influent and effluent in wetland series during the experimental time (mean ± standard deviation).

Average effluent NO_3^- in the planted FTWs was lower than found for the unplanted series ($p < 0.05$), probably due to its direct uptake by plants. Further NO_3^- concentration was found to be lower as plant density increased ($r^2 = 0.61$; $p = 0.05$). Effluent NO_3^- for the V_3 treatment (1.02 mg N L^{-1}) was significantly lower than other treatments, probably due to some denitrifying activity in the system (Table 4.4). The lower levels of NO_3^- observed in the C group was mainly attributed to a periphyton outbreak, which considerably improved NO_3^- removal compared to the shaded treatments.

Overall, accumulated NO_3^- concentrations in the treatments suggest that the nitrification was the most plausible cause of this increase versus the loss of the microbial capacity of denitrifiers, probably due to the lack of biodegradable organics (Gao et al., 2017).

4.3.1.4 Microbial Biomass and Nitrification Rates

The microbial densities in the fixed biofilm on the mat material and plant root, as well as free bacteria within the water column in the treatments, were measured using the total viable count method-TVC analysis. Microbial biomass significantly differed across the treatments during the study, with microbe quantities being more numerous at the end of the study (ANOVA, $F_{9, 30} = 61.1$; $p < 0.05$). On average, bacterial growth rates within planted treatments were 0.18 ± 0.01 Log10 CFU g^{-1} day^{-1} compared to the unplanted treatments and controls at 0.13±0.01 and 0.015±0.002 Log10 CFU g^{-1} day^{-1}, respectively (Figure 4.7).

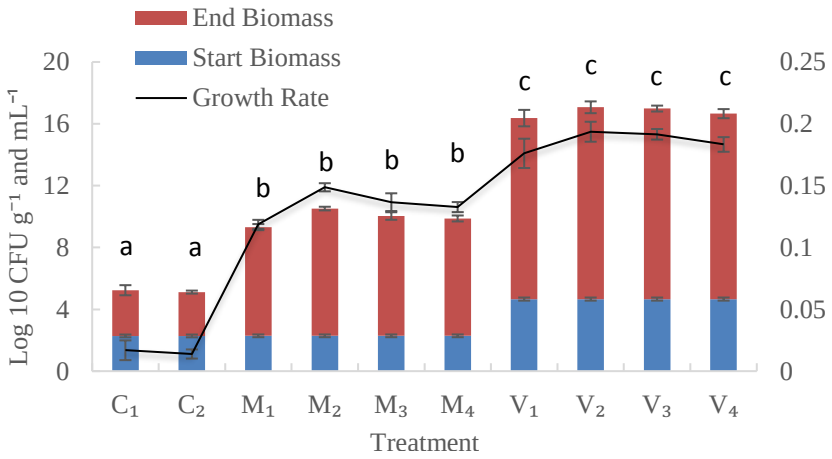

Figure 4. 8 Average microbial biomass and growth rates (± standard deviation) in wetland series at the start and end of the experiment. Upper letters denote the Tukey HSD test for multiple comparisons of means. Treatments with the same letter did not significantly differ.

Differences in the bacterial biomass in the treatments could be linked directly to the nitrification rates in these same systems. The average values for the nitrification rates in the unplanted, planted and control series throughout the study are presented in Table 4.6.

Table 4. 6 Nitrification rates (mg N day^{-1}) in the mesocosm cells.

Treatments									
C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
42.56 (±2.15)	57.7 (0±3.34)	45.89 (±0.68)	77.55 (±1.28)	45.23 (±0.73)	83.02 (±1.07)	38.24 (±0.72)	69.12 (±1.17)	27.69 (±0.62)	64.56 (±1.25)

The lower nitrification rates associated with higher ammonium removal rates in the V-series compared to M-series might be explained due to the competition between the direct uptake by the plants and nitrifying bacteria performance in removing NH_x during the experiment; this is discussed later with regards to the N mass balance.

4.3.1.5 Plant Growth and N-Tissue Content

The biomass of *Juncus effusus* in the V series was found to increase over the course of the study (Table 4.5). Plant growth rate in the V_4 treatment was greater than for other vegetated FTWs, but the differences were not significant (ANOVA, $F_{3,12} = 0.70$; $p < 0.05$). Such higher growth could be attributed to the use of proper water depth (0.4 m) that providing an adequate volume for a healthy root cover over the water column, while it is believed that beyond this depth plant roots may start to weaken (Deegan et al., 2007). Tanner and Headley (2011) reported maximum root depths ranging from 0.57 to 0.87 m for emergent wetland vegetation. The shoot system accounted for most of the plant biomass (shoot:root = 3.91±1.42). The N assimilated into the plant biomass at the end of the study was higher than the initial values. Overall, the findings obtained suggested that the treatments with higher plant abundance (V_3 and V_4) removed TIN mass to a greater extent than those with lower densities (V_1 and V_2) (ANOVA, $F_{3,12} = 7.03$; $P < 0.05$), with the associated ranking being, as follows: $V_1 < V_2 < V_3 < V_4$.

Table 4. 7 Average plant biomass, growth rate and tissue concentrations of TN (± standard error of the mean) in *Juncus effusus* per floating mat at the start and end of the experiment.

	Start biomass (g dw)	Start N content (mg N)	End biomass (g dw)	End N content (mg N)	Growth rate (g dw/day)
V_1	45.6 ± 0.4^{d}	624.9 ± 4.6^{d}	60.8 ± 3.6^{b}	924.8 ± 15.8^{b}	0.4 ± 0.1^{a}
V_2	90.1 ± 0.8^{a}	1359.8 ± 10.4^{a}	122.1 ± 5.5^{a}	1980.1 ± 54.1^{ab}	0.8 ± 0.1^{a}
V_3	105.5 ± 0.9^{b}	1487.8 ± 11.4^{b}	141.2 ± 10.4^{a}	2232.1 ± 19.3^{a}	0.9 ± 0.2^{a}
V_4	126.5 ± 1.2^{c}	2053.5 ± 15.8^{c}	170.2 ± 28.4^{a}	3044.3 ± 39.1^{a}	1.1 ± 0.7^{a}

Upper letters denote the Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

The daily mass removal rates via plant uptake were 7.72±0.47, 16.01±2.69, 19.168±4.705 and 25.55±1.08 mg N day^{-1} for V_1, V_2, V_3, and V_4, respectively. The accumulated mass of N by the vegetation at low density in the current study was consistent with the results obtained by Wang et al. (2014) who reported a TN uptake rate of pickerelweed-*Pontederia cordat*a (13 mg N day^{-1}) under low plant density (3 plants) during four experimental batches (28 days) using stormwater. Similarly, the current results with their higher plant density were consistent with the findings of Lynch et al. (2015), who demonstrated N uptake rates (25 and 26 mg N day^{-1}) for two FTW designs planted with nine specimens of *Juncus effusus* using pond water in a batch-loaded mesocosm experiment. However, the daily uptake rates of this study were lower than those obtained by Chang et al. (2012) with their recorded uptake rate of 36 mg N day^{-1} by *Juncus effusus* using lake water amended with fertilizers. Additionally, differences in the form of N showing preferential uptake by the plants may result in a more significant reduction of some sources of N than others (Marimon et al., 2013). The low NH_4^+:NO_3^- mass ratio (0.28±0.06, on average) in the effluent might indicate larger ammonium and lower nitrate uptakes in the V-series. A similar result was found by Li et al. (2015), who reported that NH_4^+ was the preferable form in terms of uptake by *Cana indica* rather than NO_3^- when both forms existed in the medium in different ratios.

4.3.1.6 Nitrogen Mass Balance

Even though the NH_x influx increased in the treatments as water depth increased, improved ammonia removal rates were observed in the FTW systems (ANOVA, $F_{9,120} = 28.96$; $p < 0.05$).

The influent applied to the wetland series contained average amounts of NH_x of 48.20 ± 0.55 and 96.41 ± 1.11 mg N day^{-1} for the shallow and deeper systems, respectively. On average, the overall NH_x removal rate in the M-series was between 45.9 ± 0.7 and 83 ± 1.1 mg N day^{-1}, and for the V-series was between 45.9±0.7 and 90±1.2 mg N day^{-1} for the total mass inflow to the system. The nitrification contributed 80-95% of the total ammonia-N removal in the M-series and 59-83% in the V-series (Figure 4.9).

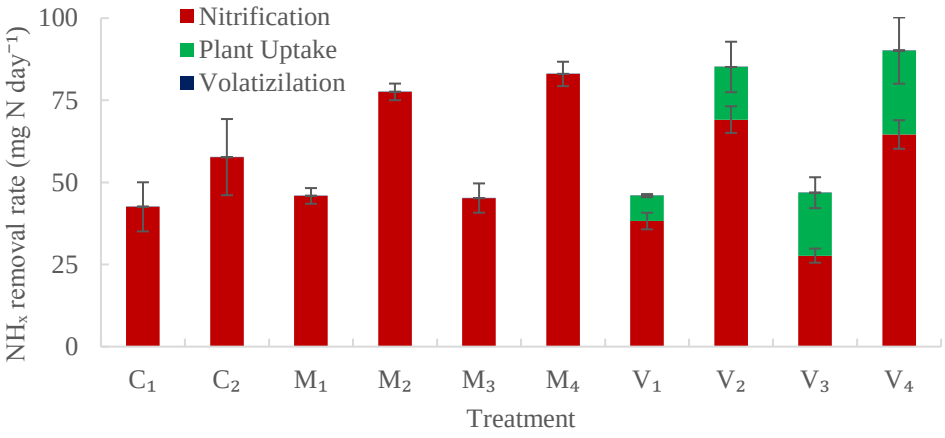

Figure 4. 9 Estimated removal pathway of NH_x in the treatments (mean ± standard deviation).

The results obtained support the concept that nitrification rates in the FTW systems increased with increasing surface area and time for microbial growth. These findings are consistent with the previous study conducted by Wang et al. (2015), who demonstrated higher ammonia removal efficiency (89.5 and 91.2%) when the attachment area for the microbial biofilm was increased through the use of two types of biological island grid in wastewater treatment. Likewise, mass removal rates via plant uptake increased with increasing plant biomass and density during the study (16-40% of the total NH_x removal). Plant related-processes may be more significant when vegetation is applied to the nutrient-rich environment in a high density (García-Lledó et al., 2011). For instance, nitrogen removal rates in FTWs planted with *Juncus effusus* with a density of 27 plants m^{-2} is 390 mg N m^{-2} day^{-1} during the growing season (White and Cousins, 2013), which is 15.3 times higher than the value found for V_4 treatment in our experiments. This significant difference in removal efficiency can be explained as the maximum plant density in the present study was only four plants per floating mat.

Although the V-series revealed higher microbial growth rates, lower nitrification rates in the planted FTWs rather than the unplanted could have been due to the competition between the developed microbial community and established vegetation on the ammonia source. Losses of NH_x in the control tanks were 88 and 59% for C_1 and C_2, respectively. The most likely reason for the greater ammonia mass removal in the control was attributed to the nutrient competition due to periphyton outbreak typical to such open systems.

Since volatilization was estimated to be negligible, the data obtained have provided direct evidence that nitrification is the main removal process for NH_x in the M and V treatments, as its products (NO_2^- and NO_3^-) were detected. This finding is consistent with previous studies which reported that the microbial activity was the main contributor to N removal (Reinhardt et al., 2006; Gao et al., 2017). In addition to the larger surface area that the floating mats offered for microbial development, it has been documented that the submerged roots of macrophytes grown hydroponically may support microbial biofilm growth (Li et al., 2010; Tanner and Headley, 2011; Wang and Sample, 2011). In addition to their role of providing a surface for the microbial growth, roots can also support aerobic respiration of the microbial population by oxygen release via radial oxygen loss (Saad et al., 2016). Wiessner et al. (2008) found a maximum release rate from *J. effusus* of 0.5 mg O_2 h^{-1}, which can support nitrifiers' activity in terms of removing NH_4^+.

4.3.1.7 Physicochemical Responses

During the experiment, pH, DO, EC and temperature were monitored. Figures 4.10 showing the dynamics of pH and DO concentration throughout the study. The pH and DO were significantly lower in the FTW systems than in the control group (ANOVA, $F_{9, 120}$ = 10.98, 35.48; $p < 0.05$) (Table 4.8). Higher nitrification rates within the FTW series, the release of CO_2 from the root systems and the possible effects of acidic rhizodeposition exudates from the plant root system within **V**-series might be the reasons for pH and DO decline (Kyambadde et al., 2004; Iamchaturapatr et al., 2007).

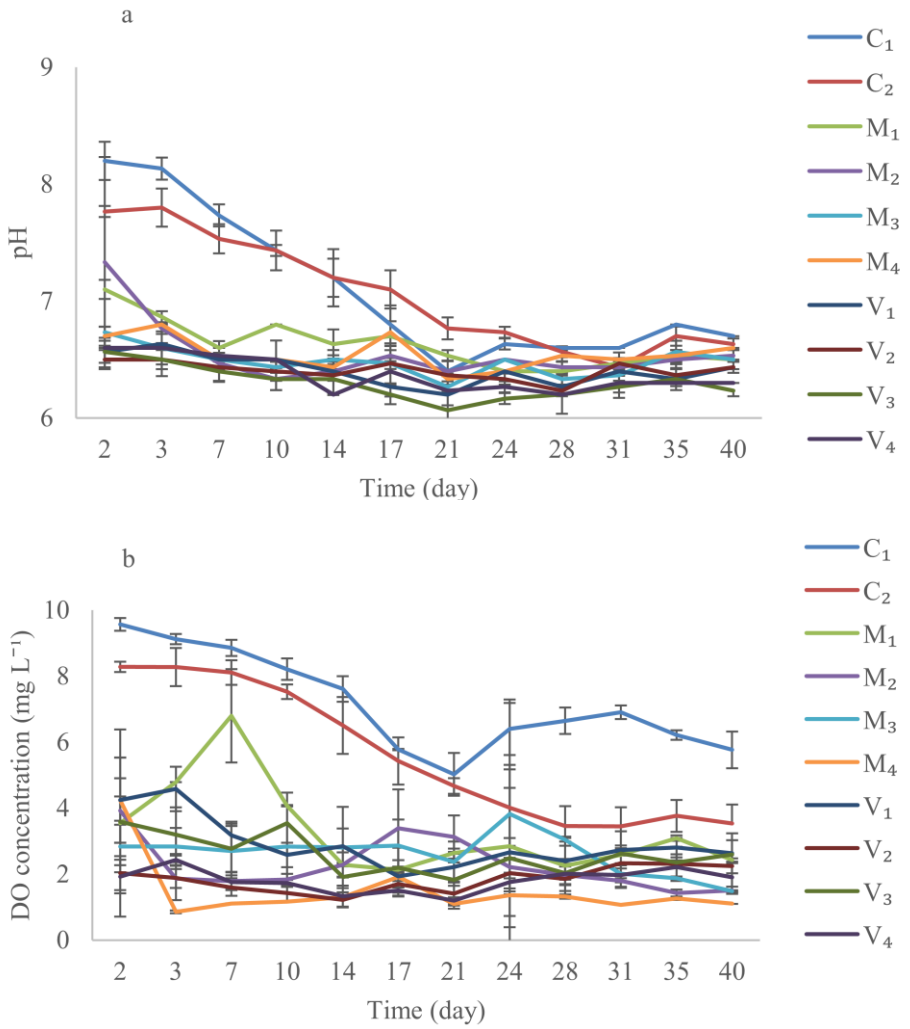

Figure 4. 10 Changes in the (a) pH and (b) DO concentration in mesocosms over time.

Table 4. 8 Average physicochemical characteristics (± standard error of the mean) of the wetland series during the experiment.

Treatments	pH	DO (mg L^{-1})	EC (mS cm^{-1})	Temperature (C°)
C_1	7.10 ± 0.18^{a}	7.17 ± 0.42^{e}	0.43 ± 0.01^{ab}	16.81 ± 0.97^{a}
C_2	7.05 ± 0.13^{a}	5.58 ± 0.58^{a}	0.47 ± 0.01^{a}	16.93 ± 0.80^{a}
M_1	6.62 ± 0.06^{b}	3.29 ± 0.39^{b}	0.44 ± 0.01^{ab}	17.50 ± 1.09^{a}
M_2	6.55 ± 0.07^{b}	2.26 ± 0.22^{bcd}	0.45 ± 0.01^{ab}	16.72 ± 0.94^{a}
M_3	6.48 ± 0.03^{b}	2.62 ± 0.17^{bcd}	0.44 ± 0.01^{ab}	17.13 ± 0.89^{a}
M_4	6.54 ± 0.04^{b}	1.48 ± 0.26^{c}	0.45 ± 0.01^{ab}	17.24 ± 0.88^{a}
V_1	6.40 ± 0.03^{b}	2.90 ± 0.22^{bd}	0.42 ± 0.01^{ab}	17.31 ± 0.88^{a}
V_2	6.40 ± 0.02^{b}	1.83 ± 0.10^{cd}	0.43 ± 0.01^{ab}	17.83 ± 1.01^{a}
V_3	6.30 ± 0.04^{b}	2.59 ± 0.17^{bcd}	0.40 ± 0.01^{ab}	17.97 ± 0.96^{a}
V_4	6.36 ± 0.04^{b}	1.81 ± 0.10^{cd}	0.42 ± 0.007^{ab}	18.13 ± 0.96^{a}

Upper letters denote the Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

On average, the pH in the M and V systems were 6.5 ± 0.03 and 6.3 ± 0.02, respectively, compared to controls (7.1±0.02). Dissolved oxygen in the FTW systems decreased to averages of 2.4±0.3 and 2.3±0.2 mg L^{-1} for the M- and V-series, respectively, compared to the control cells which achieved 6.3 ± 0.7 mg L^{-1}.

The observation of ammonia reduction in M and V systems is consistent with the expectation that the removal processes are correlated with pH and DO concentration. The rate constants for NH_x are plotted against pH and DO concentration in Figure 4.10. A negative correlation was observed between removal rate constant of ammonia and pH from one hand (r^2 = 0.53; p = 0.05) and between removal rate constant of ammonia and DO concentration from another hand (r^2 = -0.28; p = 0.05).

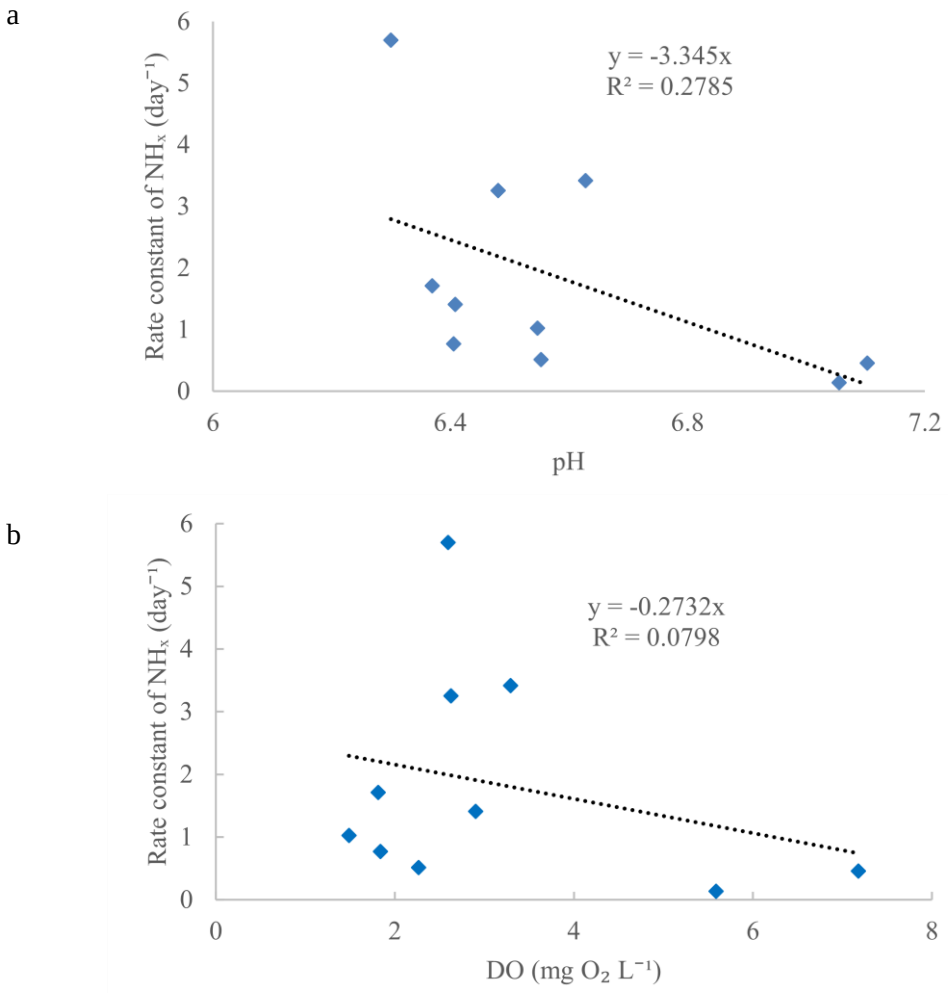

Figure 4. 11 Removal rate constants of NH_x versus (a) pH and (b) DO concentration in the treatments.

It has been documented that the root zone of *Juncus effusus* was acidified when O_2 was released from the root system (Blossfeld et al., 2011). Therefore, an extensive root system

and the exudates of *J. effuses,* and the microbial activity of the attached biofilm probably controlled the acidity of the water column in the present study. Further, the O_2 loss in the FTW systems could be attributed to the plant/biofilm respiration beneath the floating mat (Tanner and Headley, 2011; White and Cousins, 2013). Electrical conductivity variations were mainly controlled by the simulated inflow passing through the system. Figures 4.12 illustrating the changes of EC and water temperature during experimental time. The average value for EC in the wetland series was 0.439±0.006 mS cm^{-1}. Even though water temperature declined by about 11°C over the course the study, FTWs did not reflect the moderation effect on the water temperature in under 7 days of hydraulic retention time compared to the study conducted by Lynch et al. (2015).

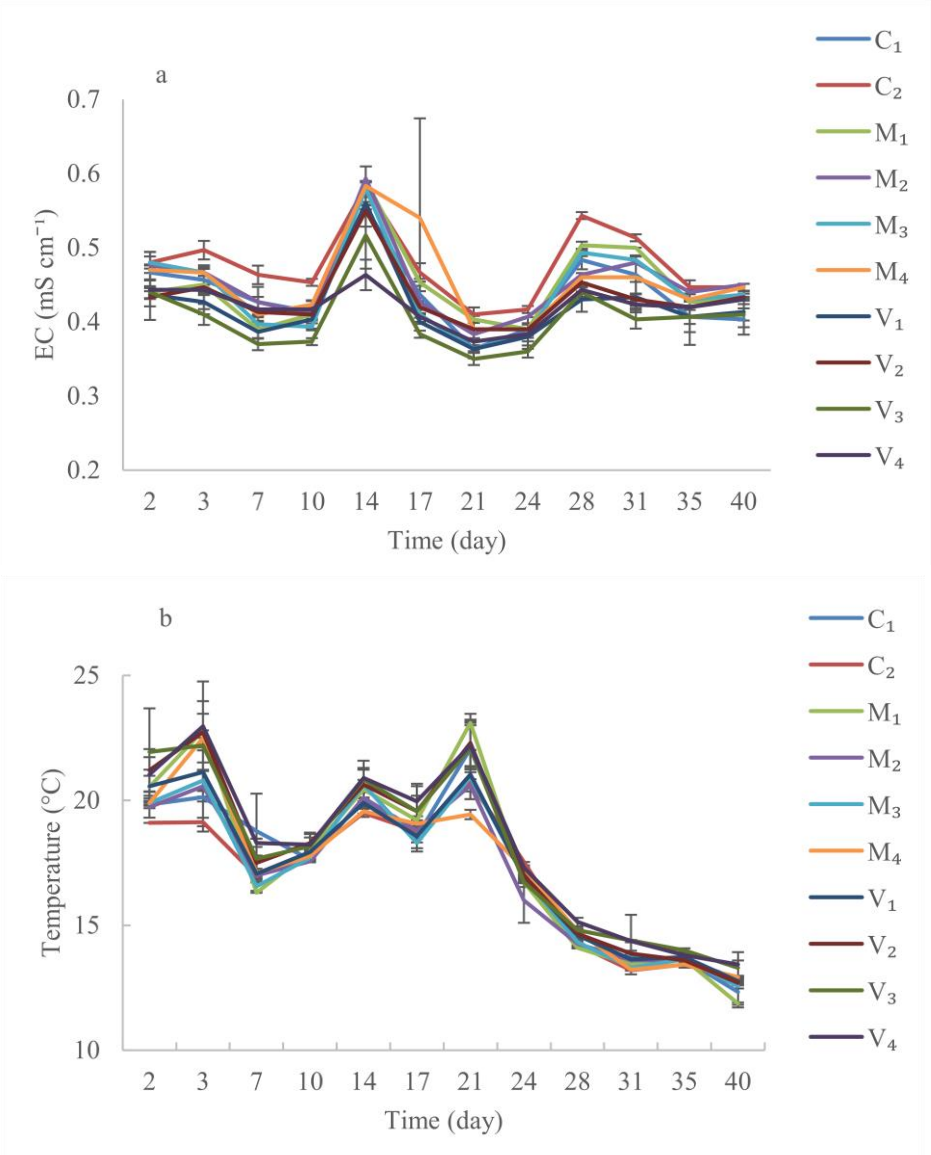

Figure 4. 12 Changes in the (a) EC and (b) water temperature in mesocosms over time.

4.3.2 Model Performance

Model performance for NH_x and NO_x is illustrated in Figure 4.13 for the V_4 treatment after calibration. The optimized RMSE values were 0.267 and 0.835 mg N L^{-1} for NH_x and NO_x, respectively, demonstrating that the model was able to reproduce the time to steady state and the steady-state concentrations of both N species reasonably well. The calibrated values of k_{nit}, k_{upNHx} and k_{upNOx} obtained were 1.4, 0.08 and 0.06 day^{-1}, respectively. These were converted into final k_{nit} and k_{up} values for the independent treatments, whereby linear scaling according to the assumptions laid out in section 2 was applied (Table 4.9).

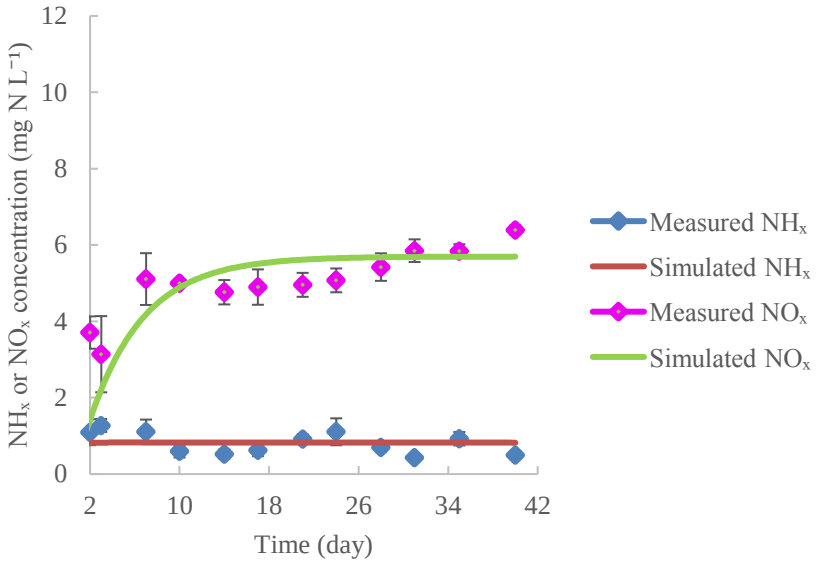

Figure 4. 13 Measured time series of the mean NH_x and NO_x concentrations in the outlet of the V_4 treatment (symbols) and simulated values of NH_x and NO_x (lines) produced using the calibrated parameters. Error bars show the standard deviation in each instance.

Table 4. 9 Calibrated kinetic parameters for nitrification (k_{nit}, day^{-1}); plant uptake for NH_x (k_{upNHx}, day^{-1}) and uptake for NO_x (k_{upNOx}, day^{-1}).

Parameter	**Treatments**								
	C_1	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**
k_{nit} (day^{-1})	0.6	0.3	1.4	0.7	2.8	1.4	1.4	0.7	2.8
k_{upNHx} (day^{-1})							0.04	0.04	0.08
k_{upNOx} (day^{-1})							0.03	0.03	0.06

Observed and simulated time series for NH_x and NO_x in the other treatments are shown in Figure 4.14. Again, there is a good agreement for most of the treatments. The model performed poorly in many treatments in terms of the time to reach steady state, although the actual steady-state concentrations were generally captured well. Possible fluctuations in treatment performance can be linked, to some extent, to daily load variations due to a range of external factors (e.g., pumping system performance) and changes in concentrations resulting from dilution from rainfall and concentration from evaporation. It should also be pointed out that mineralization of organic nitrogen to NH_x and immobilization of NH_x and NO_x in the microbial biomass were assumed to be negligible here. This is because organic N was not introduced to the influent, although clearly some ON will build up in the system from root litter and exudates, from autotrophic microbial biomass and photosynthate derived from algae. Since there is no explicit consideration of organic N, there is also no representation of the sedimentation process. These omissions could result in some model performance errors.

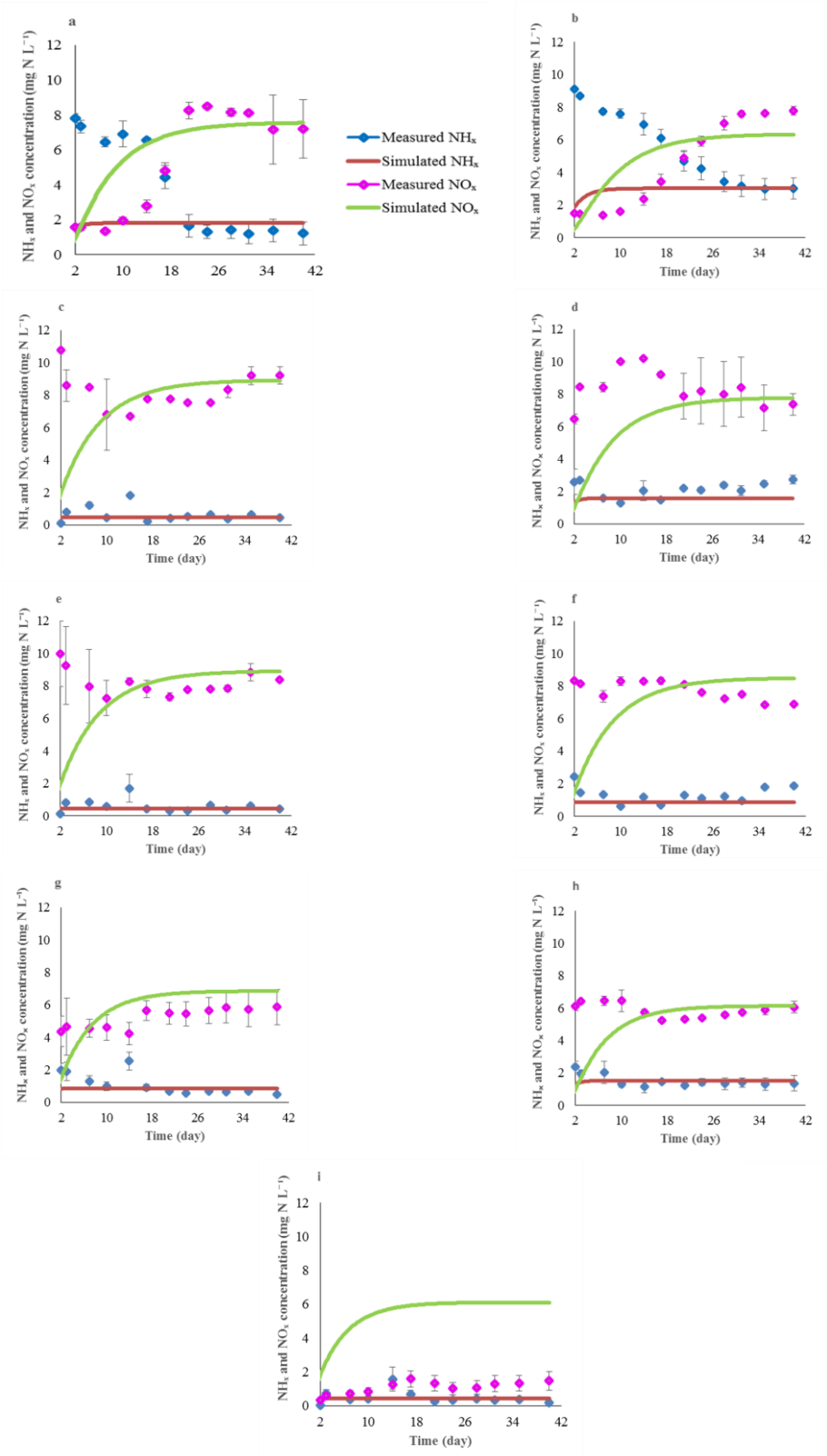

Figure 4. 14 Changes in average measured and predicted concentrations of NH_x and NO_x in the validation exercise: (a) C_1; (b) C_2; (c) M_1; (d) M_2; (e) M_3; (f) M_4; (g) V_1; (h) V_2; (i) V_3. Error bars show standard error of the mean for measured concentrations over the last 25 days of the experimental period.

The RMSEs between the measured and predicted steady-state concentrations are shown in Table 4.10. They were typically in the range 0.26-0.73 mg N L^{-1} for NH_x and 0.83-4.11 mg N L^{-1} for NO_x.

Table 4. 10 Statistical analysis for model performance

	NH_x			NO_x		
	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})	R^2	Slope (mg N L^{-1})	RMSE (mg N L^{-1})
C_1	0.953	0.699	0.471	0.398	0.318	3.697
C_2	0.914	0.875	0.367	0.569	0.510	3.427
M_1	0.366	1.398	0.464	0.418	0.346	3.097
M_2	0.740	1.268	0.730	0.596	0.536	3.153
M_3	0.388	1.309	0.400	0.440	0.346	3.000
M_4	0.584	1.498	0.650	0.509	0.379	2.732
V_1	0.427	1.299	0.670	0.811	0.531	1.437
V_2	0.675	0.903	0.414	0.482	0.367	2.130
V_3	0.337	0.104	0.362	0.919	0.212	4.117

For instance, M_1, M_3, and V_2 treatments showed good fit between measured and modelled data with lowest deviation of the observations about the regression line. Lower RMSEs were observed in these treatments compared to other ones. This is possibly due to the limited variability in measured data, therefore estimated variance of error (σ^2) was limited.

Most treatments appeared to reach an approximate biogeochemical steady state which manifested as a constant concentration over time soon after the start of the experiment, and certainly by the end of the monitoring period. The final measured steady-state mean concentrations in the last 25 days before the end of the experiment are plotted against modelled values in Figure 4.15. Overall, there is a good agreement for both determinants (particularly NH_x), and the R^2 value for the linear regression is high (0.85) and significant ($p < 0.05$) with a slope which is close to unity. A notable exception to this agreement is for treatments V_3 and V_1. Model performance for NH_x was good, but poor for NO_x (i.e., NO_x concentrations were significantly overestimated) in the V_3 treatment and to a lesser extent in the V_1 treatment.

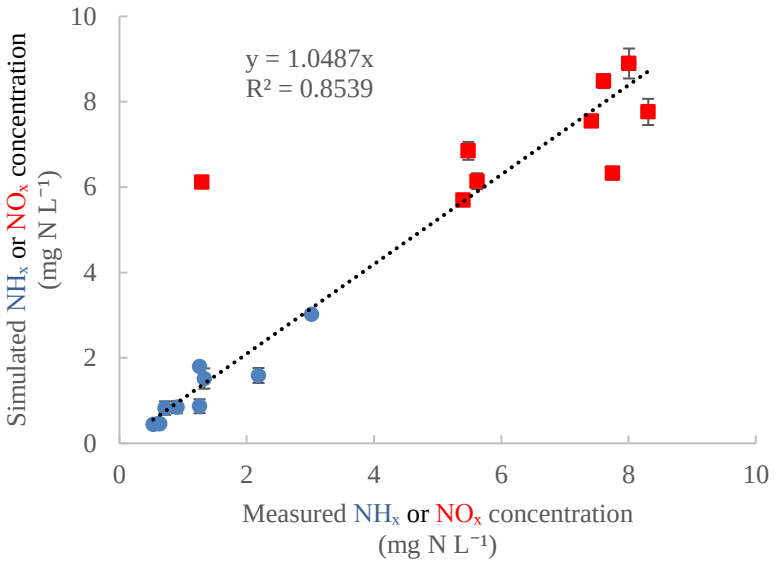

Figure 4. 15 Final average measured and simulated steady-state NH_x and NO_x concentrations in the validation treatments. Error bars show standard deviation for the measured concentrations over the last 25 days of the experimental period.

The good agreement between the modelled steady-state NH_x and NO_x concentrations and the measured data suggests that the model provides a good description of the experimental system and, importantly, that the hypotheses underpinning the conversion of rate constants obtained via calibration in treatment V_4 (i.e., k_{nit} is inversely proportional to depth and directly proportional to mat coverage and number of plants) are valid. In general, NH_x removal efficiencies tended to be higher in shallow systems than in deep ones, all other factors being equal. Since nitrification is a surface-limited process, shallow systems are characterized by a higher surface area per unit volume of water. This is then available for microbial biofilm growth and hence has the capacity to increase reaction rates (Holland et al., 2004; Matamoros and Bayona, 2006). Removal efficiencies were also higher when the mat area was increased. This confirms the postulate that nitrification is principally occurring in microbial biofilms attached to the mat material and plant roots in the planted treatments. The good agreement for outlet NO_x concentrations confirms the plausibility of this explanation (which is also supported by studies by Marimon et al. (2013) and Pavlineri et al. (2017a), who reported increased TN reduction in FTWs when surface coverage increased). Finally, increasing the number of plants increased overall removal efficiency. These results are consistent with findings obtained by García-Lledó et al. (2011).

These authors reported varied removal rate constants for ammonia and nitrate (20.9 - 369.4 m $year^{-1}$) in free surface treatment cells (8500 m^2 each, with a water depth of 0.5 m). García-Lledó et al. (2011) also reported higher removal rates in the presence of dense vegetation compared with treatments which were poorly covered. Simulation outputs in the present study broadly match experimental data obtained by Lynch et al. (2015) and by McAndrew and Ahn (2017a). These authors estimated the N uptake rate to be 0.011 g m^{-2} day^{-1} at low plant productivity in a mesocosm-based FTW with a 65% area coverage (Lynch et al., 2015) and 0.10 g m^{-2} day^{-1} at high productivity in pond-based FTW with a 25% coverage (McAndrew and Ahn, 2017a).

In general, model performance for NH_x was good but poor for NO_x (i.e., NO_x concentrations were generally overestimated) in the V_3 treatment, and to a lesser extent in the V_1 treatment. This could be denitrification (which was explicitly not accounted for in the model), but which could occur in anaerobic microbes within microenvironments of the plant biofilms even if the system is aerobic (Reddy et al., 1989; Cardon, 2007). Another possible sink for NO_x is uptake by algae which was observed in some treatments. Likewise, for the control treatments, where NH_x gradually decreased until a steady state had been achieved at the end of the study. This suggests that there was an active population of nitrifiers in both control systems. This could have been due to the development of a microbial biofilm which contained active nitrifiers on the base and sides of these tanks.

4.3.2.1 Sensitivity Analysis

Sensitivity analysis of the developed model was performed for reaction rate constants for the V_4 treatment to identify which had the greatest impact on the model predictions of NH_x loss from the system. This helps to understand the relative magnitude of the main processes in operation (nitrification, plant uptake and volatilization). The output of NH_x dynamics under the impact of varying k_{nit}, k_{up} and k_{vol} values is illustrated in Figure 4.16

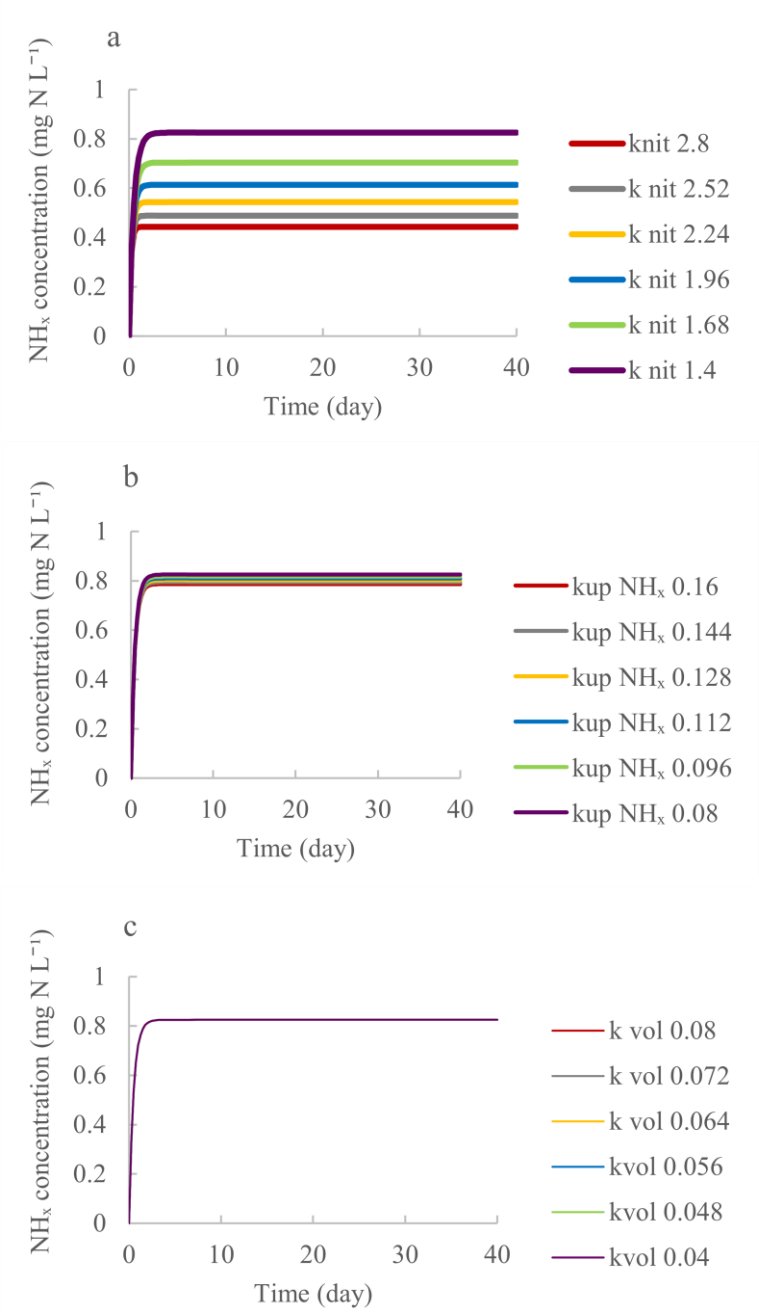

Figure 4. 16 The effect of different k_{nit}, k_{up} and k_{vol} values on the NH_x removal and model prediction.

The model was found to be most sensitive to k_{nit}. Increasing k_{nit} results in a decrease in the predicted steady-state concentration in the outflow. The model was least sensitive to k_{vol} which had relatively little overall effects on free NH_3 losses and NH_x dynamics – partly due to the relatively low pH in the experimental system and, hence, the low value for f_{FREE}. Although uptake makes clear contributions to NH_x and NO_x removal, modeled outputs were less sensitive to k_{up} than they were to k_{nit}, principally due to the lower

baseline value for this parameter. Overall, the model simulation confirmed the experimental results with regards to the magnitude of the processes contributing to NH_x removal, indicating a key contribution to the nitrification, whereas the plant uptake contribution was relatively low compared to nitrification. Volatilization was the least significant NH_x removal process in this study.

4.4 Conclusion

The contribution of FTWs to NH_x removal was investigated using experimental mesocosms under steady-state conditions of 7 days HRT. Treatments included different depths, mat coverage, and plant numbers. First-order kinetics were employed to evaluate the reaction kinetics of the ongoing FTW processes. The results suggest that the design factors tested have controlled the performance of the FTWs in removing ammonia by influencing the relative contribution of the removal processes and promoting microbial functions as the dominant process for N treatment. The results indicated that nitrification rates increased in the wetland series with shallow depths as these systems provided a larger surface area for biochemical reactions as well as the influence of their redox status on the microbial communities. Also, nitrification rates increased as the surface areas for biofilm establishment, including floating mat size and plant root systems, increased. Further, total N uptake rates increased in the treatments as plant density increased. Findings of water quality parameters was supported by microbial analysis, which revealed that microbial growth was associated with solid surfaces rather than bulk of water. The interaction between water quality parameters and biofilm revealed that the ammonia removal was higher with high surface area for microbial biomass. These results promoted shallow depth, full coverage area and higher plant abundance as a typical system design and as a potential parameter for treating ammoniacal N via microbial activity pathways.

The interpretation of the system performance was facilitated using a simple model of N dynamics in the system, assuming each treatment is behaving as a continuously stirred tank reactor (CSTR) with 7 days HRT, and loss processes occurring via first-order kinetics. This provided a mechanistic framework through which to understand the relative magnitude of the main processes operating, using rate constants as a kinetic parameters for nitrification, plant uptake and volatilization. The model was calibrated using experimental data obtained from one treatment (V_4) and validated on the other treatments after simple linear adjustment reflecting *a priori* hypotheses about the contribution of

water depth, floating mat cover and vegetation characteristics. Model performance was good overall, particularly considering the simple nature of the process representation and the fact that some processes (like organic N mineralization and denitrification) were not represented. Importantly, the model-based analysis showed that the hypotheses underpinning the conversion of rate constants obtained via calibration in treatment V_4 are valid. Final simulation results were acceptable but missed some of the dynamics observed in reality. In the control treatments and some of the V treatments, model outputs deviated from measured patterns. This might be attributed in part to the low sampling frequency employed and the fact that some processes (e.g., denitrification, mineralization, and nitrate ammonification) were not included in the modeling scheme. In the control treatments, nitrification probably occurred (in both water column and biofilms on the walls of the mesocosms). However, unexpected behaviour of N cycle in the controls as consequence of algal growth made the distinguish between the processes of floating mat and water body difficult, thus the comparison of the bulk water biofilm interactions between treatment was very difficult.

Overall, highest removal efficiencies were observed in the FTWs including vegetation. Model simulation confirmed that nitrification in fixed biofilms is the principal NH_x removal process, with maximum removal occurring in shallow systems with full mat cover. The work confirms that FTWs can enhance NH_x removal compared to the control system and illustrates the utility of a systems model as a framework for understanding the contribution of different controlling factors.

The findings obtained in this chapter will be used as a platform for wetland design on a larger scale to optimize system performance in removing ammonia. One effective design (V_4: full mat cover and higher plant density) will be extrapolated and assessed in the next pilot-scale study, including a field system of treatment chambers receiving domestic wastewater, to improve understanding of ammonia removal under less controlled conditions. Extending the application of the findings obtained from a semi-controlled mesocosm study to a lesser controlled pilot-study will be useful to confirm the kinetics of ammonia removal derived from field data.

Chapter Five – Assessment of Ammonia Removal performance in Floating Treatment Wetlands Under Different Operational Conditions

5.1 Rational

In this Chapter, a critical FTW design (full coverage of the mat and maximum plant density) from the mesocosm study was scaled up and evaluated to optimize treatment performance of a pilot-scale system in removing ammonia under different operational conditions. The removal of ammoniacal N from batch-operated static chambers receiving effluent from a small-scale domestic wastewater treatment plant was investigated using a simple mathematical model as a framework to assess potential of FTWs for enhancing ammonia removal and to improve understanding of the competing removal processes. Simple numerical system dynamic models can help to describe and interpret the overall behavior of complex systems and estimate the relative contribution of different removal processes. The contribution of the nitrification, plant uptake, and volatilization in removing ammonia from sewage was investigated using FTW system operating under different operational volumes. The prime objectives of this study are (1) to assess a critical FTW design in treating ammonia and (2) to understand ammonia removal kinetics under the effect of two operational volumes. Assessing FTW performance at the pilot-scale adds value to the understanding derived from the experimental studies of NH_x-behaviour described in Chapters 3 and 4.

5.2 Methods

5.2.1 Experimental

The study was conducted on a small wastewater treatment system serving a hotel complex near Market Harborough (52°2840.656° N, 0°5515.708° W), Leicestershire, UK. The hotel generates intermittent wastewater discharge from approximately 150 guests, along with kitchen and laundry waste. It has its wastewater treatment plant which consists of solid screening, primary settling, and a rotating biological contactor. Treated effluent from the plant was previously discharged into the adjacent river-Welland. However, a pilot-scale free-surface constructed wetland has recently been constructed to act as a "polishing" stage (tertiary treatment). This consists of four parallel chambers or cells, two of which have dimensions (2×0.46×0.8 m) and two have dimensions (2×0.70×0.8 m) (Figure 5.1).

I

II

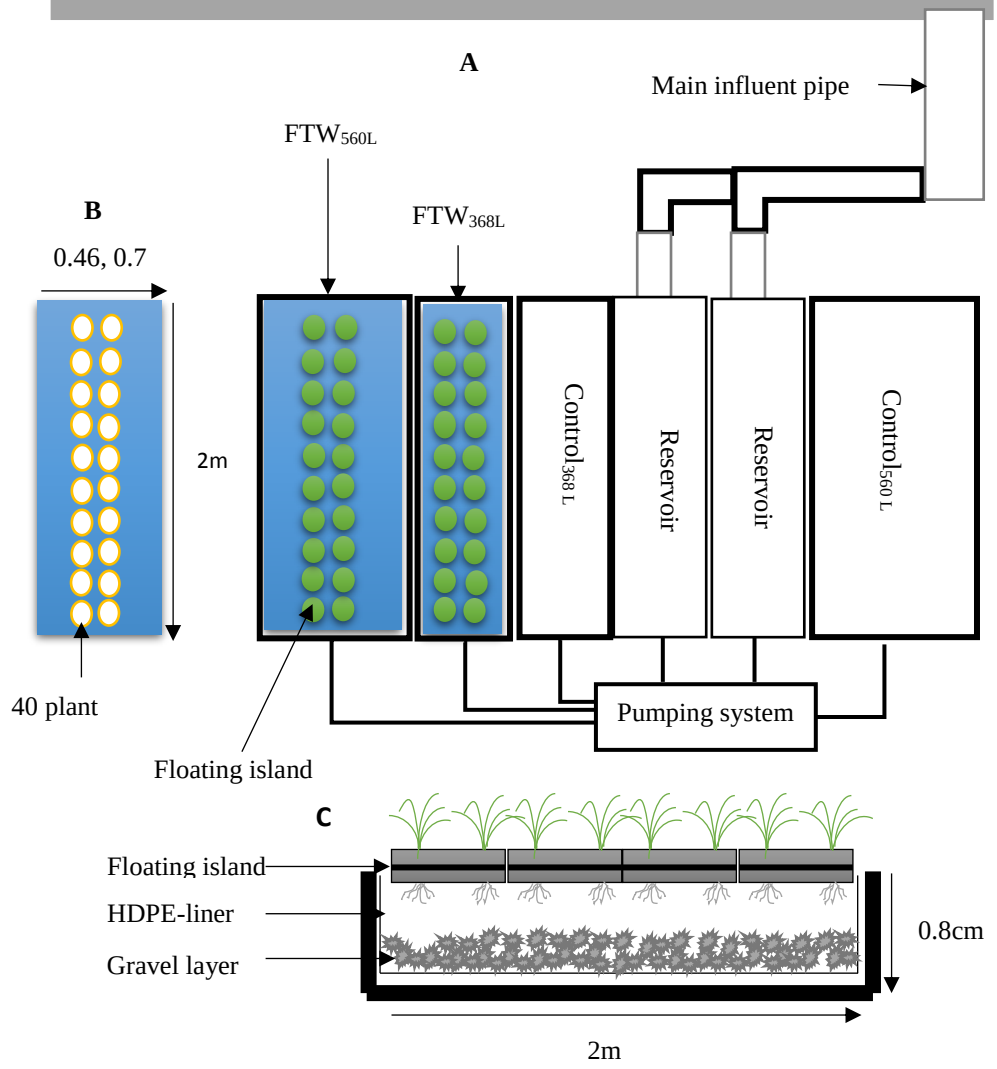

Figure 5. 1 Experimental set-up: (I) photo of the four treatments in the pilot-scale study, (II) A- top view of the treatment system, B- FTW planting zone scheme, C-side view of the treatment chamber.

These cells were constructed with gravel beds (15 cm) and lined with heavy-duty high-density polyethylene (HDPE) liner. Based on the water depth measurement, initial volumes for the treatment chambers were calculated to be 368±7.6 L and 560±9.4 L for the small and big cells. Treatment chambers will be examined for their role in treating ammonia from domestic wastewater over four experimental batches. The prime objective of tertiary treatment is the complementary removal of ammonia that was not sufficiently removed in the secondary treatment in order to minimise adverse effects when the effluent is subjected to discharge into the adjacent river body.

Four discrete 14-day trials were conducted. In each trial, each cell was filled to its operational volume with treated sewage effluent. Cells were filled in parallel and, therefore, received identical effluent. Two cells (one with 368 L; one with 560 L operational volume) were fitted with floating mats (FTW_{368L} and FTW_{560L}). Two cells (one small and one large) had no mat and were considered to act as controls from the perspective of evaluating mat performance (C_{368L} and C_{560L}). One critical design from mesocosm experiment (V_4 design), including full coverage of floating mat, high plant density and water depth of 0.4 m, was applied in this study. Extending the application of V_4 design to a larger scale (existing wastewater treatment plant) was to increase the capability of tertiary treatment in removing ammonia from domestic wastewater.

Each mat covered the whole water surface area and consisted of a buoyant structure with dimensions of 2×0.46 m and 2×0.70 m for FTW_{368L} and FTW_{560L} cells, respectively. The raw material of floating mat was consisted of a loose matrix of extruded plastic obtained from Frog Environmental Ltd, UK and injected with polystyrene foam to support the structure buoyancy. The floating mats were weighing approximately 7 and 10 Kg for (2×0.46 m) and (2×0.70 m) respectively, prior to planting. Both mats contained maximum plant density (20 individuals of *Juncus effusus* and 20 individuals of *Phragmites australis*, order: Poales, family: Juncaceae and Poaceae). After fill up the cells were left in a static condition for 14 days with no inflow or outflow, except for rainfall in and evapotranspiration out.

Water samples (50 mL) were collected from each chamber on a regular basis (0, 3, 7, 10, and 14 days) during each experimental batch. At each sampling dissolved oxygen, pH, EC, and water temperature were measured in each chamber at 15 cm from the surface. Additionally, water depth was monitored over each experimental batch using water level loggers installed in stilling wells and barometric divers for barometric compensation (Van Essen Instruments B.V., Netherlands). For a more extensive description of the sampling

regime, sample treatment, and analysis, the reader is referred to the sampling strategy and analytical methods in chapter 2, sections 2.3 and 2.4.

5.2.1.1 Water and mass balance

The water balance of the pilot-scale chambers was evaluated by measuring changes in operational volume as a function of water depth (Eq. 5.1).

$$V_{init/fin} = Z_{ini/fin} \times A_{chamber} \qquad (5.1)$$

where $V_{int/fin}$ is water volume at the start or end of each batch (L), $Z_{ini/fin}$ is water depth at the start or end of each batch (m), A_{cell} is an area of the chamber (m^2).

The change in the volume per unit area was assumed to be equal to the differences between rainfall and ET over the period.

Masses of N forms were calculated as the product of concentration (mg L^{-1}) and wastewater volume (L) in each treatment cell. Mass removal rates per unit area were calculated as (van Oostrom, 1995):

$$\frac{dM}{dt} = \left(\frac{M_{init} - M_{fin}}{\Delta t}\right)/A_{chamber} \qquad (5.2)$$

where dM/dt is mass removal rate (mg N m^{-2} day^{-1}); $M_{init/fin}$ is N mass at the start or end each experimental batch (mg N); Δt = time step (day); A = mat area (m^2).

5.2.2 Model

A system dynamics model was developed to describe the competing and interacting processes operating in the wetland. Details of the model construction can be found in Chapter 2. For the model applied to the static chambers described here, inflow and outflow discharge was set to zero. Based on empirical data (shown in the next section) water balance in the modeling scheme presumed to be in approximate equilibrium between water gain via rainfall and water loss via ET. Therefore, the effect of a change in the water budget on the N mass balance was negated.

The model was calibrated using data from cell FTW_{560L} (large volume with full mat cover and plants). Table 5.1 shows all parameters used in the. Table 5.1 lists an overview of parameters for the FTW_{560L} model. The model inputs are based on average initial masses of ON, NH_x and NO_x and rate constants in the examined system.

Table 5. 1 Symbol and parameters settings used in the pilot-scale model.

STELLA symbol	Description	Value/Units	Source
State variables			
ON_{ini}	Initial Organic-N mass	1821 mg N	Calculated, FTW_{560L} cell
$NH_{x\,ini}$	Initial NH_3+NH_4 mass	1227 mg N	Calculated, FTW_{560L} cell
$NO_{x\,ini}$	Initial NO_2^-+NO_3^- mass	1313 mg N	Calculated, FTW_{560L} cell
$Plant_{uptake}$	Total-N content in plant	mg N	Measured, FTW_{560L} cell
Volatized NH_3	Free ammonia volatilization	mg N	Estimated, FTW_{560L} cell
Flow variables			
J_{amo}	Ammonification rate	mg N day^{-1}	Calculated, FTW_{560L} cell
J_{nit}	Nitrification rate	mg N day^{-1}	Calculated, FTW_{560L} cell
J_{denit}	Denitrification rate	mg N day^{-1}	Calculated, FTW_{560L} cell
$J_{up\ NHx}$	Plant uptake rate of NH_x	mg N day^{-1}	Estimated, FTW_{560L} cell
$J_{up}\,NO_x$	Plant uptake rate of NO_x	mg N day^{-1}	Estimated, FTW_{560L} cell
J_{vol}	Volatilization rate	mg N day^{-1}	Whelan et al. (2010)
Parameters/coefficients			
[ON]	Organic-N concentration	mg N L^{-1}	Measured, FTW_{560L} cell
$[NH_x]$	NH_3+NH_4^+ concentration	mg N L^{-1}	Measured, FTW_{560L} cell
$[NO_x]$	NO_2^-+NO_3^- concentration	mg N L^{-1}	Measured, FTW_{560L} cell
k_{amo}	Ammonification rate constant	0.021 day^{-1}	Calculated, FTW_{560L} cell
k_{nit}	Nitrification rate constant	0.149 day^{-1}	Calculated, FTW_{560L} cell
k_{denit}	Denitrification rate constant	0.132 day^{-1}	Calculated, FTW_{560L} cell
$k_{up\ NHx}$	Uptake rate constant for NH_x	0.009 day^{-1}	Estimated, FTW_{560L} cell
$k_{up\ NOx}$	Uptake rate constant for NO_x	0.009 day^{-1}	Estimated, FTW_{560L} cell
k_{vol}	Mass transfer coefficient of NH_3	0.04 day^{-1}	Estimated, FTW_{560L} cell
$NH_{3\,f}$	Concentration of NH_3 gas	0.01 mg N	Estimated, FTW_{560L} cell
V	Operational volume	560 L	Measured, FTW_{560L} cell

A manual trial and error calibration procedure was performed in which the following four parameters were adjusted: k_{up}, $k_{amo,}$ k_{nit} and k_{denit}, in order to minimize the root mean square error (RMSE) between the simulated and measured values of (i) total (cumulative) measured plant uptake of N (i.e., 0.018 g N day^{-1} over the whole experimental period 25/07/2016-03/10/2016. Uptake is assumed to occur for both NH_4^+ and NO_3^-, with ammonium assumed to be more preferable to uptake than nitrate (Kadlec and Wallace, 2009b); (ii) temporal changes in ammoniacal N concentrations and (iii) temporal changes in NO_x concentrations (Rousseau et al., 2004). The model employed Euler's method of integration with a time step of 0.25 day.

The mass balance for organic N within a model environment can be written as:

$$V.\frac{d[ON]}{dt} = k_{amo}.[ON].V \qquad (5.3)$$

where k_{amo} is the rate constant for ammonification (day^{-1}), *ON* is Organic N concentration (mg L^{-1}) and *V* is the operational volume of liquid in the treatment (L).

Mass balance equation for ammonia-N (NH_3+NH_4^+) is expressed as follows:

$$V.\frac{d[NH_x]}{dt} = k_{amo}.[ON].V - k_{vol}.f_{FREE}.[NH_x].V - k_{nit}.[NH_x].V - k_{upNH_X}.[NH_x].V \qquad (5.4)$$

where $[NH_x]_{in}$ is the total ammonia concentration in the water (mg N L^{-1}), k_{vol} is the mass transfer coefficient of free ammonia across the air-liquid interface by volatilization (day^{-1}), f_{FREE} is the fraction of total ammonia which is present as free NH_3 (dependent on pH and temperature), k_{nit} is a rate constant for nitrification (day^{-1}) and $k_{up\ NHx}$ is a rate constant for ammonia uptake by plants (day^{-1}).

The average pH value during the experimental periods was 7.2. The fraction of ammonia present as NH_3 at this pH is typically very low, so volatilization was considered to be negligible in the model run.

The mass balance for oxidized-N can be written as:

$$\frac{d[NO_x]}{dt} = k_{nit}.[NH_x].V - k_{upNO_X}.[NO_x].V - k_{denit}.[NO_x].V \qquad (5.5)$$

where k_{upNOx} is the rate constant for oxidised-N uptake by the plant (day^{-1}), and $[NO_x]$ is the oxidized nitrogen concentration (mg L^{-1}), and k_{denit} is the rate constant for denitrification (day^{-1}).

Ammonia volatilization was predicted independently using the two-film resistance model (see Section 2.4.3). At the average water pH of the experimental system described here (7.2) with a water depth of 0.4 m, the mass transfer constant of NH_3 was calculated to be

0.04 day^{-1}, assuming partial mass transfer coefficients of 1 and 0.01 m h^{-1} on the air-side and water-side of the interface, respectively (Mackay, 2001). In treatments containing floating mats, volatilization was assumed to be 79-81% of the rate calculated in treatments without mats. This is due to the extra resistance imposed on phase transfer across the air-water interface by the full mat cover. However, in any case, volatilization was never predicted to be a particularly important loss process (i.e., k_{vol} << rate constants for other loss processes).

Validation was performed by comparing predicted concentrations of NH_x and NO_x with the measured data. In all cases, initial concentrations in the model were set at the initial measured concentrations in each batch. Since the two FTW treatments (FTW_{560L} and FTW_{368L}) have equivalent mat surface area per unit volume (water depth) and were both fully covered with the same plant density, no further adjustments were made to the calibrated parameters. In the control cells, uptake was assumed to be zero (k_{up} was set to zero) and k_{nit} was reduced by a factor of 3 (i.e., 0.057 day^{-1}), and k_{denit} was not changed. Since nitrification rate was hypothesized to be linearly proportional to the mat area in the micro and mesocosm systems, an assumption was made that floating mat could hold the major effect of nitrification in the pilot system. Therefore, a reduction of k_{nit} value by two-thirds (66.47%) due to the absence of the mat in the control cells could improve model performance to simulate NH_x and NO_x dynamics.

5.3 Results and Discussion

5.3.1 Experimental data

5.3.1.1 Water Balance

Average changes in the water volume of tested cells over four experimental batches are given in Figure 5.2 which shows daily water depth changes.

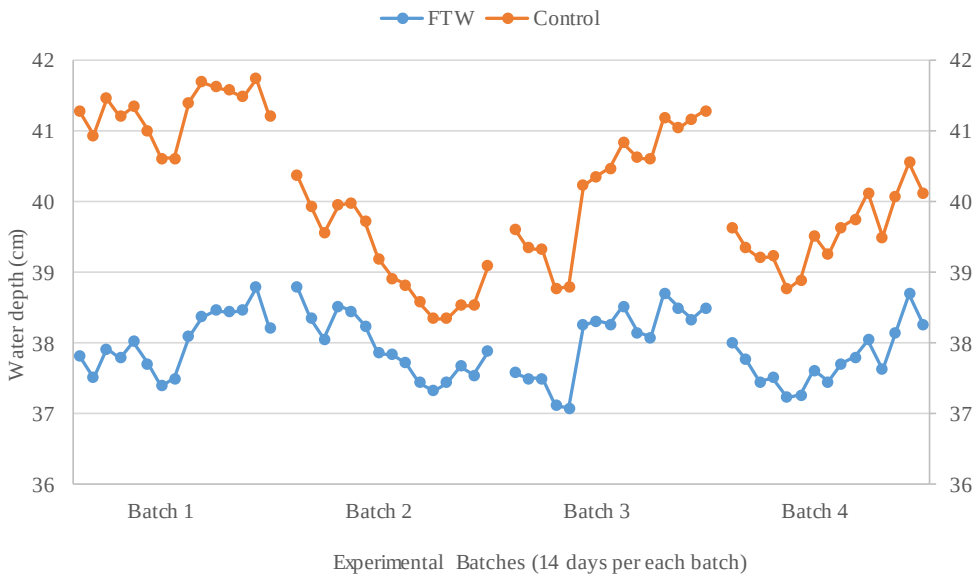

Figure 5. 2 Average water depth in the study site over four experimental periods.

The operational volumes of the FTW cells were relatively lower than those from control cells over the course of the study. As an average of four sequenced trials, water depth for FTW cells was 37.94±0.07 cm compared to control cells (40.07±0.22 cm).

Generally, the final operational volumes of the tested cells were relatively higher than the initial volumes applied to the systems except for the batch 2. Meteorological parameters measured during batch 1, 3 and 4 revealed relatively low air temperature (14.64±2.52 °C) associated with high relative humidity (59.75±22.88 %) compared to batch 2 (18.68±1.22 °C and 36.22±14.95 %) (Figure 5.3). Such meteorological conditions may be promoting low evaporation rates, but high probability for precipitations, and therefore increase water budget of the tested cells in the study site.

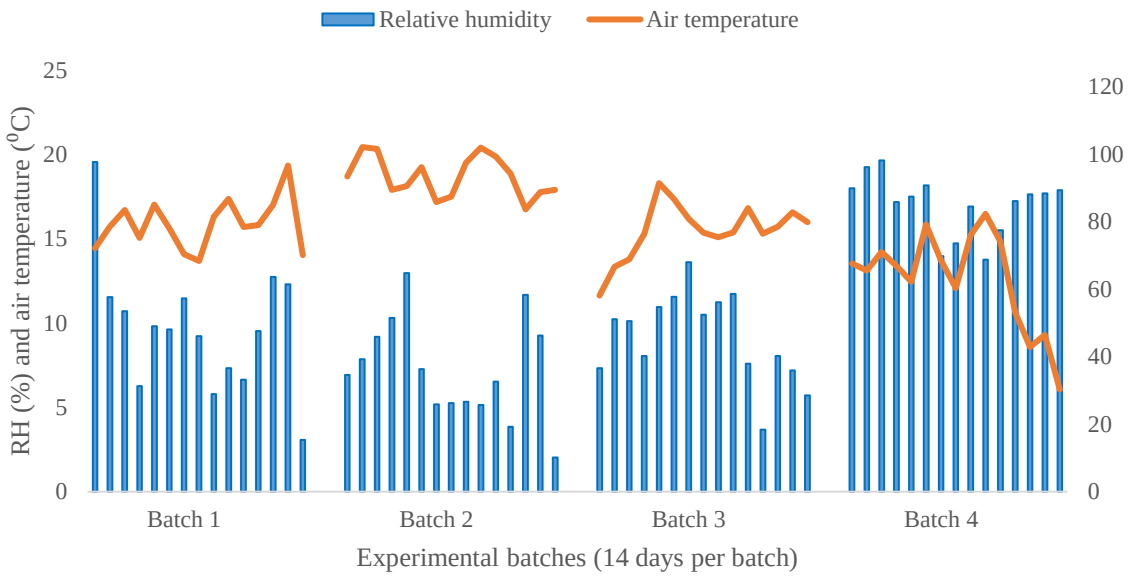

Figure 5. 3 Relative humidity (%) and air temperature (°C) in the study site during over for experimental batches.

Water budget of FTW increased by 1.5 % at the end of batch 1, 3 and 4, while the controls exhibited increase by 3 % for the same periods. Nevertheless, water volume was decreased by 3 % in FTWs and controls at the end of batch 2 (Table 5.2).

The differences in the water budget between treatments could be resulted from the effect of evapotranspiration in the planted cells compared to unplanted, although water loss in the FTWs could be restricted in some extent by full coverage surface area of the floating systems, which relatively restrict water evaporation. However, water gain or loss in the examined systems have not indicated significant differences in the water budget.

Table 5. 2 Average water depth, initial and final volume in the FTW and control over four experimental periods.

Batches	Water depth (cm)		Initial water volume (L)		Final water volume (L)	
	FTW	**Control**	**FTW**	**Control**	**FTW**	**Control**
Batch 1	38.03	41.28	548.39	598.61	553.97	597.53
Batch 2	37.93	39.18	562.39	585.23	549.25	566.76
Batch 3	38.02	40.24	544.87	574.37	558.18	598.65
Batch 4	37.77	39.57	550.97	574.76	554.59	581.63

It can be concluded that the dilution effects and concentration effects of the precipitation and evaporation have not substantial impacts on the nitrogen concentrations in the treatments. Therefore, the effect of water volume changes on the N mass balance was, neglected.

5.3.1.2 Organic Nitrogen Removal

The concentrations of organic-N (ON), ammonia-N (NH_x) and oxidised-N (NO_x) in the starting material (effluent from the WWTP) in across the four experimental batches are summarised in Table 5.3.

Table 5. 3 Initial concentrations of ON, NH_x and NO_x in the wastewater applied to the experimental cells over four experimental periods.

Batches	N variables	Concentration (mg N L^{-1})			
		FTW_{368L}	**C_{368L}**	**FTW_{560L}**	**C_{560L}**
Batch 1	**ON**	3.17±0.38[a]	2.93±0.53[a]	2.85±0.21[a]	3.41±0.13[a]
	NH_x	2.20±0.06[a]	2.52±0.04[b]	2.41±0.05[a]	2.27±0.06[b]
	NO_x	2.32±0.27[a]	2.58±0.07[a]	2.48±0.31[a]	1.84±0.33[a]
Batch 2	**ON**	4.15±0.22[a]	3.74±0.62[a]	3.97±0.19[a]	4.54±0.75[a]
	NH_x	1.84±0.11[a]	1.87±0.05[b]	2.35±0.10[a]	1.85±0.13[b]
	NO_x	2.07±0.34[a]	2.15±0.21[a]	2.25±0.60[a]	2.21±0.39[a]
Batch 3	**ON**	3.78±0.54[a]	3.90±0.30[a]	3.31±0.31[a]	3.36±1.54[a]
	NH_x	1.52±0.03[a]	1.57±0.04[b]	1.61±0.02[a]	1.94±0.01[b]
	NO_x	2.24±0.60[a]	2.25±0.44[a]	2.05±0.07[a]	2.72±0.36[a]
Batch 4	**ON**	3.26±1.41[a]	3.12±1.25[a]	2.86±0.50[a]	3.76±0.82[a]
	NH_x	2.35±0.04[a]	2.13±0.02[b]	2.38±0.02[a]	2.29±0.03[b]
	NO_x	2.62±0.42[a]	2.51±0.47[a]	2.58±0.14[a]	3.06±0.32[a]

Changes in the concentration of ON in the four chambers over the four batch periods is shown in Figure 5.4. Analysis of variance was performed to explore if ON concentrations were differed by experimental time or by treatments. There was a general decrease in ON concentration over time in all treatments (ANOVA, $F_{3, 80} = 5.02$; $P < 0.05$), although there appeared to be some occasional increases. At each sampling, there was a high degree of variability in ON concentrations between batches for the same treatment. Decreased ON concentrations in the water column reflect a dominance of loss processes (net mineralization (ammonification) and sedimentation) over processes supplementing the ON pool (N uptake by growing algae, immobilization by the microbial biomass and sediment resuspension). Occasional increases in ON concentrations may reflect periods of algal growth or the sloughing of organic material from mat surfaces, chamber walls,

and gravel bed and to some extent sampling method (five sampling intervals per each batch) where variations of measured concentrations might be less pronounced.

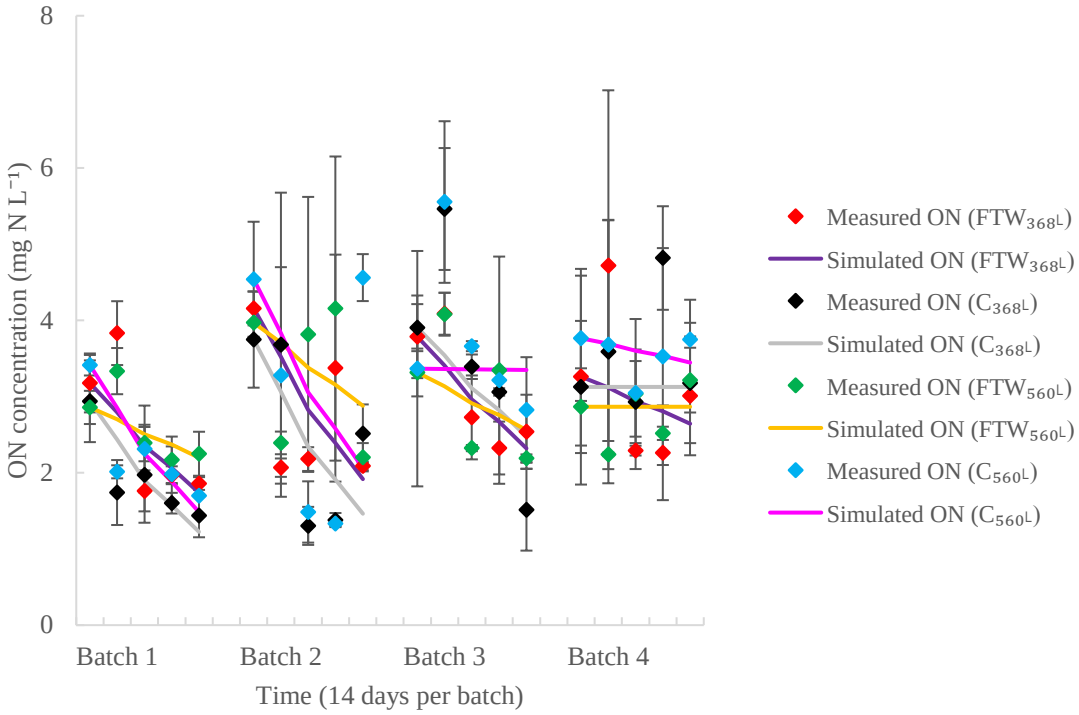

Figure 5. 4 Changes in the concentration of ON in the treatment system with (FTW_{368L} and FTW_{560L}) and without (C_{368L} and C_{560L}) mats for the four experimental batch periods (mean ± standard deviation).

The temporal patterns were similar in all four chambers, and no significant differences were detected between them (ANOVA, $F_{3, 80} = 0.448$; $P = 0.05$). In other words, the presence of FTW mats had no significant impact on ON removal compared to the controls. The average net ON removal over each 14-day batch period in each treatment is shown in Table 5.4, along with the estimated removal efficiency (*RE*). The final average ON concentrations in the FTW_{368L} and FTW_{560L} for all four experimental periods were 2.37±1.22 and 2.46±0.25 ON-N L^{-1} respectively. Final ON concentrations in the C_{368L} and C_{560L} treatments were 2.16±1.26 and 3.20±0.56 mg N L^{-1}, respectively.

First order rate constants and corresponded removal half-life from the tested cells were statistically the same indicating insignificant variations of the removal kinetics of ON in treatments (Table 5.4). The relatively limited loss of ON in all the treatments could reflect a reduction in ammonification due to low dissolved oxygen (DO) levels (<<2 mg L^{-1}). Organic matter decomposition (and associated ammonification) can proceed under low partial pressures of DO and even under anaerobic conditions but at reduced rates

(Vymazal, 2010; Marimon et al., 2013). Furthermore, there is always some generation of new ON in wetland systems as well as losses due to primary production (plants and or algae) and subsequent senescence, which reduces the net loss rate (Hammer, 1989).

Table 5. 4 Average values (± standard deviation) of ON removal (mg N L^{-1}), first order mineralization rate constant (k_{amo}, day^{-1}), half-lives ($T_{1/2}$, day), and removal efficiencies (*RE*, %) over the four experimental batches.

Treatment	Extents (mg N L^{-1})	k_{amo} (day^{-1})	$T_{1/2}$ (day)	*RE* (%)	R^2
FTW_{368L}	1.22 ±0.37[a]	0.03±0.01[a]	21.35±3.36[a]	32.96±9.09[a]	0.66
C_{368L}	1.28±0.49[a]	0.04±0.01[a]	31.85±18.68[a]	40.15±16.43[a]	0.58
FTW_{560L}	0.87±0.37[a]	0.02±0.01[a]	37.56±9.03[a]	24.92±9.56[a]	0.42
C_{560L}	0.56±0.40[a]	0.02±0.01[a]	48.14±23.92[a]	16.70±11.79[a]	0.43

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other (α =0.05).

Exponential equations (first-order kinetics) were fitted to the data from each treatment (also shown in Figure 5.3). Comparison of the modeled and measured ON-concentrations shows that 66 and 42 % of the variances in the FTW_{368L} and FTW_{560L} was explained by the model, but 58 and 43 % of the C_{368L} and C_{560L} data (Table 12).

5.3.1.3 Ammonia Removal

The initial wastewater contained an average of 2.07±0.09 mg N L^{-1} as total ammonia N (NH_x). Changes in NH_x concentration over time in each batch and each treatment are shown in Figure 5.5. There were significant differences between treatments (ANOVA, $F_{3, 80} = 46.08$; $P < 0.05$). NH_x concentration decreased in all treatments, but the rate of decrease was much lower in the controls compared with the FTW cells in all experimental batches. Figure 5.5 also shows first-order kinetic fits to the data, which suggest that NH_x loss can be reasonably described with first order assumptions, particularly in the case of the FTWs.

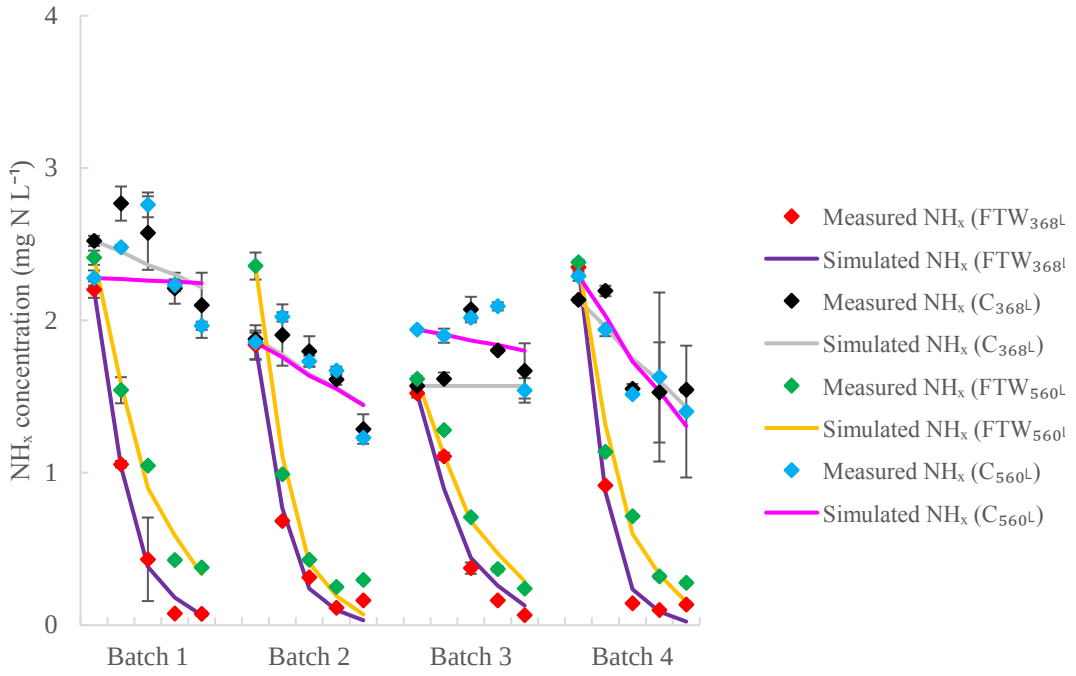

Figure 5. 5 NH_x behavior in the treatment system with (FTW_{368L} and FTW_{560L}) and without ($C_{368}L$ and C_{560L}) mats during four experimental periods (mean ± standard deviation).

A gradual reduction was observed in NH_x concentrations in the FTWs over four experimental batches. Experimental data indicated similar trend of NH_x dynamics in all batches with no obvious differences in treatment performance in both FTW systems. Although NH_x concentrations in the controls reduced slightly from their initial values, NH_x behaviour were relatively differed over the sequenced batches. Such variation could be due to the interference of some other factors, e.g. activity of some autotrophic microorganisms such as algae. As shown in the figure 5.5, the four cluster of ammonium concentrations were modeled using 1st order kinetics model. Based on the calculated correlation coefficient (R^2), comparison of the modelled and measured NH_x concentrations during study periods shows that 0.99 percent of the variances in the FTW_{368L} and FTW_{560L} can be explained by the model. While 72 and 68 percent of the controls data only be described by the model (Table 5.5). Although modeled and measured data of the NH_x concentrations from control treatments have not shown best fit on the regression lines, the performance of the retrofitted treatments has illustrated goodness of fit of the trend's lines. This suggest that the change in the NH_x within the tested chambers is systematically faster in the presence of the FTW systems than without (Figure 5.5). However, a decrease in treatment performance was observed in the control

treatments when the measured data were outside the regression line. Obviously, FTW treatments have demonstrated higher levels of NH_x removal and treatment performance compared to the control treatments with no floating systems. Even though, there were not significant differences between FTW treatments in removing NH_x during the study, 1st order kinetics model has well described the comparable reduction trend of the two treatments and confirming insignificant differences between the two groups.

First order rate constants (shown in Table 5.5, along with equivalent half-lives) were much higher for the FTW treatments compared with those for the controls, suggesting that the mats provide effective enhancement of NH_x removal. This could have been due to a combination of ammonia volatilization, plant uptake, and additional nitrification. An attempt was made to estimate the relative contributions of these processes using the system dynamic model later in this Chapter. Higher removal rate constants were associated with lower half-lives of NH_x concentrations in the FTW systems. The final averages of NH_x concentrations in the FTW_{368L} and FTW_{560L} of the experimental periods were 0.11±0.02 and 0.29±0.02 mg N L^{-1} respectively. Final average NH_x concentrations in the C_{368L} and C_{560L} were 1.65±0.16 and 1.53±0.15 mg N L^{-1}, respectively.

Table 5. 5 Average values (± standard deviation) of NH_x removal (mg N L^{-1}), first order nitrification rate constants (k_{nit}, day^{-1}), half-lives ($T_{1/2}$, day), and removal efficiencies (*RE*, %) over the four batches.

Treatment	Extents (mg N L^{-1})	k_{nit} (day^{-1})	$T_{1/2}$ (day)	*RE* (%)	R^2
FTW_{368L}	1.86±0.18[a]	0.22±0.01[a]	3.08±0.19[a]	94.42±1.18[a]	0.99
$C_{368\ L}$	0.40±0.13[b]	0.01±0.01[b]	61.75±23.18[b]	18.96±7.04[b]	0.72
FTW_{560L}	1.89±0.17[a]	0.14±0.01[c]	4.64±0.10[a]	86.28±0.95[a]	0.99
C_{560L}	0.55±0.12[b]	0.02±0.01[b]	49.67±14.23[b]	26.73±5.78[b]	0.68

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

Ammonia loss via NH_3 volatilization in the system was also studied. The average rate constants of NH_3 loss over four experimental batches were 5×10^{-4} and 6×10^{-4} day^{-1} in the FTW and control systems. The average concentration of NH_3 were 0.01 and 0.03 mg N L^{-1} in the FTW and control groups (Figure 5.6).

The low rate of volatilization in all treatments could be due to the dominant low pH values (7.2) throughout the study, which pushed the equilibrium of the ammoniacal nitrogen towards NH_4^+ rather than NH_3 generation.

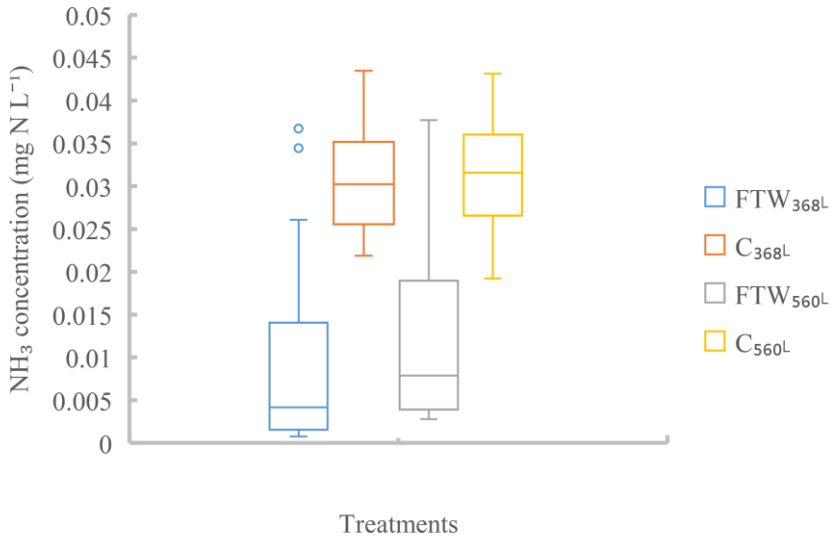

Figure 5. 6 Concentration of NH_3 in the pilot-scale treatments

Overall, the FTW systems showed enhanced removal rates for ammonia and the observed temporal patterns and differences between treatments were well predicted by the model. The results presented in Chapter 3 (benchtop experiments) show that nitrifying bacteria colonize the mat material such that overall nitrification rate is proportional to submerged surface area. Nitrification is also likely to have been enhanced by the relatively high temperatures observed over the experimental period (Zhang et al., 2009) and may also have been promoted by the production of root exudates such as organic carbons (Cardon, 2007) and oxygen release from plant roots growing in the presence of mat material (Blossfeld et al., 2011; Borne et al., 2013). It is known that nitrifier activity can be inhibited by UV light. The opaque nature of the mat material used in the experiments ensured that the material in contact with the water column would have been relatively dark, thus, preventing such light inhibition effects (Lynch et al., 2015). Overall the enhancement of ammonia removal in the presence of FTWs reported here exceeds that reported by Chang et al. (2013) who observed a 51% improvement in NH_4^+ removal in a stormwater retention pond fitted with floating island. This may be because Chang et al. monitored stormwater which will have limited retention time compared with the batch

arrangement employed in our work. Winston et al. (2013) are also report a significant enhancement of treatment efficiency for NH_4^+ in a stormwater retention pond when fitted as FTWs.

5.3.1.4 Oxidized Nitrogen Removal

Concentrations of oxidized forms of nitrogen (NO_2^- and NO_3^-) were relatively at high levels at the start of each batch and tended to decrease over each 14-day period. Wastewater applied to the treatment system contained an average of 2.3 mg N L^{-1} as total oxidized N (NO_x). Changes in NO_x concentration alongside with first order fits over time in each batch, and each treatment is shown in Figure 5.7. A general decrease of NO_x concentration was observed in all treatments.

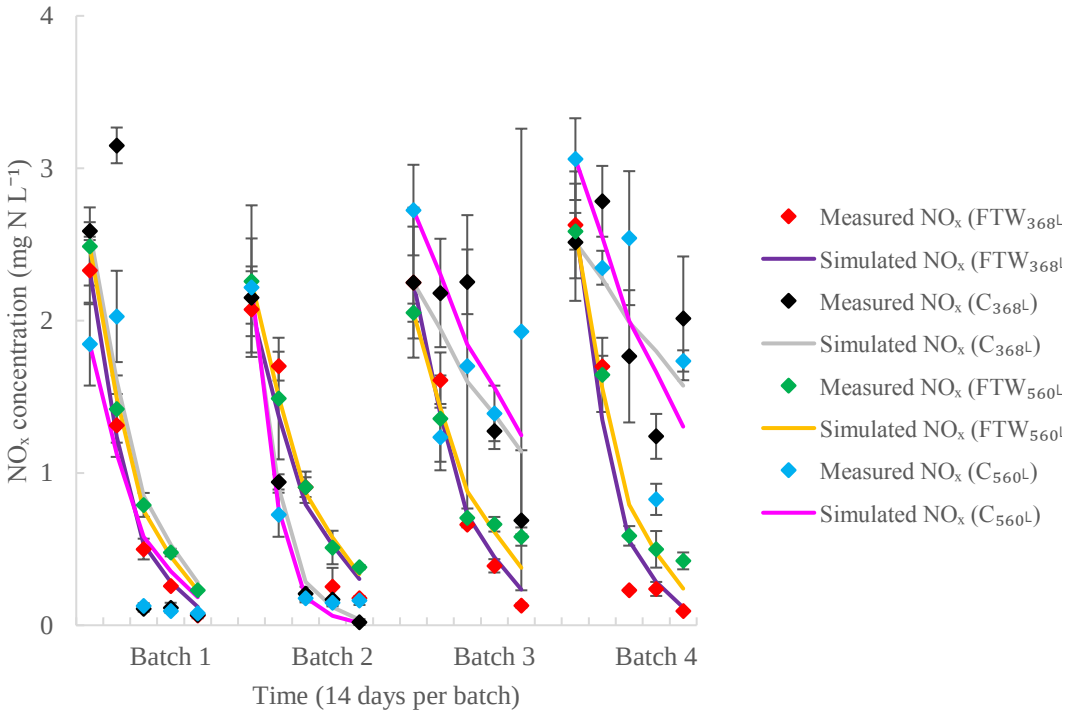

Figure 5. 7 NO_x behavior in the treatment system with (FTW_{368L} and FTW_{560L}) and without (C_{368L} and C_{560L}) mats during four experimental periods (mean ± standard deviation).

Changes in NO_2^- concentrations are shown in Figure 5.8. On average NO_2^- concentrations decreased by 0.77±0.02 mg-N L^{-1} in the FTW treatments and 0.59±0.01 mg-N L^{-1} in the controls. These losses represent net removal efficiencies for NO_2^- of approximately 98% for the FTW treatments and 87% for the controls, respectively. Final average NO_2^- concentrations in the FTW treatments and controls were 0.01±0.01 and 0.07±0.02 mg N

L^{-1}, respectively. There were no significant differences between the two different operational volumes for either treatment (ANOVA, $F_{3, 80} = 0.527$; $P < 0.05$).

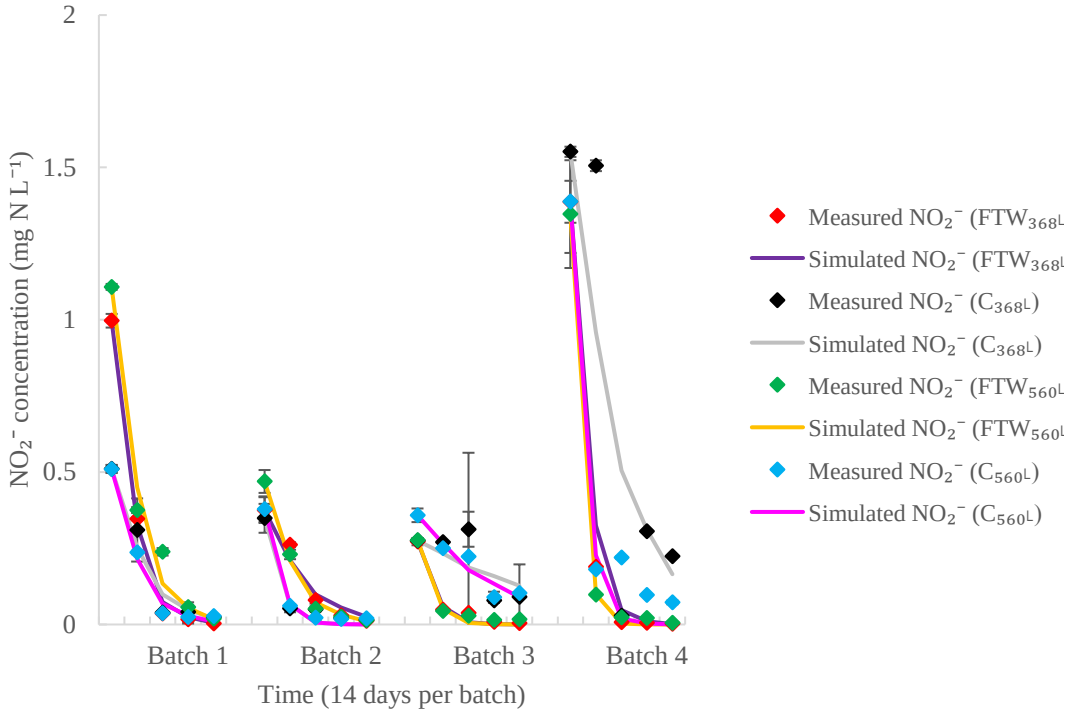

Figure 5. 8 Changes in NO_2^- concentrations in the treatment system with (FTW_{368L} and FTW_{560L}) and without (C_{368L} and C_{560L}) mats over the four experimental batches (mean ± standard deviation).

First order kinetics were fitted to the data from each treatment (also shown in Figure 5.8). The rate constants for NO_2^- loss were 0.36 and 0.27 day^{-1} for the FTW_{368L} and FTW_{560L} cells, respectively and 0.17 day^{-1} for the controls. Corresponding half-lives are shown in Table 5.6, along with the r^2 values for the fits, which suggest that the first order model is reasonable for representing net NO_2^- dynamics. It should be noted, however, that NO_2^- is an intermediate species in two-step nitrification, so concentrations represent the net balance between NH_4^+ oxidation and NO_2^- oxidation. The fact that concentrations of NO_2^- were relatively high at the start of each experimental batch period suggests that the main secondary treatment stage (a rotating biological contactor) in the wastewater treatment plant installed at the hotel complex is not effective at completely converting NH_4^+ to NO_3^- Since NO_2^- is toxic to aquatic organisms at high concentrations (Colt et al., 1981; Kamstra et al., 1996; Nyerges et al., 2010), its removal during wastewater treatment prior to discharge to surface waters is important.

Table 5. 6 Average values (± standard deviation) of NO_2^- removal (mg N L^{-1}), first order nitrification rate constants (k_{nit}, day^{-1}), half-lives ($T_{1/2}$, day), and removal efficiencies (*RE*, %) over the four batches.

Treatment	Extents (mg N L^{-1})	k_{nit} (day^{-1})	$T_{1/2}$ (day)	*RE* (%)	R^2
FTW_{368L}	0.75±0.26^a	0.36±0.05^a	2.04±0.29^a	98.74±0.65^a	0.99
$C_{368\ L}$	0.58±0.25^a	0.17±0.02^b	4.45±0.93^b	85.91±6.68^a	0.90
FTW_{560L}	0.78±0.25^a	0.27±0.03^{ab}	2.66±0.36^{ab}	97.25±1.21^a	0.99
C_{560L}	0.60±0.24^a	0.17±0.02^b	4.31±0.90^b	88.82±5.81^a	0.98

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other (α =0.05).

The dynamics of nitrate concentrations over time in the four experimental batches is shown in Figure 5.9. Initial concentrations (average 1.65±0.12 mg L^{-1}) in each batch were approximately twice those of NO_2^-. In all four batches, there was a general decrease in NO_3^- concentrations – but most markedly in the first two batches for all treatments and most notably for FTW treatments.

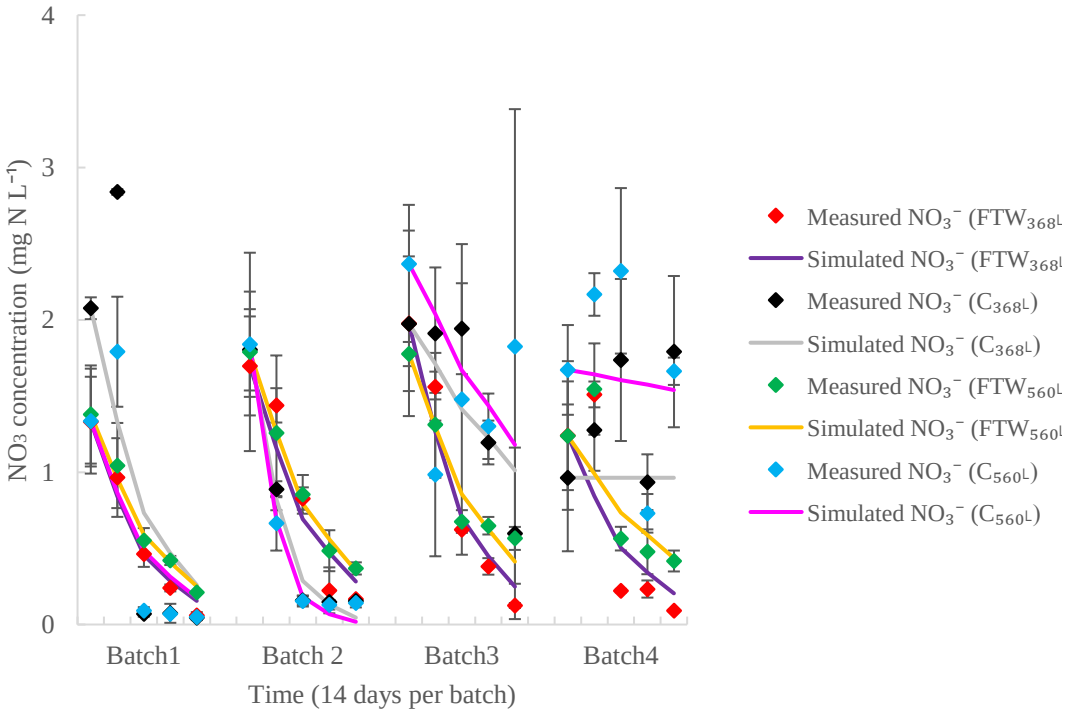

Figure 5. 9 Changes in NO_3^- concentrations in the treatment system with a system with (FTW_{368L} and FTW_{560L}) and without (C_{368L} and C_{560L}) mats over the four experimental batches (mean ± standard deviation).

The average total losses of NO_3^- were higher for the FTW treatments compared with those for the controls (Table 5.7). The final averages of NO_3^- concentrations in the FTW_{368L} and FTW_{560L} of the experimental periods were 0.11±0.02 and 0.39±0.07 mg N L^{-1} respectively. Final average NO_3^- concentrations in the C_{368L} and C_{560L} were 0.64±0.34 and 0.92±0.48 mg N L^{-1}, respectively. There were no significant differences between the two different operational volumes for either treatment (ANOVA, $F_{3, 80} = 2.41$; $P = 0.05$). However, FTW-systems exhibited higher capacities to remove NO_3^- compared to the control particularly during 3rd and 4th trials (Figure 5.9).

The loss of NO_3^- from the experimental chambers could be due to uptake by plants and or algae and immobilization by the microbial biomass (Reddy et al., 1989; Cardon, 2007; Karpuzcu and Stringfellow, 2012). Losses could also be due to the process of denitrification. The first order reaction rate constants shown in Table 5.7 represent a combination of these processes. An attempt is made to disentangle their relative contributions later in the Chapter using the system dynamic model.

Table 5. 7 Average values (± standard deviation) of NO_3^- removal (mg N L^{-1}), first order denitrification rate constants (k_{denit}, day^{-1}), half-lives ($T_{1/2}$, day), and removal efficiencies (*RE*, %) over the four batches.

Treatment	Extents (mg N L^{-1})	k_{denit} (day^{-1})	$T_{1/2}$ (day)	*RE* (%)	R^2
FTW_{368L}	1.45±0.15[a]	0.20±0.01[a]	3.44±0.11[a]	92.99±1.10[a]	0.95
$C_{368\ L}$	1.26±0.44[a]	0.15±0.06[a]	9.77±5.22[a]	64.76±22.41[a]	0.53
FTW_{560L}	1.15±0.12[a]	0.11±0.01[a]	6.59±0.65[a]	74.66±4.42[a]	0.95
C_{560L}	0.88±0.37[a]	0.13±0.06[a]	16.93±8.55[a]	52.99±24.28[a]	0.65

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

The rate constant for the FTW_{368L} treatment was slightly higher than that for the respective control. However, for the FTW_{560L} chamber, the rate constant was slightly less than that in its control. This relatively small difference between the FTW treatments and

the controls, together with the fact that all four chambers demonstrated high loss rates for NO_3^-, suggests that the major loss process in these systems was denitrification. In each batch period, the wastewater in chambers was static. Since primary-treated domestic wastewater applied in this study is expected to contain reasonably high concentrations of organic matter, a high rate of oxygen consumption is likely, resulting in anaerobic conditions which facilitate denitrification. Lower DO concentration (1.26±0.25 mg L^{-1}) observed in tested cells during experimental periods could support such an explanation. Denitrifiers may have developed on the FTW material itself, but are more likely to be present in biofilms on the side walls and the bed substrate, where DO concentrations are likely to have been lower (Zhang et al., 2016). Nitrification and denitrification can proceed simultaneously, particularly in the presence of DO gradients (higher DO near the water surface; lower DO in the detritus on the bed where decomposition provides an important oxygen sink. Plant exudates also introduce dissolved organic carbon (rhizodeposits) to the water column which can act as electron donors (Saad et al., 2016). Removal efficiencies of NO_x observed in this study were substantially higher than those reported by Zhang et al., (2016) (7.3% - 18.2% in semi-static systems containing synthetic wastewater retrofitted with FTWs). Although systematic reduction, initial and final NO_x concentration in the current study had also appeared much higher than the findings observed by Chang et al. (2013) when total oxidized N decreased from 0.06 to 0.03 mg N L^{-1} in a detention pond retrofitted with floating island for 11 months. Similarly, was observed in a study conducted by (Winston et al., 2013), who found that NO_x concentration decreased in two stormwater retention pond from pre-to-post floating mat-retrofits from 0.3 to 0.06 mg N $L^{-1}$and from 0.17 to 0.06 mg N L^{-1} than without (from 0.20 to 0.08 mg N L^{-1} and 0.12 to 0.06 mg N L^{-1}) for 14 months.

Corresponding half-lives are shown in Table 5.7, along with the r^2 values for the fits, which suggest that the first order model is reasonable for representing net NO_3^- dynamics, particularly in the case of the FTWs. There is some possible reasons for discrepancies between the model predictions and the observed concentrations. These include: (a) the assumption of 1st order kinetics which may be an oversimplification of reality. In many systems zero order or mixed (Michaelis-Menten) kinetics can be observed; (b) the measured data have associated sampling errors (they were obtained from only five samplings in each batch); (c) the measured data also have associated analytical errors and uncertainties about preservation.

Overall, model prediction suggests that the NO_3^- treatment performance could be improved by the FTW treatments through extending the incubation period of the floating system. Much biofilm development alongside with robust plant growth over the time could resulted in reliable cumulative plant/microbe's uptake processes by the floating system to mitigate NO_3^- levels.

5.3.1.5 Total Nitrogen Removal

The TN concentration at the start of each batch period was approximately 8 mg N L^{-1}. This decreased systematically in all treatments and in all four batches (Figure 5.10).

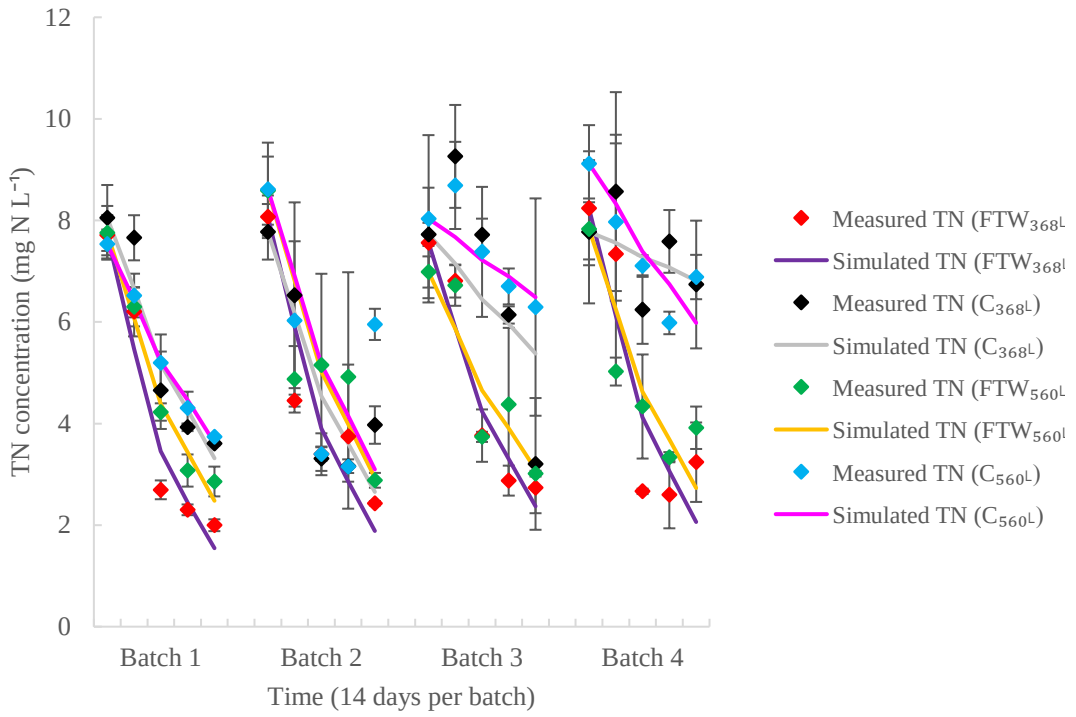

Figure 5. 10 Changes in the concentration of TN in the treatment system with (FTW_{368L} and FTW_{560L}) and without (C_{368L} and C_{560L}) mats for the four experimental batch periods (mean ± standard deviation).

The average total losses of TN were higher for the FTW treatments compared with those for the controls (Table 5.8). The final averages of TN concentrations in the FTW_{368L} and FTW_{560L} of the experimental periods were 2.60±0.26 and 3.16±0.25 mg N L^{-1} respectively. Final average TN concentrations in the C_{368L} and C_{560L} were 4.54±0.73 and 5.71±0.68 mg N L^{-1}, respectively.

First order rate constants (shown in Table 5.8, along with equivalent half-lives) were relatively higher for the FTW treatments compared with those for the controls, suggesting that the mats provide an effective improvement of TN removal. The extent of the TN removal within treatment systems was almost a result of the reduction of the total inorganic N-forms (TIN) rather than organic-N which demonstrated lower removal rates. This could have been attributed to a combination of N removal pathways including microbial transformations, plant uptake, and physicochemical processes. Although, the TN removal rates of the treatments exceeded those from controls. There was no significant difference (ANOVA, $F_{1, 40} = 12.53$; $P = 0.05$), however.

Table 5. 8 Average values (± standard deviation) of TN removal (mg N L^{-1}), first-order rate constants (k_{denit}, day^{-1}), half-lives ($T_{1/2}$, day), and removal efficiencies (*RE*, %) over the four batches.

Treatment	Extents (mg N L^{-1})	k (day^{-1})	$T_{1/2}$ (day)	*RE* (%)	R^2
FTW_{368L}	5.29 ± 0.22^{a}	0.08 ± 0.01^{a}	8.18 ± 0.59^{a}	67.12 ± 3.01^{a}	0.94
$C_{368\,L}$	3.28 ± 0.76^{b}	0.05 ± 0.01^{bc}	22.32 ± 11.19^{a}	44 ± 10.40^{bc}	0.81
FTW_{560L}	4.62 ± 0.42^{ab}	0.06 ± 0.01^{ac}	11.33 ± 0.99^{a}	59.10 ± 3.62^{ac}	0.92
C_{560L}	2.60 ± 0.43^{b}	0.03 ± 0.01^{b}	23.20 ± 4.45^{a}	31.84 ± 6.47^{b}	0.85

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other ($\alpha = 0.05$).

Estimated R^2 values for the measured and simulated data suggest that the first order model is reasonable for describing net TN dynamics in all treatments (Figure 5.10). Data from first order fits showed that 94 and 92% of the measured data in FTW_{368L} and FTW_{560L} were represented by the first order model, while 81 and 85% of the overall TN dynamics in C_{368L} and C_{560L} were described.

5.3.1.6 Microbial Biomass

The distribution of the culturable bacterial population in different compartments of the treatment system at the start and end of the study is presented in Figure 5.11. Total viable counts (TVCs) were generally much higher in the chambers fitted as FTW treatments

than in the controls. Bacterial densities in the microbial biofilm established on plant roots and the mats themselves were generally much higher than the densities of freely suspended bacteria in the water column.

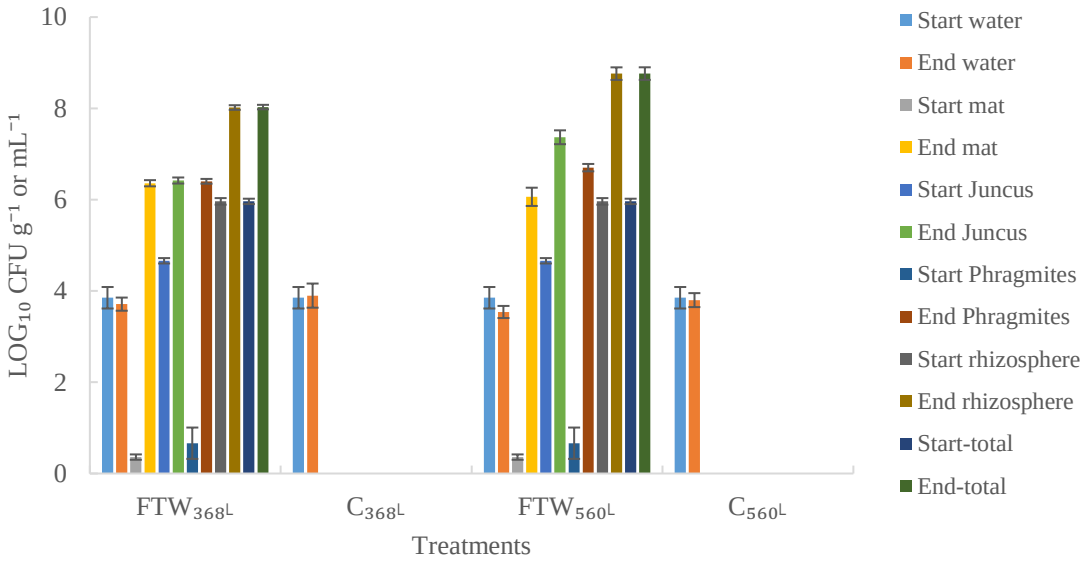

Figure 5. 11 Viable plate counts of bacteria present in each treatment cell at the start and end of the experimental duration. Each bar represents a different sample medium including water, mat material, and epiphyton at different treatment chambers investigated in the study. Each bar represents the average ± standard deviation.

TVC's on the mat and plant root materials increased throughout the study. The number of colonies forming units on the floating mat material increased from 0.359±0.058 log10 CFU g^{-1} at the start of batch 1 to 6.36±0.06, 6.06±0.19 log10 CFU g^{-1} at the end of the batch 4. A similar trend was observed with microbial abundance on plant roots over the same period with a total epiphytic increase from 5.96±0.05 log10 CFU g^{-1} to > 8.01±0.05 and 8.76±0.13 log10 CFU g^{-1} in the FTW_{368L} and FTW_{560L}. However, free bacteria in the wastewater column have only achieved 3.71±0.14 and 3.53±0.26 log10 CFU mL^{-1} for the small and large volumes, respectively.

The ratio of bacterial density per unit volume of water sample was 40 and 46% of the density per unit weight of floating island in FTW_{368L} and FTW_{560L}, respectively. On average, bacterial growth rates per island in the FTW_{368L} and FTW_{560L} cells were 6.34±0.06 and 6.83±0.24 log10 CFU g^{-1} m^{-2} day^{-1}, respectively. These results suggest that FTW systems contain higher microbial densities compared with the controls. Since most of the transformation processes for N in wetland systems are mediated by micro-

organisms, this finding supports the observations that most of the salient processes are operating at higher rates in the presence of floating mats. In addition to the quantitatively increase of the microbial density, different microbial populations could be developed, as biofilm built, leading to more diverse community that capable to transform different contaminants. However, it should be noted that plate counts are a relatively crude measure of the size and composition of the microbial biomass. Many micro-organisms found in environmental systems cannot be cultured using traditional plate counting procedures (Mincer et al., 2007; Truu et al., 2009). Furthermore, plate counting does not give any indication about the size of the fungal population (Bisen et al., 2012). Finally, plate counts do not tell us anything about the composition of the microbial consortium present (i.e., the abundance and diversity of different organisms or functional groups of organisms). Although the changes in concentrations of different N-species dynamics in the studied systems is indicative of the presence of competent organisms (e.g., nitrifiers) in the consortium, it would have been useful (and be useful in future studies of this kind) to attempt to quantify aspects of the microbial population composition.

Molecular technologies and genetic techniques such as polymerase chain reaction followed by denaturing gradient gel electrophoresis-PCR DGGE or fluorescence in-situ hybridization-FISH probes could be applied to quantify these aspects (Harmsen et al., 2000; Urakawa et al., 2006; Wertz et al., 2008). Further, activity assays for microbial communities could add valuable information about active microbial groups present, and therefore identify predominant processes in the study area. Collectively, this could help to better-interpret the N dynamics in the tested systems.

5.3.1.7 Plant Growth and Nitrogen Uptake

The biomass (dry weight) of *Juncus effusus* and *Phragmites australis* increased over time in both chambers containing FTWs. Maximum vegetative biomass was observed at the end of the four experimental batches (56 days in total) in the FTW_{368L} (357.60 g dryweight m^{-2}) (ANOVA, $F_{1, 12} = 6.806$; $P = 0.05$). The mean growth rate for *Juncus spp* and *Phragmites spp* were 3.67±0.76 and 0.61±0.57 g dry weight m^{-2} day^{-1}. For FTW_{560L} wetland, the total biomass was 202.23 g dry weight m^{-2} associated with total growth rate of 2.23±0.64 g dry weight m^{-2} day^{-1} (1.41 g dry weight m^{-2} day^{-1} for *J. effuses* and 0.81 g dry weight m^{-2} day^{-1} for *P. australis*). The increase in the *J. effuses* biomass was mainly the result of an increase in shoots. However, the biomass increases in *P. australis* were

observed in both the roots and the shoots (Table 5.9). For *J. effusus*, although N concentration decreased over experimental time (from 18.16±1.49 to 12.04±0.35 mg N g^{-1}), there was still a net accumulation of N on the plants because biomass increased faster than N concentration decreased. In contrast, in *P. australis*, TN content increased from 19.52±0.68 to an average of 23.97±1.41 mg N g^{-1} in both FTW cells. These differences might indicate different N-accumulation capacities by selected plants and, hence, variability in their ability to remove N from wastewater (White and Cousins, 2013). Overall, FTW_{368L} showed higher N-uptake rate (28.7±8.4 mg N m^{-2} day^{-1}), which accompanied with greater total biomass production. In comparison, the daily uptake rate of N was lower in FTW_{560L} (18.8±8.5 mg N m^{-2} day^{-1}) associated with lower biomass production (Table 5.9). The net amount of N absorbed by the plants established on the FTW_{368L} was 1611±472.2 mg m^{-2}, while the assimilated N-mass by FTW_{560L} was 1055±479.6 mg m^{-2}. These results are much less of the findings from Keizer-Vlek et al. (2014) who reported TN uptake rate of *Iris* (18.6 g N m^{-2}) during the 91-day experimental trial with groundwater maintained with 4 mg N L^{-1}. Such variations might be due to the differences in the bioavailability of N for vegetation at different media, where plant uptake is accepted to be more efficient in rich- N environment. Also, the wastewater quality conditions (e.g., sewage discharge) are expected to exert an influence on biomass production, which might influence the accumulated amount of N in plant tissues.

	Time	Species	Root/Shoot ratio	Total biomass (g m^{-2})	Growth rate (g m^{-2} day^{-1})	Total N (mg N g^{-1})	Uptake rate (mg N m^{-2} day^{-1})
FTW_{368L}	Start	*Junc.**	0.3±0.04	100.6±9		37.6±1.6	
		*Phrag.***	3.5±0.65				
	End	*Junc.*	0.7±0.12	357.6±50[b]	4.45±1[a]	35.4±2.1[a]	28.7±8.4[a]
		Phrag.	1.9±0.19				
FTW_{560L}	Start	*Junc.*	0.3±0.04	66.1±6		37.6±1.6	
		Phrag.	3.5±0.65				
	End	*Junc.*	0.5±0.07	202.2±28[a]	2.23±0.6[a]	37.1±1.7[a]	18.8±8.5[a]
		Phrag.	1.8±0.34				

Table 5. 9 Mean plant biomass characteristics and TN tissue content (±standard deviation) of the two-species established on the FTWs at the start and end of the experiment (a total of 56 days). * *Juncus effuses*; ** *Phragmites australis*.

5.3.1.8 Nitrogen Mass Balance

The N-mass balance consists of quantifying the total mass of nitrogen in different forms in each treatment over the time course of each batch. The N mass balance for the $FTW_{368L,}$ FTW_{560L} treatments and corresponding controls are illustrated in Figures 5.12 and 5.13. Average N mass loads applied to the treatments over four experimental batches and the percentage of each constituent is shown in Table 5.10.

Table 5. 10 Nitrogen mass loads in the wastewater applied to the experimental cells over four experimental periods (batches).

	FTW_{368L}		**C_{368L}**		**FTW_{560L}**		**C_{368L}**	
	mass load (mg N)	**(%)**	**mass load (mg N)**	**(%)**	**mass load (mg N)**	**(%)**	**mass load (mg N)**	**(%)**
TN	2905±58		2882±27		4362±183		4662±193	
TIN	1582±46	54.44[(a)]	1620±48	56.20	2541±75	58.24	2550±75	54.69
ON	1323±147	45.55[(b)]	1262±86	43.79	1821±147	41.75	2112±152	45.30
NH_x	728±68	46.03[(c)]	746±74	46.03	1227±108	48.30	1171±63	45.92
NO_x	854±43	53.96[(d)]	874±38	53.97	1314±67	51.69	1379±150	54.07

(a) Percentage of total inorganic N mass in the total N presented in the domestic wastewater applied.
(b) Percentage of org-N mass in the total N presented in the domestic wastewater applied.
(c) Percentage of NH_x mass in TIN presented in the domestic wastewater applied.
(d) Percentage of NO_x mass in TIN presented in the domestic wastewater applied.

The daily average TN mass removal rates from the $FTW_{368L,}$ FTW_{560L} and corresponded controls in each 14-day batch were 151±6, 132±12, 94±22 and 75±13 mg N m^{-2} day^{-1}, respectively. Thus, on average, TN-mass removal in FTW chambers were 67 and 59% of the total-N mass inflow to the treatment system, whereas, controls achieved 41.84 and 31.84% of the total-N mass within the same time frame. For comprehensive process analysis of FTWs performance, an attempt was made to separate the performance of floating mat from other treatment processes ongoing within their respective water body. The performance of floating mat was estimated by taking the difference of mass removal rates between FTW (with mat) and control cells (without mat). Accordingly, estimated performance of the floating mat was by 37.95% and 43.59% of the whole $FTWL_{368L}$ and FTW_{560L} performance, with a net daily removal rate of 57 mg N m^{-2} day^{-1} for both treatments. Therefore, water body processes were estimated to be responsible for 62% and 56% of the total performance of FTW_{368L} and FTW_{560L} cells, with a mass removal rate of 93.9 and 74 mg N m^{-2} day^{-1}, respectively. Likewise, the contribution of the microbial activity developed on the floating mat was estimated as a product of the difference between the performance of the floating island and the measured plant uptake.

Microbial transformation onto a floating system, including root zone, has characterized 18.9% and 29% of the total microbial performance in the FTW_{368L} and FTW_{560L} systems. Overall, Microbial transformations contribute to between 80 and 85% of the total-N removal in the FTW treatments. Likewise, plant uptake rates revealed a contribution of 19% and 14% of the overall performance of FTW systems, respectively. Uptake by vegetation accounted for 28.8±8.43 and 18.85±8.56 mg N m^{-2} day^{-1} in FTW_{368L} and FTW_{560L} cells, respectively (Figure 5.12a, b).

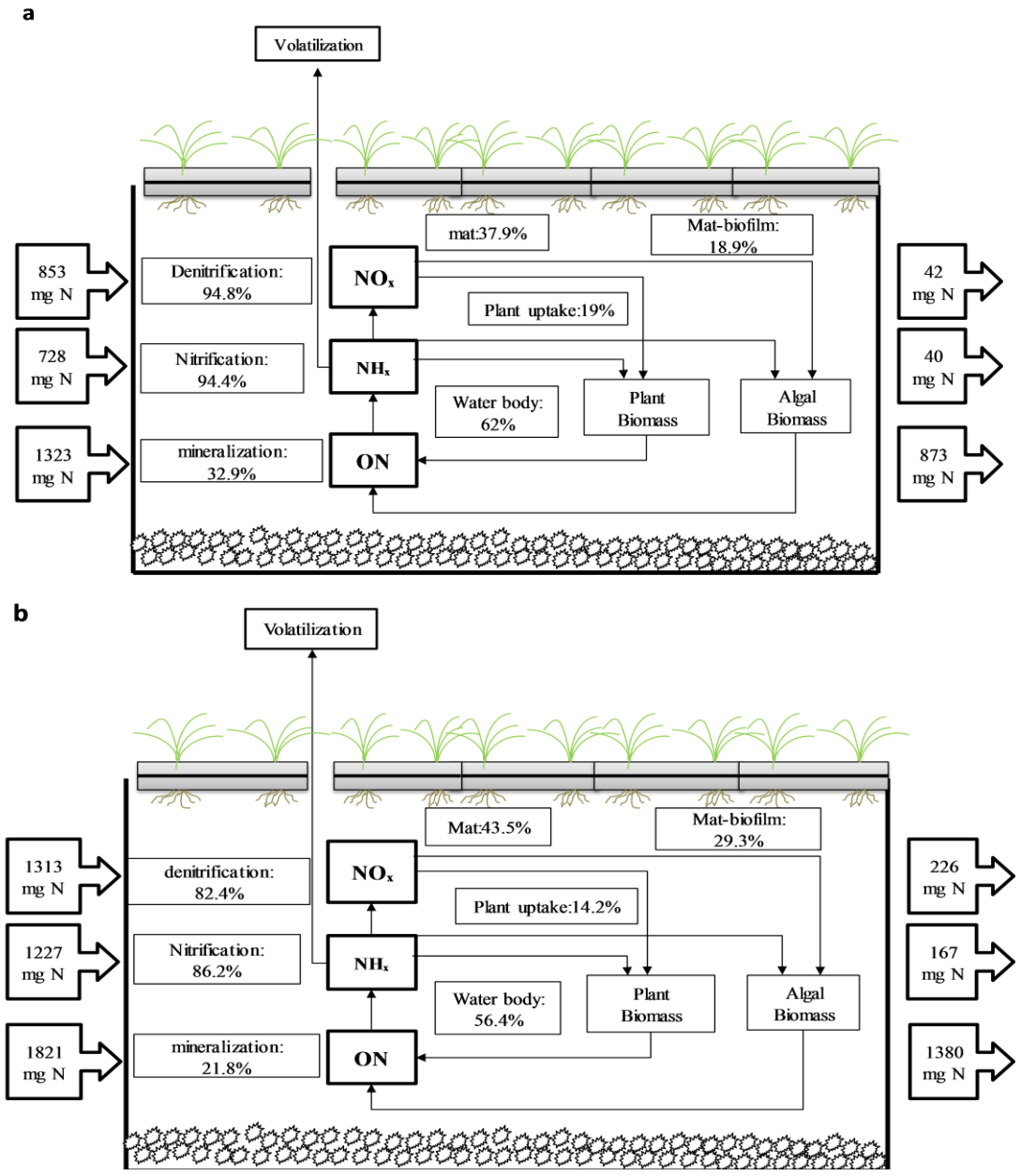

Figure 5. 12 Summary of N mass balance and the contribution of different removal processes in (a) the FTW_{368L} chamber (b) FTW_{560L} chamber. The data represent average values over four experimental batches.

Losses in the control chambers include NH_3 volatilization, inorganic-N immobilization by microorganisms, settling out of particulate N and adsorption of NH_4^+ to the system liner and gravel bed. Since the average water pH during the experimental phase was 7.2, volatilization was calculated to be negligible (see Section 5.3.2). Subsequently, the microbial pathway was estimated to be responsible for 62% (93.8 mg N m^{-2} day^{-1}) and 56% (74.4 mg N m^{-2} day^{-1}) of the overall N-removal of C_{368L} and C_{560L} cells, respectively (Figure 5.13a, b).

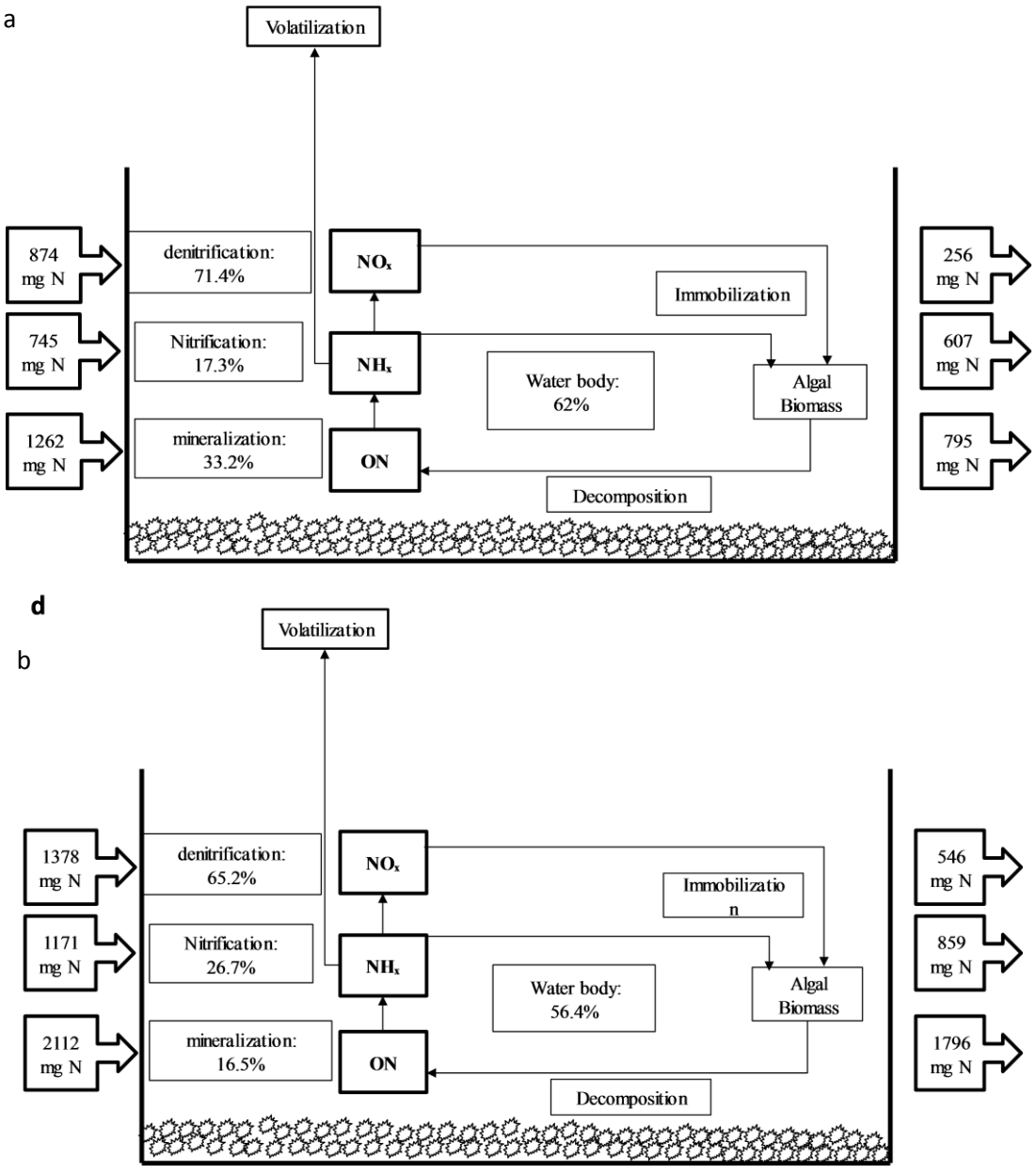

Figure 5. 13 Summary of N mass balance and the contribution of different removal processes in (a) the C_{368L} chamber (b) C_{560L} chamber. The data represent average values over four experimental batches.

In terms of N-species fate, the average daily loss of the ON in FTW_{368L} and FTW_{560L} were 35±10.6 and 22.4±12.7 mg N m^{-2} day^{-1}, while C_{368L} and C_{560L} showed removal rates of 35.2±13.6 and 16.1±11.5 mg N m^{-2} day^{-1}, respectively. Removal efficiency by FTW_{368L} and FTW_{560L} systems were 32.9% and 21.8% compared to the corresponded controls (33.2% and 16.5%, respectively). NH_x behavior within FTW systems has revealed greater mass removal rate compared to the controls. Average NH_x losses for the FTW_{368L} and FTW_{368L} were 53.4±5.17 and 54.1±4.9 mg N m^{-2} day^{-1} (94% and 86.2% of input, respectively). However, mass removal rates in C_{368L} and C_{560L} systems were 10.7±4.67 and 15.9±3.6 mg N m^{-2} day^{-1} (17% and 26.73% of input, respectively). Thus, on average, removal of NH_x was 40.5 mg N m^{-2} day^{-1} (i.e., a factor 4) higher with FTWs than in the controls.

There was also a reduction in NO_x concentrations in all chambers over time in each batch. On average, removal of NO_x in the FTW_{368L} and FTW_{560L} were 63±3.83 and 55.48±5.05 (94.9% and 82.29% of input), whereas C_{368L} and C_{560L} exhibited 47.9±12.5 and 42.47±7.84 mg N m^{-2} day^{-1}, respectively (71.4% and 65.257% of input) (i.e., losses were 20% higher with the FTWs compared to the controls).

On average, the final ON mass remaining in the FTW_{368L} and FTW_{560L} were 873 and 1380 mg N (91 and 77.8% of TN remaining), while C_{368L} and C_{560L} revealed 795 and 1796 mg N (48 and 56% of TN remaining), respectively. Final TIN mass remaining in the FTW_{368L} and FTW_{560L} were 83 and 393 mg N (9 and 22% of TN remaining), while corresponded controls showed 864 and 1405.4 mg N (52 and 43.8% of TN remaining).

Based on the obtained data, it suggests that the FTW-systems influenced N removal. N-removal in the FTW systems compared to the control treatment observations indicated that treatment performance for N was enhanced.

5.3.1.9 Physicochemical Responses

During each of the four experimental batches pH, dissolved oxygen, electrical conductivity, and temperature were monitored. Figures 5.14 showing the dynamics of pH and DO concentration over four sequenced batches. pH was slightly depressed in the presence of FTWs compared with the controls (ANOVA, $F_{3, 80} = 35.53$; $P < 0.05$) (Table 5.11). pH values in the FTWs decreased from 7.4 to 7.2 during each experimental batch. This was probably due to the well-known phenomenon of protonation during

nitrification which results in the generation of 2 H^+ ions for every molecule of NH_4^+ which is oxidized to NO_3^- (Housecroft, 2010), although they may have been some buffering by the wastewater (Cervantes, 2009). There may also have been some release of organic acids in root exudates from the established plants (Cardon, 2007). Such releases are expected to increase by increasing plant density (e.g. maximum plant density used in the study). Zhai et al. (2013) reported that fluxes of soluble root exudates from *P. australis* (9±0.9 µg C g^{-1} dry weight root biomass) were higher than from *J. effuses* (4.3±0.4 µg C g^{-1} dry weight root biomass).

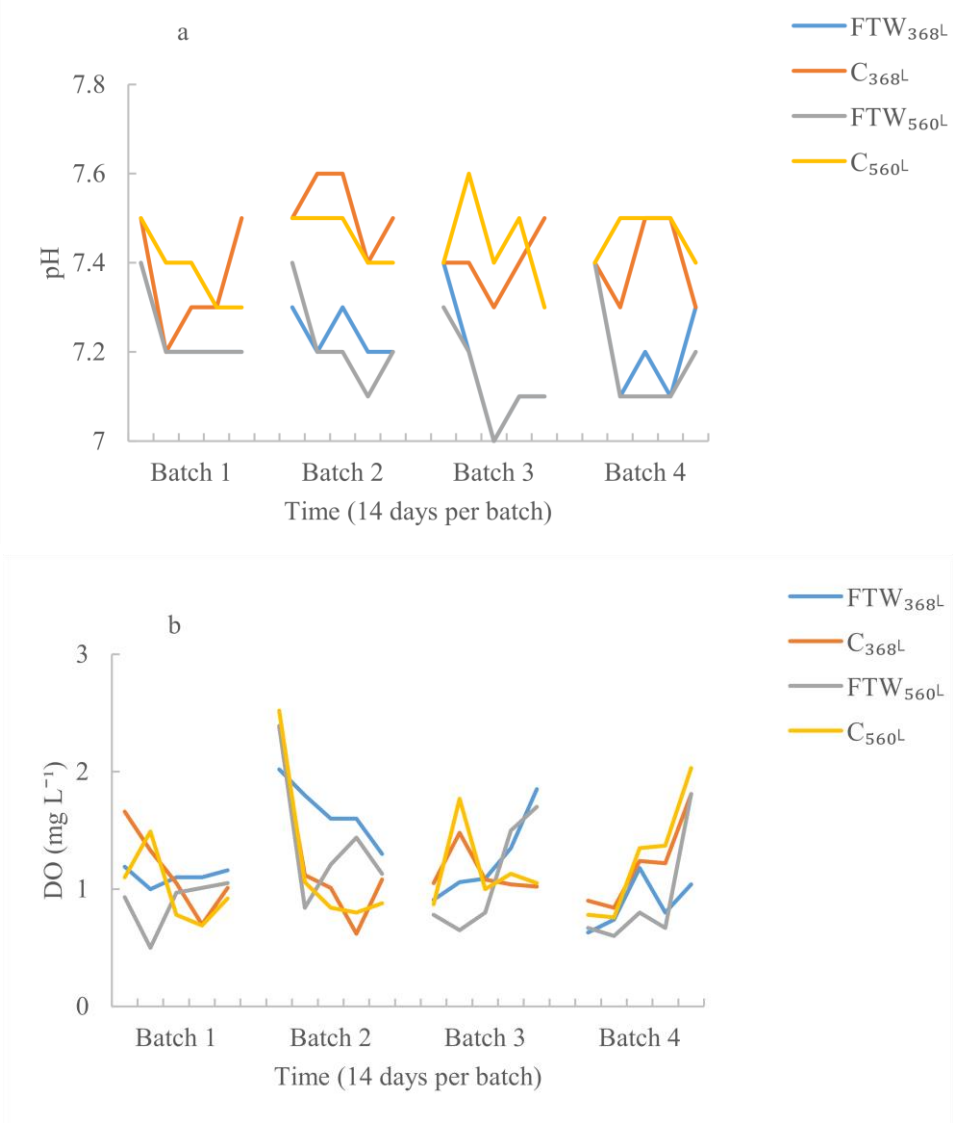

Figure 5. 14 Changes in the (a) pH and (b) DO concentration in treatment chambers over four experimental periods.

Table 5. 11 Mean (standard deviation) pH, DO concentration, Electrical conductivity (EC) and temperature in each chamber over the whole experimental period.

Treatment	pH	DO ($mg\ L^{-1}$)	EC ($mS\ cm^{-1}$)	Temp (°C)
FTW_{368L}	7.21 (0.04)^{a}	1.63 (0.24)^{a}	1.23 (0.02)^{ab}	17.57 (0.66)^{a}
C_{368L}	7.42 (0.04)^{b}	1.18 (0.17)^{a}	1.20 (0.02)^{a}	17.07 (0.71)^{a}
FTW_{560L}	7.19 (0.04)^{a}	1.07 (0.20)^{a}	1.20 (0.04)^{a}	17.40 (0.64)^{a}
C_{560L}	7.43 (0.03)^{b}	1.15 (0.21)^{a}	1.30 (0.01)^{b}	17.49 (0.71)^{a}

Upper letters denote Tukey HSD test for multiple comparisons of means. Treatments with the same letter are not significantly different from each other (α =0.05).

The rate constants for NH_x are plotted against pH in Figure 5.15. A negative correlation was observed between removal rate constant of ammonia and pH (r^2 = 0.92; p = 0.05).

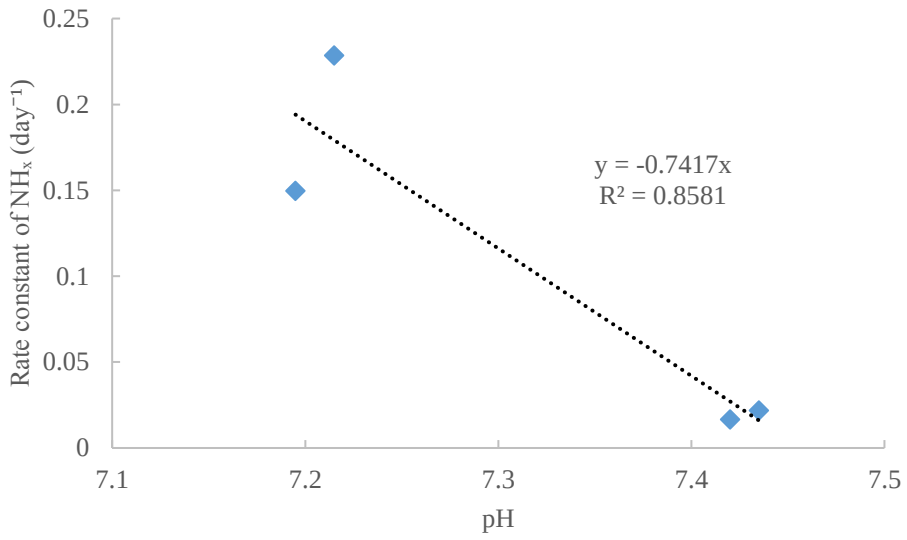

Figure 5. 15 Removal rate constant of NH_x versus pH in the treatment chambers during the study.

The DO concentrations fluctuated from day to day, but average concentrations in all chambers were consistently relatively low (<< 2 mg L^{-1}). There were no significant differences between treatments (ANOVA, $F_{3,\ 80}$ = 2.665; P < 0.05). Oxygen can be depleted by aerobic respiration and augmented by photosynthesis, passive diffusion (reaeration) and root-mediated radial oxygen loss (Saad et al., 2016). Brix et al. (1996) and Wiessner et al. (2008) estimated the net flux of oxygen from *P. australis* and *Juncus effusus* to below ground tissues to be up to 5.7 and 12 mg O_2 L^{-1} day^{-1}.

There was almost limited variability in EC over four experimental batches. EC values in all treatments ranged between from 1.3 to 1.2 mS cm^{-1} (Figure 5.16). Again, there were no significant differences between treatment means (ANOVA, $F_{3, 80} = 7.654$; $P < 0.05$). Water temperature decreased from 20 to 13 °C throughout the study, in response to meteorological variations. On average, the water temperature in the FTW-treatments was not significantly different from the controls (i.e., there was no evidence of shading effects).

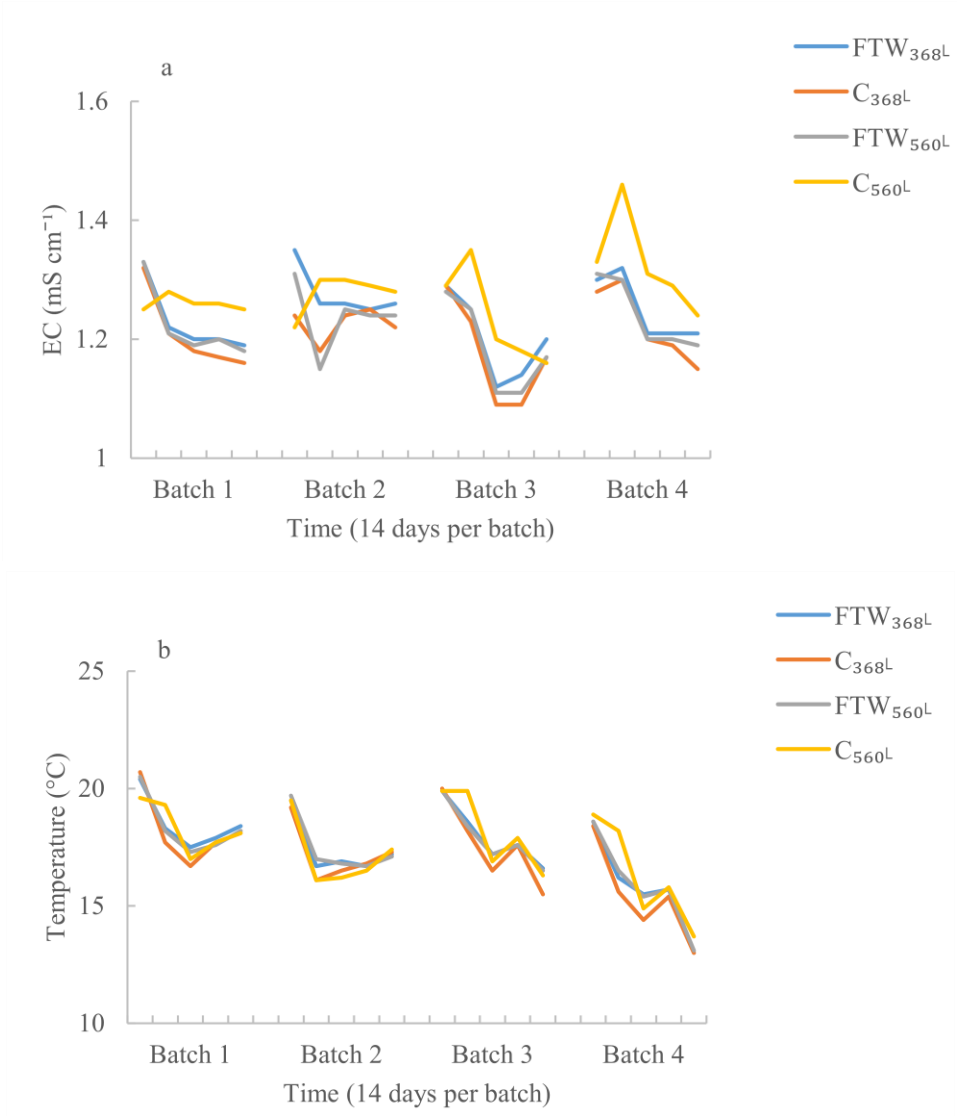

Figure 5. 16 Changes in the (a) EC and (b) water temperature in treatment chambers over four experimental periods.

5.3.2 Model Performance

The numerical model described in Chapter 2 was applied to the experimental system. Four parameters (k_{up}, k_{amo}, k_{nit} and k_{denit}) from cell FTW_{560L} were optimised within effort of model calibration in order to get best fits between simulated and measured values of ON, NH_x and NO_x. Model performance for ON, NH_x, and NO_x for the calibration on the FTW_{560L} treatment is illustrated in Figure 5.17. The optimized RMSE values were 0.174, 0.145 and 0.118 mg N L^{-1} for ON, NH_x and NO_x, respectively. These values are relatively low, reflecting that the model was able to reproduce the pattern of measured concentrations reasonably well (also illustrated in good visual fits between measured and modeled concentrations). Calibrated values of k_{amo}, k_{nit}, k_{denit}, k_{upNHx} and k_{upNOx} were 0.0198, 0.17, 0.26, 0.015 and 0.015 day^{-1}, respectively. The model was validated against independent data from other treatments (with no further optimization of parameters, except that k_{up} values were set to zero in controls with no vegetation and k_{nit} was reduced from 0.17 to 0.057 day^{-1} in the controls to represent the absence of mat-associated nitrifiers), see section 5.2.2.

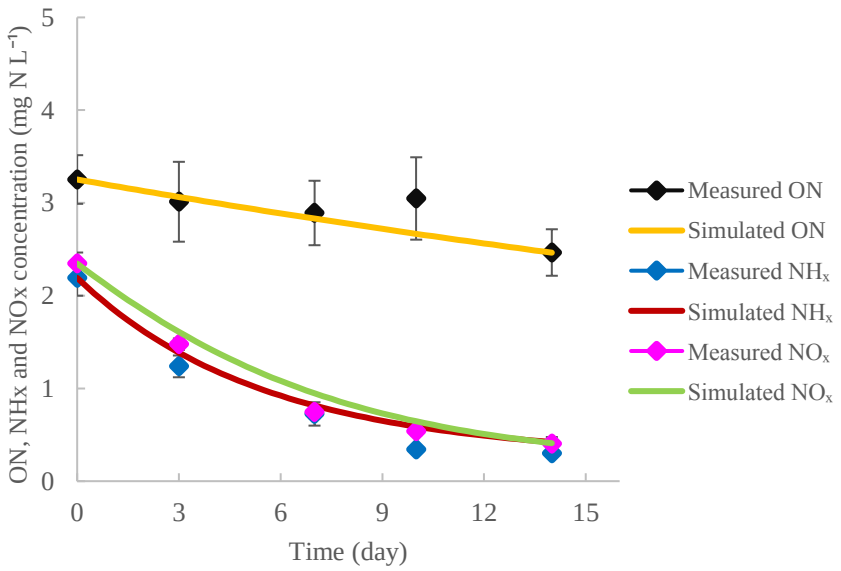

Figure 5. 17 Measured time series of mean ON, NH_x and NO_x concentrations in the FTW_{560L} treatment (symbols) and simulated values (lines) produced using the calibrated model. Error bars show standard deviations.

Measured and simulated time series for ON, $NH_{x,}$ and NO_x in the other treatments are shown in Figure 5.18. R^2, slope, RMSE values for concentrations are shown in Table 5.12. There was a good agreement between predicted and measured data in the FTW_{368L} treatment. In general, ON, NH_x and NO_x removal efficiencies for equivalent systems (FTW or control) tended to be slightly higher in those chambers with lower volume than in those with higher volume. In part, this could result from the higher plant uptake rate in the FTW_{368L} that associated with higher biomass growth (28.78±8.43 mg N m^{-2} day^{-1}, 4.30 g dry weight m^{-2} day^{-1}) compared to FTW_{560L} (18.85±8.56 mg N m^{-2} day^{-1}, 2.20 g dry weight m^{-2} day^{-1}, respectively).

Higher measured uptake in the FTW_{368L} treatment (by a ratio of 1.53) compared to the FTW_{560L} treatment could explain some of the deviation model predictions from observations for NH_x and NO_x. Model performance was poor for NH_x in the controls. This could result from the higher value of k_{nit} that consisted the majority of the nitrification in the system, that hold by floating mat, which is absent in the control systems. Figure 5.18b_1,c_1 shows model performance when k_{nit} reduced by factor 3 in the control treatments.

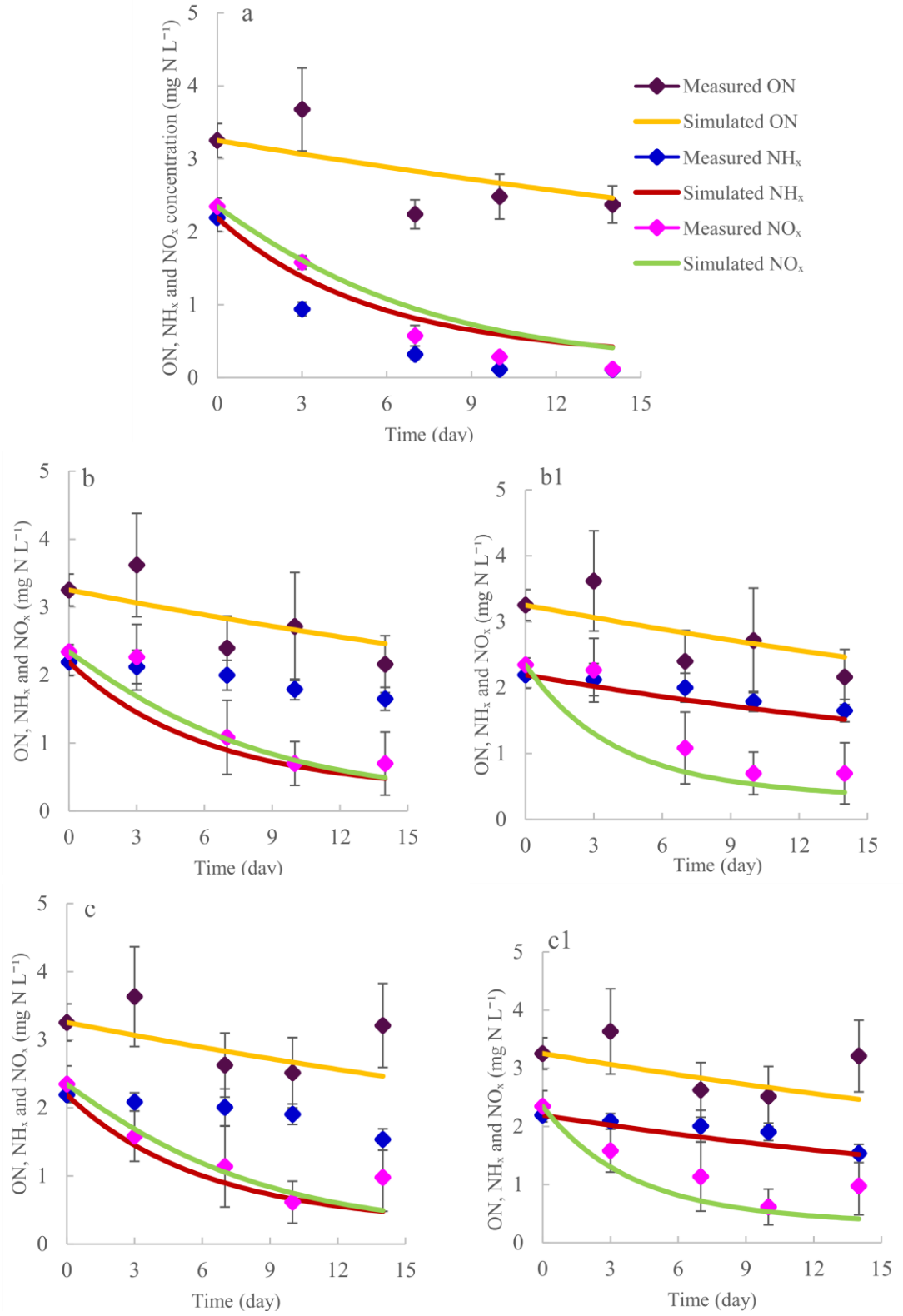

Figure 5. 18 Changes in measured and predicted values of ON, NH_x and NO_x in the validation exercise: (a) FTW_{368L}; (b) $Control_{368L}$, (b1) $Control_{368L}$ without mat effect, (c) $Control_{560L}$ and (c1) $Control_{368L}$ without mat effect. Each point represents average concentration over four batches ±standard deviation.

Table 5. 12 Comparison of statistical indexes for different variables (ON, NH_x and NO_x, mg L^{-1}) in different treatments. Slope (mg N L^{-1}); RMSE (mg N L^{-1}).

	ON			**NH_x**			**NO_x**		
	R^2	Slope	RMSE	R^2	Slope	RMSE	R^2	Slope	RMSE
FTW_{368L}	0.77	1.54	0.39	0.99	1.21	0.39	0.99	1.20	0.26
$Control_{368L}$	0.83	1.60	0.34	0.91	0.29	0.92	0.95	1.04	0.27
$Control_{560L}$	0.43	0.64	0.43	0.82	0.30	0.92	0.95	0.83	0.23

By reducing calibrated k_{nit} (0.17) to 0.057, model performance for NH_x dynamic in the control treatments was enhanced. RMSE values between measured and predicted NH_x concentration were 0.121 and 0.136 mg NH_x L^{-1} for FTW_{368L} and FTW_{368L}, respectively. In another word, reducing the effect of floating mat by factor 3 improved model performance in the controls.

These results have supported the idea that the majority of nitrification is generated on the floating mat. Microbial transformations are mainly fixed film processes in which bacteria are immobilized in a viscoelastic layer of biofilm that is attached on the solid surfaces rather than in the bulk of the water (Chen et al., 2006; Boltz et al., 2017). These findings have also boosted our hypothesis underpinning the conversion of rate constants obtained via calibration in treatment FTW_{560L}.

Overall, there was good agreement between modeled and measured concentrations for all N species in the validation for both the FTW (R^2 = 0.82, slope 0.92) and the controls (R^2 = 0.94, slope 0.91) (Figure 5.19a,b). This suggests that the model is able to describe the temporal patterns of N transformations in each treatment and explain the differences between treatments. In this way, the model can be seen as an extended hypothesis (representing the set of assumptions upon which it is based) which is "tested" via the validation process.

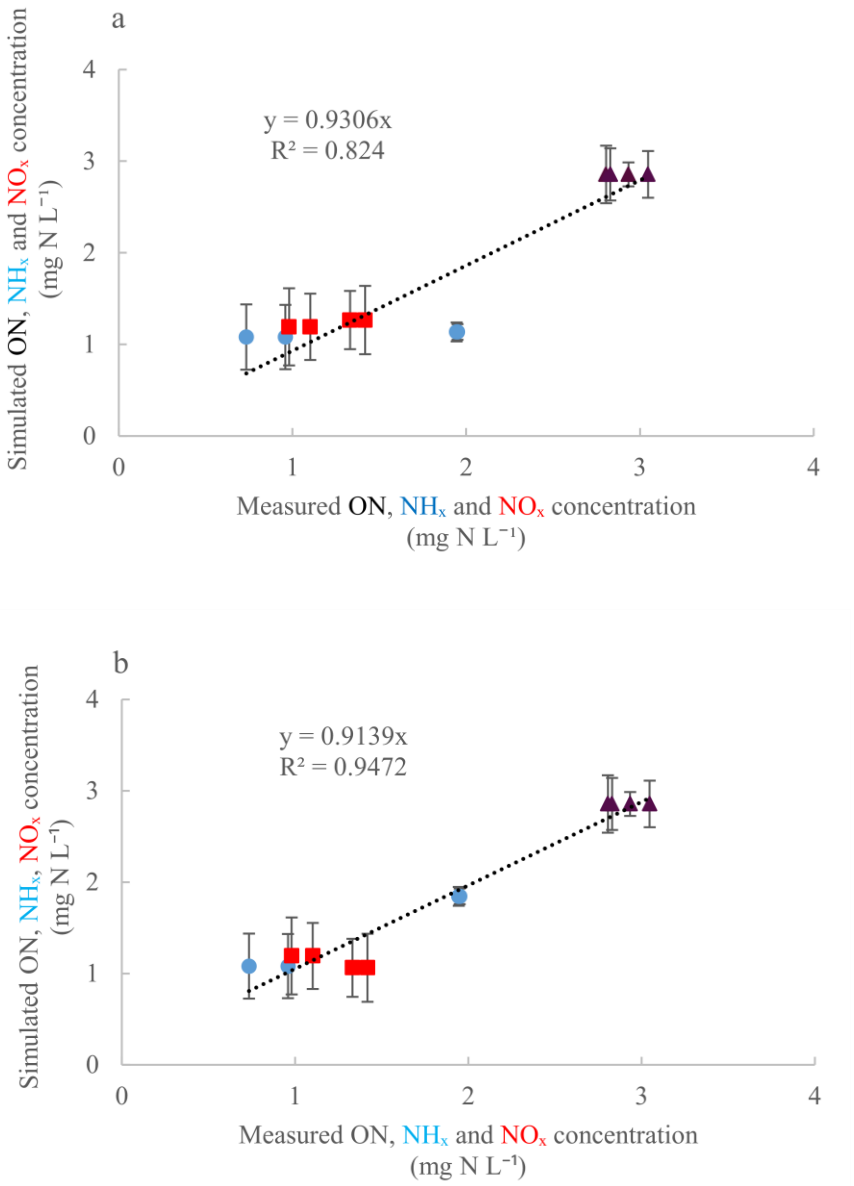

Figure 5. 19 1:1 lines showing the final average measured and simulated ON, NH_x and NO_x concentrations with (a) the effect of floating mat and (b) without. Error bars show standard deviation for measured concentration.

5.3.3 Sensitivity Analysis

Figure 5.20 shows the magnitude of the nitrification, plant uptake, and volatilization processes on the model prediction of NH_x removal under different k_{nit}, k_{up} and k_{vol} values. Of the reaction rate constants investigated, model simulations revealed that NH_x behaviour was most sensitive toward k_{nit} which control biochemical reaction within the system, confirming the potential of nitrification as loss process. By increasing k_{nit}, predicted NH_x concentration were sensitively decreased over time.

Though plant uptake makes clear contributions to NH_x and NO_x removal; model outputs were less sensitive to k_{up} than they were to k_{nit}. Model prediction was least sensitive to k_{vol} which had a relatively little impact on free NH_3 losses and NH_x dynamics overall.

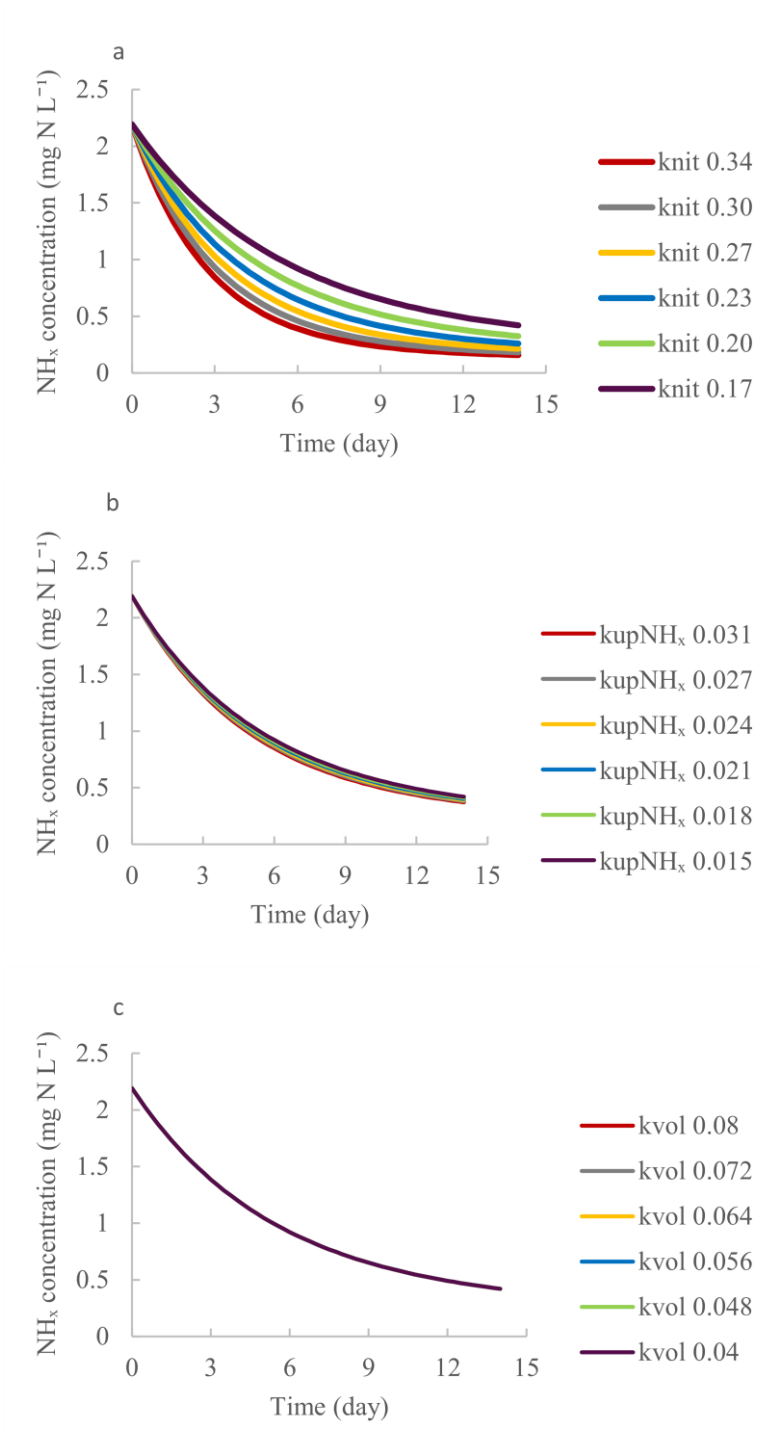

Figure 5. 20 Predicted NH_x removal in the FTW_{560L} with systematic changes in (a) k_{nit}, (b) k_{up} and (c) k_{vol} for FTW_{560L} treatment.

In this study, nitrification was shown to be the most important loss process for NH_x, particularly in the FTWs. This supports the findings from Chapters 3 and 4 which show higher nitrification rates, particularly by increasing the surface of mat materials for more biofilm establishment. Hence, it should be pointed out that the k_{nit} values derived in the present study (e.g., 0.14 day^{-1}) were mostly comparable to those obtained from bench-top experiment (e.g., 0.03 day^{-1}), but lower than mesocosm study (e.g., 1.71 day^{-1}). Such variation could be due to the differences in the oxygen availability in these systems and its effect on nitrification, where the continuous flow-system promotes high oxygenated conditions rather than batch systems. A high ammonia oxidation rate in constructed wetlands with the aerobic condition is often likely because the amount of oxygen available for oxidizing bacteria is usually unlimited (Kadlec and Wallace, 2009b). The contribution of uptake was relatively low compared to nitrification. Plant uptake of nitrogen is typically seen as a less significant nitrogen removal mechanism (Tunçsiper, 2009). Kumar et al. (2016) and Xuan et al. (2012b). These authors estimated the contribution of nitrification rate to be 65 % of the overall removal performance in pilot-scale systems received farm and domestic wastewater. Improved model performance, particularly for NH_x, was observed in the controls by reducing the effect of a floating mat by factor 3.

5.4 Conclusions

In this Chapter, the benefits of FTWs were evaluated in a pilot-scale batch operated tertiary treatment system for real wastewater. This complements the investigations conducted in Chapters 3 and 4 and confirms that FTWs can, indeed, enhance ammonia removal significantly in a semi-operational context. As in the previous Chapters, a simple model was used as an integrating explanatory framework. This allowed the relative contributions of different removal processes to be quantified.

The main conclusions are:

i. A critical FTW design (full coverage of floating mat, maximum plant density and water depth of 0.4 m) from the mesocosm study was scaled up and evaluated to optimize treatment performance of a pilot-scale system in removing ammonia from domestic wastewater under different operational volumes.
ii. A repeated “batch” type experimental design was developed to overcome the shortcomings of low treatment replications. The data obtained from the four

sequenced batch trails represent pseudo replication and allow general conclusions to be drawn from what might otherwise be a compromised experimental setup.

iii. Over the four experimental batches, reductions of 21-32 % of ON, 86-94 % of NH_4^+, 97-98 % of NO_2^-, 74-92 % of NO_3^- and 59-67 % were achieved in the two FTW systems operated with 368 and 560L of wastewater, respectively. The FTW_{368L} system had higher apparent removal efficiency compared with the FTW_{560L} system. All treatments systems behaved similarly.

iv. A simple model was used as an explanatory framework. This employed first order kinetics to describe the rate of reactions. A reasonable agreement was observed between modeled and measured data for all N species, with some exceptions. This supports the hypotheses which underpin the model assumptions. High removal rate constants were derived for NH_x. NH_x removal rate constants were approximately 3x higher with FTWs than without, confirming the outcomes from Chapters 3 and 4 and adding more evidence to support the hypothesis that the mat material provides an effective support matrix for nitrifiers in fixed biofilms.

v. The apparent rate constant for nitrification was much lower than that derived from the mesocosm experiment (Chapter 4). This is probably due to dissolved oxygen limitations on nitrification.

vi. NO_x losses can best be explained by denitrification. The apparent rate constant for denitrification was the same in treatments with FTW and the controls. This suggests that denitrification is not associated with the mat material. Rather, it suggests that denitrifiers inhabit the bed sediments – which are likely to have a lower concentration of dissolved oxygen.

vii. TN-mass removal ranged between 59 and 67 % in the FTW_{368L} and FTW_{560L} chambers, compared with 41-31 %, respectively, in the C_{368L} and C_{560L} chambers. Microbial transformations contribute to between 80 and 85 % of the total-N removal in the FTW treatments, with plant uptake contributing 12-14 %. TN removal was approximately by 8% higher in the smaller chambers than in the larger ones.

viii. Upscaling the application of an effective design from a semi-controlled mesocosm study to a pilot-scale under lesser controlled conditions was a valid approach to optimize system performance in removing ammonia and to verify the removal kinetics.

Chapter Six - Discussion and Synthesis

Floating treatment wetland - a variant of constructed wetland technology – emerged recently and is considered as a tool for water purification with varied applicability. Gradually however, experience with full-scale systems and innovative experimental set-ups led to sometimes changes in design and operation and ever-increasing application of this technology. A list of applications was given in Chapter 1 and encompassed agricultural, stormwater and domestic wastewaters, often containing mixtures of organic and inorganic, substances, e.g. nitrogen, in varying concentrations. Removal of nitrogen in these systems can be accomplished by array of biological, chemical and physical processes, as was also explained in detail in Chapter 1.

Although the number of published literatures on FTW systems for wastewater treatment, many knowledge-gaps still exist. In fact, until recently, field-scale research focused on nitrogen removal efficiencies and performance observations by adopting black-box (input – output) approaches without much speculation on the basic processes behind the observations. Recent works do concentrate more and more on nitrogen dynamics, but this research still tends to be very fragmented and is often carried out on a lab-scale, making it difficult to extrapolate the outcomes to a larger scale. As a result, many quantitative data have been assembled without the necessary theoretical foundation. A well-structured approach is therefore needed to optimise the design and efficiency of these natural wastewater treatment systems.

This research adopted an integrated approach that integrates three scaled up studies within the domain of FTW technology to improve design and operation. The findings from a simple experimental design are used as a platform for designing more complex systems with the aim of optimising treatment performance of ammonia. The role of FTWs in ammonia removal from domestic wastewater was investigated under different design criteria in an attempt to better understand the contributed processes and to identify most effective system design. A system modeling approach was developed to provide an interpretive framework for systems performance. A better design and system performance could come from better understanding of basic processes controlling treatment performance.

In view of this, the following scientific research questions were addressed within each of the results chapters:

1. Do the contribution of FTW processes in removing ammonia is influenced by the impact of different design characteristics (e.g. mat surface area, plant density and water depth)?
2. To what extent dose ammonia removal from domestic wastewater enhances by using a critical FTW design under different operational conditions?
3. How the implementation of the model approach can improve mechanistic understanding of the removal processes and system performance in terms of ammonia removal?
4. To what extent dose the use of ammonia kinetics from simple experimental design to support kinetics relationships obtained from complex systems in the field?

This chapter unifies and synthesis the obtained data and discussion points presented in the results chapters (3, 4, and 5) to address the research questions and contextualise the findings within relevant literature. This research is providing insight into the dynamics of ammonia in FTWs and provide information that helps for advanced wetland design. Thus, this study gives a platform for further research. Recommendations for further research directions are presented later in this chapter.

6.1 Conclusions of present work

This research evaluated FTW behaviour and its ammonia removal performance from domestic wastewater through three study topics. First, Laboratory experiments were conducted under different treatments in order to quantify the potential of microbial transformation in ammonia removal. The effect of different experimental designs including different mat surface area, aeration and ammonia concentration were examined. A model-based analysis was employed to improve mechanistic understanding of ammonia removal. While most published studied considered FTW systems as a single unit, the floating mat influence has received little attention and is less understood. Second, mesocosm study utilised a multiple treatments approach to investigate the effect of different design criteria on the magnitude of different removal mechanisms of ammonia and to determine most effective design for treatment performance. Mesocosm study was conducted outdoors, which simulated a domestic wastewater treatment system. Some mesocosms contained mat material only; some mesocosms contained mats with plants.

All mesocosms were operated under approximately steady state conditions. The role of water depth, floating mat area, and plant density in altering ammonia removal were examined. Again, process-based modeling was used to interpret N dynamics in each experimental treatment. Third, the field (pilot-scale) study focus was to evaluate treatment performance of a critical FTW design, scaled up from the mesocosm study, in treating ammonia from domestic wastewater. Experimental data were used to illustrate the contribution of different processes of ammonia removal in wastewater obtained from the effluent of a small rotating biological contractor sewage treatment plant serving a hotel complex. The treatment chambers were operated under batch-mode (i.e., static) conditions. The kinetics of the ammonia removal with two different operational volumes with and without floating islands were evaluated. Behavior was analyzed using the modeling approach.

6.1.1 *Magnitude of Different Processes in Removing Ammonia Under Different Design criteria.*

6.1.1.1 Effects of Mat Surface Area, Free ammonia concentration and Aeration (Microcosm study)

The results of the laboratory study provide clear evidence that an increased amount of biofilm (introduced as an increased area of the mat) can increase ammonia removal rates. Microbial removal pathways, particularly nitrification, are mediated by fixed biofilms that occur on solid surfaces such as bed sediment, plant roots, and floating mats (Boltz et al., 2017). The results are broadly comparable with the findings obtained by Stewart et al. (2008). These authors looked at ammonia removal in water containing a high concentration of 200 mg N L^{-1} under full cover of a floating matrix. They found a reduction in ammonium concentration of ~ 70% compared to controls over 16 days. In the experiments reported in this thesis, increased NO_2^- concentrations in the treatments, suggests that (i) nitrification was the most plausible cause of ammonia removals, and (ii) microbial capacity for converting NO_2^- to NO_3^- was inhibited. This was probably due to the toxic effect of unionized ammonia (NH_3) on *Nitrobacter*. Philips et al. (2002) showed that NOB organisms could be inhibited at an NH_3 concentration of 0.6 mg N L^{-1} in the batch reactors (i.e., total ammoniacal concentration of 13 mg N L^{-1} at 20 °C and pH 7.8).

The explanation of ammonia loss by accelerated nitrification rates by increasing attachment surface area for the microbial biofilm (via increased submerged mat material)

was also supported by the reduction of dissolved oxygen. Oxygen concentration was negatively correlated with the apparent nitrification rate (i.e., higher DO consumption by the increased microbial activity). The loss of oxygen was proportional to the loss rate of ammonia and the rate of NO_2^-+NO_3^- production.

There was a reasonable agreement between observed and predicted N dynamics in different treatments (calibrated using data from the T_5 treatment and validated on the other treatments with rate constants adjusted in accordance with specific hypotheses, e.g., nitrification rate constant will be directly proportional to mat surface area and therefore with the amount of established biofilm). The relationship between ammonia removal and increase in surface area for microbial growth, including T_5 as best design, was further investigated in a larger mesoscale system to confirm this concept and to determine critical design for treatment performance.

Nitrification rates were higher in treatments subjected to lower ammonia concentrations, This is in agreement with other published studies, e.g., Andersson (2005) who reported lower nitrogen removal in free water surface wetland receiving a concentration of 37 mg N L^{-1} of ammonia-N such as municipal or domestic wastewater. Slower nitrification rates observed at high initial concentrations might be due to the toxic effect of NH_3 on nitrifiers' growth. There is a general agreement that free ammonia has an inhibitory effect on both AOB and NOB (Ciudad et al., 2005; Sun et al., 2015). NOB tend to be more sensitive than AOB (Park et al., 2015). Initial inhibiting concentrations of NH_3 for *Nitrosomonas* and *Nitrobacter* activity are typically between 10-150 and 0.1-1.0 mg L^{-1}, respectively (Anthonisen et al., 1976). Here, NH_x oxidation was slower in the treatment with an initial concentration of 60 mg N L^{-1}, compared with an initial concentration of 15 mg N L^{-1}. Nitrite accumulation was also high when NH_x concentrations were high which *Nitrobacter* growth inhibited. Peng and Zhu (2006) found that NH_3 concentrations higher than 3.5 mg NH_3-N L^{-1} were sufficient to inhibit NO_2^- oxidation. The results did not suggest an important role for aeration in ammonia removal (i.e., removal rates were not significantly different in aerated and non-aerated treatments). This was probably due to the fact that dissolved oxygen concentrations were generally > 1 mg L^{-1}. Nitrification is known to be inhibited only at DO concentrations below 1 mg L^{-1} (Van Hulle et al., 2010). Nitrification was clearly a sink for DO: DO concentrations decreased in unaerated treatments in a classical "oxygen sag" but recovered in treatments where ammonia was exhausted.

6.1.1.2 Effects of Water Depth (Mesocosm study)

In the mesocosm study, nitrification rates were 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 (2009b). Observed overall ammonia removal efficiencies were 57, 93, 88, 93 and 94 % for shallow treatments: C_1, M_1, V_1, M_3 and V_3, and 39, 77, 83, 85 and 91 % for deep treatments C_2, M_2, V_2, M_4 and V_4. 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 (Kadlec and Wallace, 2009b). 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 (Holland et al., 2004; Matamoros and Bayona, 2006). Here, the dissolved oxygen concentrations were higher in the shallower systems than in the deeper ones (by typically 1 mg O_2 L^{-1}) over the course of the study. Differences in DO between two systems may be also be explained due to the competition between O_2 consumption by microbial activity and O_2 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 O_2 L^{-1}) (Park and Noguera, 2004; Van Hulle et al., 2010). 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.

6.1.1.3 Effects of mat surface area (Mesocosm study)

Nitrification rates were higher in treatments where mat area was 100% surface area coverage compared to 50% and no mat treatments. This supports the results from the benchtop experiment and reinforces the idea that biofilm development (onto the floating mat material and roots) enhances nitrification (Headley and Tanner, 2012). Observed NH_x removal efficiencies increased from 93 and 77 % in M_1 and M_2 to 93 and 85 % in M_3 and M_4. Pavlineri et al. (2017a), 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 NH_3 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.

6.1.1.4 Effects of plant density (Mesocosm study)

Overall ammonia removal was 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 NH_4^+. When plant density increased from 2 individual plants to 4, removal efficiencies increased from 88 and 83 % in V_1 and V_2 cells to 94 and 91 % in V_3 and V_4 cells, respectively in the mesocosm study.

As well as increased uptake, plants can influence the removal of ammonia via nitrification by providing additional surfaces for biofilm development (Bissegger et al., 2014; Coban et al., 2015a). Some wetland plants can also enhance dissolved oxygen levels by transferring air through their root systems (Wiessner et al., 2008). 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 (Cardon and Whitbeck, 2007).

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 NH_3 via volatilization is estimated to be negligible of removed NH_x 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 NO_x dynamic was observed in V_3 and to a lesser extent in the V_1 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, V_4 design was selected to be evaluated for ammonia removal from domestic sewage in wastewater treatment system serving a hotel complex receiving visitors equating to 150 pe (resident population equivalent).

6.1.2 *Effectiveness of FTW in Removing Ammonia Under Different Operational Conditions*

In the pilot-scale study, the potential of FTWs for removing ammonia from real domestic wastewater was assessed in order to improve understanding of removal kinetics derived from microcosm and mesocosm studies. The pilot system consisted of static (batch) chambers, which imposed some limitation on this component of the project (discussed later) but the fact that real wastewater was used is considered to be a major advantage

which led to some important facts of ammonia behavior, as a part of N cycle in a pilot system under less controlled conditions. One of the mesocosm designs (V_4) was scaled up in the pilot study (i.e., full mat cover and higher plant density). Two FTW treatments and controls were operated under two volumes (368 and 560 L). Ammonia removal rates in small volume treatment (FTW_{368L}) were higher than that in the large volume (FTW_{560L}) constant depth and with full vegetated mat coverage.

Over four experimental batches, removal efficiency for ammonia in FTW_{368L} was 18% higher than in the FTW_{560L} treatment. Equivalent losses of nitrate were 28% higher in the low volume versus high volume treatment. Biomass growth and N uptake were higher in the FTW_{368L} treatment (4.3 g dry weight m^{-2} day^{-1} and 28.7±8.4 mg N m^{-2} day^{-1}), compared to the FTW_{560L} treatment (2.2 g dry weight m^{-2} day^{-1} and 18.8±8.5 mg N m^{-2} day^{-1}, respectively). Higher uptake, therefore, partly explain the differences between treatments. However, process analysis using the numerical model as a tool indicated that microbial transformations were much more important contributing between 80 and 85 % of the TN removal in the FTW_{368L} and FTW_{560L} treatments. Microbial activity was responsible for 94% of ammonia losses in both FTW_{368L} and FTW_{560L} and between 82-86% for total oxidized N losses in these treatments. These results are higher than other published studies including those of Chang et al. (2013) and Wang and Sample (2014). Both of these studies report improved removal efficiency of N by 15-18 % in the stormwater retention ponds retrofitted with FTW. However, no detailed information about the relative contribution of N removal processes was presented in these papers.

Furthermore, it is difficult to compare removal fractions in a static (batch mode) system with dynamic ponds or wetlands operating in flow-through mode due to difference in the hydraulic characteristics, e,g. hydraulic residence time (HRT) and flow rate. Absolute removal will be closely linked with HRT because biogeochemical reactions (e.g., nitrification and denitrification) can be strongly controlled by the short or long water and solute residence times (Clilverd et al., 2008; Pinay et al., 2009). For example hydraulic regime of static systems often tend to have longer HRT compared continous flow systems (Karpuzcu and Stringfellow, 2012). There is a general agreement that higher removal efficiency is often associated with long HRT of the system. However, long HRT is also associated with lower redox condition, where higher O_2 consumption by microbial activity is expected. Lower redox condition in system with longer HRT could also be reulted from limited diffusion of atmospheric O_2 in water column due to low flow rate

that facilitates reaeration with the air. Therefore, higher nitrification rates can be promoted at high redox conditions where water turbulence of high flow rate facilitates O_2 bioavialability for aerobic reactions (Gu et al., 2007; Zarnetske et al., 2011). From other hand, denitrification environment can be encoraged as DO declines due to the limited aeration and O_2 consumption by aerobic processes at longer residence time (Morrice et al., 2000; Böhlke et al., 2004). This could explain the high rate of nitrification in the continuous-flow mesocosms operated under 7 days, where O_2 suppliment via reaeration with air under flow condition compensated microbial consumption, compared to pilot-static chambers operated under 14 days resented in this research. Further, different water quality applied, tap water with ammonia in which organic N was not introduced in the mesoscale systems, and real wastwater in which contain organic N and other constituens are represented, and thus magnitude of processes presented could explain differences of removal fractions in both systems. The microbial biomass analysis conducted in the pilot study system was consistent with the other data. Accumulated bacterial biomass in the FTW_{368L} and FTW_{560L} treatments were 6.34±0.07 and 6.84±0.24 log10 CFU g^{-1} m^{-2} day^{-1}, respectively. These compare with 3.71±0.14 and 3.54±0.26 log10 CFU mL^{-1} for the $Control_{368L}$ and $Control_{560L}$ chambers, respectively. The findings and interpretations are also supported by the measurement of physicochemical parameters; lower pH might result from the higher nitrification rates, was observed in both treatments containing mats compared with controls. Low pH observed could be explained by the generation of H^+ ions from the enhanced nitrification rates in these treatments. Temperature is known to be a major factor in controlling microbial activity (Kadlec and Wallace, 2009b). The results obtained here suggest that the moderate water temperature observed during the experimental period (average 17 ºC) supported microbial growth on underwater surfaces and was adequate for the processes involved. The contribution of uptake was relatively low compared to microbial pathways (14-19% in the FTW_{368L} and FTW_{560L} treatments, respectively).

As in the laboratory study and the mesocosms, the system dynamics model was a useful framwork to improve understanding of N behavior in the pilot system. Develop design knowledge of the treatment systems through quantifying contributed processes (e.g. using kinetics parameters) can help to optimise system performance. Process-based modelling approach facilitates a comprehensive understanding of these processes through provide insight into the back-box FTWs function and give indulgent information which helps for

the design purpose. Overall agreement between model predictions and observations was reasonable (after calibration and making an adjustment for system dimensions etc.). Importantly, the model suggested that nitrification rate constants in the pilot system were lower than those in the mesocosm (0.13 and 1.55 day^{-1} for full mat cover). Again, this could reflect a number of limitations – most likely the differences in the residence time between the two systems in which aerobiosis reactions declined versus the increase in the anaerobiosis processes in the bed of the pilot system as a consequence of DO limitation (Mulholland et al., 2008; Böhlke et al., 2009). Sensitivity analysis confirmed that nitrification is likely to be the principal control on ammonia removal within the tested systems. While a relatively minor contribution of plant uptake.

6.1.3 *Use of removal kinetic parameters of microcosm to support kinetics relationships measured in the field*

In this research, a scaled approach employing lab, mesocosm and pilot-scale studies was employed in order to improved understanding of ammonia removal kinetics in FTW systems. The objective is to use the measured kinetic parameters from a well-controlled laboratory microcosm study to confirm kinetic parameters derived from field data. Here, the outcomes of our best characterized treatment from the controlled laboratory experiment are compared with the ammonia removal kinetics derived at mesoscale and pilot-scale. Data from T_5 in the microcosm study was compared with the kinetic measurements made in the mesocosm and pilot-scale experiments. The corresponding treatment in the mesocosm experiment was V_4 (highest mat area and plant density), and in the pilot-scale system, FTW_{560L} was used (higher mat area and plant density). Table 6.1 shows kinetic parameters of ammonia removal obtained from T_5, V_4, and FTW_{560L} treatments.

Table 6. 1. Rate constants for overall ammonia removal (k_{tot}), nitrification (k_{nit}), plant uptake (k_{up}), and volatilization (k_{vol}) in T_5, V_4, and FTW_{560L} treatments.

Treatment	k_{tot} (day^{-1})	k_{nit} (day^{-1})	k_{up} (day^{-1})	k_{vol} (day^{-1})
T_5	0.033	0.032	No vegetation	0.001
V_4	1.711	1.561	0.150	0.0002
FTW_{560L}	0.149	0.129	0.02	0.0005

The *k* value for T_5 represents removal nitrification and volatilization only as there was no vegetation. The *k* values for V_4 and FTW_{560L} represent the combined effects of nitrification, plant uptake, and volatilization. The *k* values for T_5 and FTW_{560L} were quite similar but markedly differed from V_4 in the mesocosm study (1.711 day^{-1}; 1.56 day^{-1} for nitrification and 0.15 day^{-1} for plant uptake). Removal efficiency was higher in the mesocosm system compared to the microcosm and pilot-scale systems. This could be attributed, in part, to the differences in the hydraulic design of these systems. The microcosm and pilot-scale experiments were essentially batch reactors (static), while the mesocosms were operated under continuous flow at steady state. Therefore, differences in the HRT in addition to dissolved O_2 bioavailability between examined systems could explain the differences in the removal rates (as discussed in Section 6.2). There was good agreement between observed removal kinetics at pilot-scale system and the microcosm at similar long HRT (14 day). However, higher first-order removal rate constant was observed in the mesocosm system which was operated under 7-day HRT. The higher nitrification rate observed at the mesoscale treatment could be explained by high DO in this system (average of oxygen content was 2 mg L^{-1} over the experimental period). Under open air environment and continual flow condition, reaeration of water column with atmospheric O_2, and therefore increase in DO is expected. Increasing DO enhances oxidative environment, which in turn promote high rates of ammonia oxidation via microbial activity. However, lower *k* in both the microcosms and at pilot-scale could be attributed to lower dissolved oxygen in these systems. DO concentration was less than 2 mg L^{-1} in T_5 and FTW_{560L} over most of the experimental period. In the microcosm, this was due to nitrification activity which consumed oxygen. In the pilot-scale system, DO depletion may have also been due to BOD from organic matter. When DO concentration decrease below 2 mg L^{-1} nitrification can be inhibited (Park and Noguera, 2004). Overall, these results indicate that removal kinetics obtained from laboratory microcosms could be reasonably scaled up to the pilot-scale system, particularly in terms of scaling of the surface area of floating mat.

6.1.4 *Design Implications*

During the last few decades, treatment wetlands were designed, and great attempts to assess their performance were performed (Rousseau et al., 2004; Kadlec and Wallace, 2008). However, there are a number of questions about the applicability of data collected in one wetland for use in designing subsequent wetlands (Kadlec, 2000).

A well-structured research approach or tools for establishing basic design criteria, such as methods for determining scale and sizing of treatment wetlands, are still needed (Stringfellow and Jain, 2010). This research adopted an integrated approach that combines a scaled-up methodology and modelling approach within the domain of FTW technology to improve design and operation. The findings from a simple experimental design are used as a platform for designing larger complex systems with the aim of optimising treatment performance of ammonia. It is clear from the findings that design factors such as mat surface area, plant density, water depth, and operational volume control performance of the FTWs in predictable ways – supporting various a *priori* hypotheses. Finding presented in this research indicates that examined FTW design (shallow water depth, full coverage area of mat material and maximum plant density) can achieve optimal system performance in removing ammonia. Higher redox condition associated with the shallow depth, higher biofilm establishment associated with high surface area per unit volume and dense hydroponic vegetation considered critical design criteria from ammonia removal. Accordingly, a design code of 0.4 m water depth, full coverage of floating mat (2 × 0.70 m), and plant density of 40 specimen can be regarded as best design for a treatment system performance with volume of 560 L (2 × 0.70 × 0.8 m, L × W × H) and total surface area of 1.4 m^2 in treating ammonia from of sewage effluent of 150 pe (resident population equivalent). Despite increased number of investigations on FTWs for water purification and other ecosystem services, however information about the use of kinetics parameters for sizing and estimating the amount of land area that would need to be devoted in order to mitigate domestic wastewater are rare. For example Karpuzcu and Stringfellow (2012) studied the kinetics of nitrate removal in wetlands receiving agricultural drainage from irrigated cropland with the objective of establishing design criteria for incorporation of ecosystem services in agricultural watersheds. In this study, a well-controlled laboratory microcosm study was used to confirm kinetics parameters derived from field data obtained from full-scale free water surface wetland. The wetland microcosm system consisted of a completely mixed flow-through reactor with volume of 227 L (86 × 42 × 61 cm, L × W × H), water depth of 30 cm and a total surface area of 0.75 m^2 receive synthetic influent media of agricultural drainage. Free water surface wetland (80.93 m^2) with an average depth of 0.6 m receive agricultural drainage were included in this study. There was good agreement between observed removal efficiency at full-scale wetland and the microcosm at similar hydraulic residence times (3.1±0.6 day).

The first-order constant *k* was calculated as 12.97 cm day^{-1} of which was in good agreement with the first-order removal rate *k*-value of 12.07 cm day^{-1} calculated from the field data. Accordingly, the authors suggested that supplementing field study with a microcosm study was an effective approach for confirming and complementing kinetics parameters derived from field data. The authors also suggested that the close agreement of nitrate removal kinetics from field and laboratory studied may allowing of land area requirements for treatment wetlands in agricultural watersheds.

Consistently with the finding of Karpuzcu and Stringfellow (2012), there was also a good agreement between removal efficiency at microcosm and pilot-scale system at similar HRTs (14 day) as discussed above. Estimations of the ammonia removal kinetics from laboratory and field studies were in close agreement, indicating that extrapolate findings from small scale system to larger scale system was a useful approach to confirm lab-based removal kinetics in the field scale. Application of such approach may allow the estimation of land area requirements that need to be dedicated to FTW systems in order to mitigate ammonia from domestic wastewater on larger scale in the future studies.

6.1.5 *Application of FTWs and treatment objectives*

Designing and operating constructed wetlands for optimal treatment performance is the rather narrow-minded "engineering approach" where the system boundaries are clearly defined by constructed wetland itself. However, from an economical and ecological point of view, the objective should rather be to have a good ecological quality in receiving water course, and this at a minimum cost. Depending on the use of receiving water course (e.g. recreation, potable water production, fishing etc.), different quality standards apply which in turn can be translated into different effluent standards.

It is well known that statutory powers provide the means of enforcing remedial action to reduce many pollution risks. However, the power provided by current legislation is still limited in different parts of the world including developing countries and even in number of developed countries. For example, the European Community Urban Wastewater Directive (UWWD) was proposed back in 1991 and sets stringent criteria for sewage effluent discharged to surface water, depending on both the sizes of sewage treatment works and the sensitivity of the receiving water (as discussed in section 1.4). As such, this Directive potentially has special importance in the area of wastewater purification, as attempt to deal with nutrient enrichment by sewage effluent (EU91/271/EEC, 2002).

However, it has still not been fully implemented by many EU countries, and the selection of sites for designation as sensitive areas has been left to member states (Vymazal, 2010). In some EU countries, there is still controversy between the Government's assessment and relevant bodies which deal with the conservation of freshwater that are subject to eutrophication. Moreover, it resulted in insufficient development of policy and legislation in the area of nutrient enrichment, although currently being a water-quality issue (Englande et al., 2013). Such problematic situation is clearly demonstrated in large number of developing countries. Therefore, it is not surprising that nutrient removal has not been studied using contemporary treatment technologies, e.g. constructed wetlands, to a great deal in these countries (Zhou et al., 2010).

In general, constructed wetlands can be found in rural or remote areas with small-scale communities where limited sewage treatment capabilities (using primary processes) are presented and where people are thus - legally - obliged to treat their own wastewater. An upper population limit of 2,000 individuals is defined as a small community (WHO, 2012). Pollution sources of such communities may involve domestics sewage, agricultural processing activities or some small scale industerial activities (Kuai et al., 2000). The treated effluent will have to be of a high quality to allow safe discharge to nearby watercourse. The quality of any discharge is measured in terms of the demand that it makes for oxygen from the watercourse and its lack of suspended solids, and the amounts of ammonia and other poisoning substances present, all of which are critical to the well-being of river life (Karri et al., 2018). In such designated areas, advance treatment level of sewage and, in sensitive areas in particular, for nitrogen removal is required. An effective treatment system that meet desired standards, limited energy consumption, minimal-maintenance, the capability to resist flactuation of flow, environmental friendly and attactive appearance are the desirable features of an effective treatment facility desiged for small community structure (Jones and Silva, 2009). Therefore, the application of FTW systems seems to be one of the potential technologies to improve wastewater quality in these areas.

FTWs are typically designed as polishing technique during tertiary treatment of wastewater treatment systems. They are more efficient as a compliment for treatment systems to treat wastewater effluent with low loading rates. Therefore, the prime objective of such treatment method is the complementary removal of ammonia that was not

sufficiently removed in the primary (or secondary) treatment in order to minimise adverse effects when the effluent is subjected to discharge into the adjacent watercourse.

Despite increased applications of FTW as a new approach for water purification, comprehensive performance information relating to its role for tertiary sewage treatment in the EU and UK has not been reported. In most parts of Europe, the use of subsurface vertical flow treatment wetlands for tertiary treatment has been standard practice since the mid – 1990s (Schönerklee et al., 1997). However, in the UK, tertiary sewage treatment by wetlands has almost solely used subsurface horizontal flow treatment wetlands, first introduced in the mid – 1980s by the statutory water companies (Weedon, 2017). A comparison between systems performance, including removal efficiencies and life expectancy, of these systems is difficult because of the differences in operational parameters such as the loading rate, age of the system, hydraulic design (subsurface, overland or vertical flow), type of pre-treatment unit used, conditions of the vegetation, and physical and chemical properties of the substrate (Faulwetter et al., 2009; Kadlec and Wallace, 2009b).

Another way to surpass the strict engineering approach has been briefly touched in Chapter 1, i.e. to incorporate wastewater management in the FTW projects and to make use of the so-called ancillary benefits like landscape enhancement and habitat restoration. Especially in developing countries, this subject received attention, but given the increasing water demand and scarcity, it will become a crucial issue in developed countries as well.

By being both environmentally sustainable and economically viable, FTW technology may provide an appropriate solution for meeting wastewater management objectives and may have a strong potential for application in developing countries since their warm tropical and subtropical climates stimulate biological treatment and productivity. However, these systems have not yet found widespread use, due to lack of awareness and local expertise to develop these technologies on a local scale.

6.2 Limitation of the research approach

6.2.1 *Process-based analysis*

Processes analysis involved in N removal via FTWs are discussed in Chapters 3, 4 and 5, respectively. The predominant N removal mechanisms in FTWs is nitrification of

ammonia, with subsequent denitrification of the nitrate to nitrogenous gas which can escape to the atmosphere. Ammonia removal from domestic wastewater was a prime objective of this thesis and as such was measured through three experimental systems using planted and unplanted settings. As the three scaled-up wetland systems proved to have high peak-shaving efficiency, although low sampling frequencies in the order of several days. Because of the time limitation, no experiments were conducted to measure activity assay for microbial community established onto FTW materials, and this has yet to be investigated. However, the production of NO_x in both microcosm and mesocosm experiments from one hand, while reduction of NO_x concentration in the pilot-scale study from another hand indicates rapid rates of nitrification in the former and denitrification in the latter. In this study, the microbial biomass in the FTWs was measured based on TVC analysis. However, as pointed in Section 5.3.1.6, TVC is a relatively crude measure of the size and composition of the microbial biomass and cannot represent microbial community as there are many micro-organisms found in environmental systems cannot be cultured using traditional plate counting procedures. It was not possible in the current study to quantify microbial uptake of N because it was empirical to sterilise the large quantities of bed substrate and volume of wastewater that were used for the experiments. It is important to conduct such an experiment in the future. This would help to determine the relative amount of N which are removed via incorporation to the microbial biomass compared to other measured parameters in the FTW. Most of nitrogen which could be taken by immobilization can be re-released to the water column. It should also be taken into account that other processes such as adsorption onto the bed and wall surfaces of examined FTWs should also be considered as potentially important mechanisms for ammonia removal.

6.2.2 *Model-based evaluation*

System dynamics modelling approach was adopted to simulate the measured performance of the examined FTWs, already discussed before. The one major advantage of this that it uses routinely measured variables like NH_x, NO_x and Organic-N as inputs. However, when collection data for the purpose of model calibration, one should make sure to measure, if reasonably possible, all required model inputs. One should anyhow be aware that the model incorporates only the major (expected) processes. Model performance was mainly based on the relatively simple nature of process representation such as plant uptake, nitrification, denitrification and volatilization. Lack of representation of some

other important processes (e.g. immobilization, adsorption ANAMMOX) could regard a potential limitation of model performance. Aquatic ecosystem, including FTWs, are so complex that probably dozens of processes have not been covered. Investigation on the biota of FTWs for instance revealed the presence of significant quantities of macro-invertebrates such as oligochaetes, springtails, beetles etc. which are thought to play an important role in the food-web by ingestion larger organic particles, grazing then biofilm etc. (Kato et al., 2009). Further, no explicit representation of the effects of other parameters such as pH, DO, microbial biomass, and temperature may explain, in some extent, the variability of the observed from predicted data. Moreover, lack of represent some observed dynamics (e.g. effect of lag phase of microbial growth on N dynamics in the lab experiments) caused some error in the model performance.

6.2.3 *System Performance assessment*

In this research, three FTW systems with different hydraulic designs (static and continuous flow systems) were examined as discussed in Chapters 3, 4 and 5. Lab and pilot-scale systems were operated under static condition, while mesocosm system was operated under continuous flow regime. Differences in the hydraulic regimes (including HRT, flow rate, volume etc.) renders interpretation of the N cycle in the examined systems quite difficult. Static systems with similar HRT (14 day) revealed similar NH_x dynamics, although NO_x behaviour was differed due to the denitrification observed in the pilot-scale system. Combined effects of low DO level resulted from aerobic processes and possibly high organic matter associated with sewage influent could encourage anaerobic environment for denitrification in the pilot-scale system. From another hand, continuous-flow regime of the mesocosm with shorter HRT (7 day) have a higher removal efficiency compared to the static systems. This is may be due to the enhanced DO as a result of reaeration with ambient air, and thus improve nitrification rates (as discussed in Section 6.3). In addition to hydraulic design and physicochemical properties, differences in the water quality, type of pre-treatment unit used, and conditions of the vegetation makes comparison between treatment performance of examined systems is challenge.

Although the performance of the microcosm and pilot-scale FTW demonstrated similar NH_x behaviour, with comparable measured kinetics parameters, it is still difficult to say whether the results obtained from pilot-scale study are representative of full-scale systems. The performance of constructed wetlands mainly depends on the characteristics

of the applied wastewater, substrate and loading rates (Vymazal, 2013). Many authors have stated that very often full-scale systems are not able to achieve or maintain the performance that was extrapolated from the studies on small-scale researches (Hammer, 1989; Kadlec and Wallace, 2009b; Sanchez-Ramos et al., 2017). For example, small-scale systems have favourable area:volume ratio, making them difficult to reproduce at full-scale. The latter systems are also characterised by greater irregularities in substrate size, shape and placement, resulting in different hydraulic conditions (García et al., 2005). Further, in full-scale systems, the time required for plants to reach their full growth is extended and therefore these systems take longer to mature (García-Lledó et al., 2011).

6.3 Recommendations for Further Research

6.3.1 *Process-based analysis*

To understand more about the behavior of nitrogen in FTWs, further research is needed. This should include a long-term field study to investigate temporal variations in process rates including diurnal and seasonal changes in eco-hydrological factors, e.g., temperature, dissolved oxygen, pH, evapotranspiration, and rainfall which could all interact with treatment performance. Better knowledge of diurnal and seasonal variations would improve our understanding of system behavior overall. A larger term study would also tell us about whether processes change in time (e.g., does treatment efficiency changed over time as biofilms developed or as plants die?). Future research should also attempt to tease out the contributions of other microbially-mediated transformation processes such as N-mineralization, ANAMMOX (anaerobic ammonia oxidation) and nitrate ammonification (conversion of nitrate to molecular ammonia under anaerobic conditions). These processes need to be more adequately understood and quantified in order to optimize FTWs performance.

A knowledge about the composition of the microbial communities associated with FTWs and their responses to seasonal variations in environmental conditions is required in order to advance understanding of FTWs performance. This would include identification of community composition and structural diversity of the microbial biomass and analysing different aspects of microbial activity. There are multitude of different novel methods available for characterising microbial communities in the FTWs. More recent studies have taken advantage of new molecular technologies and genetic techniques in order to

improve understanding of systems performance (Limpiyakorn et al., 2011; Gao et al., 2013; Sinthusith et al., 2015).

6.3.1.1 Molecular techniques for microbial diversity

These are the most employed techniques to assess microbial diversity and relative abundance over the last 10 years. Polymerase chain reaction followed by denaturing gradient gel electrophoresis (PCR-DGGE) seems to be the most popular technique to estimate global diversity of bacteria or to estimate bacterial communities involved in N transformation (Faulwetter et al., 2009). These DNA-based methods typically rely on PCR amplification of genetic markers using different sets of primers for ribosomal operon genes (universal and group specific primers) and functional genes (Truu et al., 2009). Florescence in-situ hybridization (FISH) probes enable both the isolation and enumeration of specific bacteria populations. By choosing adapted FISH probes, it is possible to stain specific populations of bacteria differently, e.g. nitrifying and denitrifying, and to observe the results within a biofilm using fluorescent microscopy (Faulwetter et al., 2009). This method has been successfully used to determine the population structure and dynamics of bacteria communities in different wastewater treatment systems (Schleifer, 2004).

6.3.1.2 Microbial activity assays

Microbial activity can be measured *in situ*, often by estimating a specific gas production, e.g. CO_2, N_2 or CH_4, or *ex situ* where the term potential activity is usually employed (Faulwetter et al., 2009). Potential respiration can be estimated either by soil basal respiration (the sample is placed in closed environment without any additional substrate) or by placing a sample in a respirometer (Nurk et al., 2005). Both techniques can be applied to anaerobic and aerobic activities by measuring anaerobic gaseous by-products (Caselles-Osorio et al., 2007). Measured potential values can be compared to water quality measures of treatment wetland. Enzymatic activity of different enzymes (e.g. dehydrogenase, urease, protease, phosphatase, etc.) is well proved indicator of microbial activity (Truu et al., 2008). An estimation of the production of enzymes used in various biological processes important to water treatment can shed light on those processes, therefore better understanding degradation mechanisms of a variety of pollutants and specific pathways within the N cycle (Kang et al., 1998). Organic molecules with unnatural isotopes ratios of important elements, e.g. ^{15}N, can be introduced to the

treatment wetland and tracked through various metabolic pathways to determine activity of various microbial consortia (Faulwetter et al., 2009). Use of the stable isotope ^{15}N has provided useful insight into N cycling processes in wetlands (Fry, 2006).

6.3.2 *Model-based Evaluation*

Improvement can also be made in how the dynamic complexity of N cycle processes are represented in models. For example, considering the effects of different parameters (e.g. microbial biomass, pH, DO, and temperature) on nitrification rates within modelling scheme is critical. As discussed before, development of microbial biomass is quite connected to the removal processes and activity quantitatively and qualitatively. Therefore, rate constants of these processes are strongly controlled, being other factors are not limiting, by variation in microbial growth over time. Similarly, increase or decrease in pH, DO, temperature levels could significantly interfere with rate constants of removal processes (as discussed in Chapters 3,4 and 5). Therefore, relating the effects of these parameters on the rate constants of the removal processes in the modelling scheme could potentially reduce uncertainty of model performance. Also, reducing the model uncertainty can be achieved by more explicit representation of mineralisation of organic-N as well as denitrification of NO_3^- (supported by measurements of N_2O and N_2 emissions). Other important processes such as immobilisation, adsorption and ANAMMOX should also be incorporated into modelling approach. Further, better differentiation between the uptake of nitrate and ammonium should be attempted. Although there is no well established analytical method for the determination of ammonia and nitrate content in the plant tissues yet, a reliable calculation of NH_x:NO_x ratio within water column could indicate tissue content of these forms indirectly.

An overarching aim of the research presented in this thesis was to develop a mechanistic understanding of ammonia removal in FTWs to improve design and operation. Better understanding of treatment mechanisms can lead to improvement in system design and therefore optimisation of treatment performance in removing ammonia. The application of a combination of studies at different scales combined with the use of modeling approach as an interpretive framework for the experimental data allows FTW performance in treating ammonia to be critically evaluated. Findings presented in this thesis demonstrate that FTWs can enhance ammonia removal. Comparison between removal kinetics from a lab and a field studies revealed close agreement which confirmed

the similarity of ammonia behaviour at different scaled systems. However, speculation of ammonia dynamics in larger systems (e.g. full-scale) is still challenge as discussed in Section 6.6.3. Further studies, including full-scale studies, are needed to confirm ammonia kinetics and dynamics obtained in this research.

6.3.3 *System Performance assessment*

As mentioned in the Section 6.5, the contribution of FTW systems to the removal is best fitted as a polishing technique and they could be functioned as a sort of backup system in the wastewater treatment systems for domestic sewage. It has been suggested that the concept of multistage treatment system (e.g. employing constructed wetlands with conventional treatment systems) could optimise treatment performance (Belmont et al., 2004). This concept is consisted with the statement that the effluent quality appears to improve with the complexity of the facility (Vymazal, 2013). Results from pilot-scale system (Chapter 5) indicates that integration of FTW system to the existing primary treatment stage yielded the highest possible removal efficiencies for ammonia and oxidised-N. Some recommendations are nevertheless given on the design of these combined systems which could help to optimise treatment performance in treating domestic wastewater.

1. Preliminary treatment unit should be included: the objective of treatment is removal of coarse solids, settable solids and part of organic matter. treatment methods in which physical forces for are predominant (e.g. screening, mixing, sedimentation, filtration).
2. Aerobic biofilm reactor unit (e.g. rotating biological contractor) should be included: BOD can be stabilised aerobically by biomass grows adhered to a support medium, which is usually composed by a series of discs.
3. On or more sealed FTW systems laid out in parallel, planted with mixed culture of plants (e.g. *phragmites* and *Juncus*) with gravel or sediment bed.

In such multistage system design, it is possible to regulate the loading pattern in the different units of the system in accordance with sewage production and composition. Application of these suggestions would be as a forward step to fit within the tasks resulting from the implementation of the Water Framework Directive which, by imposing a good ecological quality for every water body.

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Appendices

Chapter Three: Microcosm Experiments

Effect of mat area (Experiment 1)

Appendix 3.1. Mean± Standard deviation of ammonia concentration in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average NH_x concentration (mg N L^{-1})					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	72.99	74.24	73.23	70.21	72.08	71.25
1	72.99	73.66	70.42	72.05	69.58	69.76
2	73.21	72.69	73.41	70.86	72.21	71.37
3	67.30	67.76	68.09	66.26	62.44	62.73
4	72.93	71.37	69.14	72.96	69.36	66.48
6	68.67	69.58	70.19	68.66	61.47	62.75
9	72.02	74.09	72.83	68.78	56.31	59.16
12	70.63	69.80	66.73	60.11	52.86	54.36
14	69.51	63.46	59.20	48.64	43.62	44.62
Time (day)	Standard deviation					
0	1.63	3.66	3.53	1.37	1.28	3.54
1	1.63	2.83	2.67	1.62	1.02	0.80
2	2.33	1.68	3.17	2.46	2.42	2.09
3	0.59	1.79	0.46	1.97	0.56	1.77
4	2.15	0.67	1.09	4.33	4.22	1.44
6	0.50	1.61	1.82	1.11	3.62	15.15
9	4.86	1.67	3.23	4.07	2.03	2.31
12	3.39	0.72	1.93	6.76	3.43	1.98
14	1.99	1.03	2.82	1.01	2.21	1.18

Appendix 3.2. Analysis of variance (ANOVA) for the change in ammonia concentrations in the lab-scale treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1546.2	8	193.27	12.520	.05
Treatments	715.9	5	143.19	9.276	.05
Error	617.5	40	15.44		

Appendix 3.3. Post hoc multiple comparison (Turkey's HSD test) of ammonia concentr ation (mg N L^{-1}) among treatments subjected to different amount of mat areas (4, 8, 12, 16, and 20 cm^2, for T_1, T_2, T_3, T_4, T_5, respectively). No mat material was introduced to c ontrol.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
T1-Control	-0.3981481	-5.940105	5.1438091	0.9999316
T2-Control	-1.8891852	-7.431142	3.6527721	0.9084692
T3-Control	-4.6336852	-10.175642	0.9082721	0.1478850
T4-Control	-8.9214815	-14.463439	-3.3795242	0.0002892
T5-Control	-8.6406481	-14.182605	-3.0986909	0.0004622
T2-T1	-1.4910370	-7.032994	4.0509203	0.9649367
T3-T1	-4.2355370	-9.777494	1.3064203	0.2232424
T4-T1	-8.5233333	-14.065291	2.9813760	-0.0005614
T5-T1	-8.2425000	-13.784457	-2.7005427	0.0008911
T3-T2	-2.7445000	-8.286457	2.7974573	0.6774509
T4-T2	-7.0322963	-12.574254	-1.4903390	0.0060549
T5-T2	-6.7514630	-12.293420	-1.2095057	0.0092397
T4-T3	-4.2877963	-9.829754	1.2541610	0.2120185
T5-T3	-4.0069630	-9.548920	1.5349943	0.2771734
T5-T4	0.2808333	-5.261124	5.8227906	0.9999879

Appendix 3.4. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), removal rate (RR, mg N day^{-1}), and removal efficiency (RE, %) of ammonia-N in treatments with different mat area (4, 8, 12, 16 and 20 cm^{-2} for control. T_1, T_2, T_2, T_3, T_4 and T_5, respectively.

Parameters	**Treatments**					
	Control	**T_1**	**T_2**	**T_3**	**T_4**	**T_5**
k	0.003	0.011	0.015	0.026	0.036	0.033
$T_{1/2}$	198.7	61.9	45.6	26.4	19.3	20.7
RR	0.25	0.77	1.00	1.54	2.03	1.90
RE	4.77	14.52	19.17	30.73	39.49	37.38

Appendix 3.5. Volatilization rate (day^{-1}) in the treatments during experimental time.

Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	0.001285143	0.00124367	0.00124367	0.00124367	0.00124367	0.001203514
1	0.00124367	0.00124367	0.00124367	0.001164636	0.001164636	0.001164636
2	0.00124367	0.00124367	0.001126996	0.001126996	0.001203514	0.001090555
3	0.001164636	0.001126996	0.001126996	0.001126996	0.001203514	0.001203514
4	0.001126996	0.001126996	0.001126996	0.001090555	0.00124367	0.001164636
6	0.00124367	0.00124367	0.001126996	0.001090555	0.001090555	0.001126996
9	0.001285143	0.001126996	0.001203514	0.001203514	0.001090555	0.001203514
12	0.00124367	0.001164636	0.001090555	0.00124367	0.001164636	0.001203514
14	0.00124367	0.001203514	0.001203514	0.001164636	0.001203514	0.001203514

Appendix 3.6. Mean± Standard deviation of nitrite concentration in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average NO_2^- concentration (mg N L^{-1})					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	0.004	0.001	0.00	0.00	0.00	0.00
1	0.001	0.25	0.64	0.98	2.01	2.47
2	0.01	0.30	0.60	1.10	1.91	2.46
3	0.002	0.35	0.72	1.24	4.90	5.81
4	0.003	0.54	1.29	1.86	5.39	9.25
6	0.003	0.39	0.79	1.68	10.36	12.00
9	0.004	1.74	3.86	9.91	20.45	18.55
12	0.005	3.75	6.84	12.28	24.48	30.04
14	0.01	7.28	11.31	19.37	25.51	27.09
Time (day)	Standard deviation					
0	0.002	0.005	0.005	0.005	0.005	0.005
1	0.001	0.04	0.11	0.05	0.16	0.19
2	0.00	0.09	0.09	0.06	0.34	0.12
3	0.004	0.06	0.04	0.22	0.25	0.30
4	0.001	0.05	0.21	0.23	2.37	1.54
6	0.001	0.02	0.05	0.32	0.38	1.36
9	0.002	0.12	0.50	1.20	1.16	1.64
12	0.005	0.43	0.86	3.68	4.53	3.30
14	0.003	0.80	1.11	15.27	0.18	2.12

Appendix 3.7. Analysis of variance (ANOVA) for the change in nitrite concentrations in the lab-scale treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1487.3	8	185.92	8.732	.05
Treatments	1073.9	5	214.79	10.088	.05
Error	851.7	40	21.29		

Appendix 3.8. Post hoc multiple comparison (Turkey's HSD test) of nitrite concentratio n (mg N L^{-1}) among treatments subjected to different amount of mat areas (4, 8, 12, 16, and 20 cm^2, for T_1, T_2, T_3, T_4, T_5, respectively). No mat material was introduced to cont rol.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
T1-Control	1.618326	4.89035526	-8.127007	0.9750171
T2-Control	2.892233	-3.61644786	9.400915	0.7670234
T3-Control	5.378270	-1.13041082	11.886952	0.1568082
T4-Control	10.552011	4.04332992	17.060692	0.0002601
T5-Control	11.959974	5.45129288	18.468655	0.0000337
T2-T1	1.273907	-5.23477378	7.782589	0.9914408
T3-T1	3.759944	-2.74873674	10.268626	0.5217876
T4-T1	8.933685	2.42500400	15.442366	0.0024803
T5-T1	10.341648	3.83296696	16.850329	0.0003511
T3-T2	2.486037	-4.02264415	8.994718	0.8603657
T4-T2	7.659778	1.15109659	14.168459	0.0129504
T5-T2	9.067741	2.55905955	15.576422	0.0020691
T4-T3	5.173741	-1.33494045	11.682422	0.1882770
T5-T3	6.581704	0.07302251	13.090385	0.0461242
T5-T4	1.407963	-5.10071823	7.916644	0.9865131

Appendix 3.9. Mean± Standard deviation of total oxidized N concentration in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average NO_x concentration (mg N L^{-1})					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	0.09	0.12	0.27	0.35	0.40	0.62
1	0.07	0.25	0.50	0.80	1.73	2.25
2	0.07	0.56	1.10	1.63	4.83	6.31
3	0.06	0.31	0.71	1.10	3.61	4.11
4	0.03	0.28	0.76	1.31	4.22	5.12
6	0.07	0.53	1.19	3.03	7.51	7.94
9	0.07	1.08	2.74	6.91	12.94	13.18
12	0.05	3.35	5.46	12.07	17.30	18.72
14	0.07	5.22	8.17	16.74	19.25	21.20
Time (day)	Standard deviation					
0	0.05	0.01	0.05	0.04	0.02	0.01
1	0.00	0.02	0.11	0.03	0.31	0.32
2	0.00	0.07	0.06	0.04	0.71	0.51
3	0.00	0.05	0.07	0.09	0.24	0.25
4	0.01	0.04	0.09	0.02	0.27	0.58
6	0.00	0.06	0.07	0.68	0.18	0.86
9	0.01	0.09	0.12	0.72	0.89	0.87
12	0.00	0.40	0.33	0.46	2.99	0.69
14	0.01	0.46	1.01	2.10	0.77	1.48

Appendix 3.10. Mean± Standard deviation of dissolved oxygen concentration (mg L^{-1}) in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average DO concentration (mg L^{-1})					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	9.23	9.22	9.21	9.18	8.75	8.69
1	9.00	8.90	8.78	8.64	5.95	5.35
2	8.87	8.75	8.60	8.43	5.16	4.75
3	8.90	8.54	8.44	8.11	3.04	2.70
4	8.87	8.63	8.25	7.62	2.91	2.46
6	8.90	8.54	7.94	6.15	2.07	2.22
9	8.88	7.50	5.82	1.28	1.46	1.85
12	9.01	5.90	4.30	0.88	1.36	1.70
14	8.90	2.73	2.13	0.81	1.22	1.53
Time (day)	Standard deviation					
0	0.02	0.00	0.02	0.00	0.09	0.17
1	0.02	0.00	0.02	0.02	0.35	0.79
2	0.04	0.00	0.02	0.03	0.37	0.56
3	0.06	0.24	0.04	0.08	0.27	0.63
4	0.04	0.02	0.11	0.27	0.58	0.51
6	0.07	0.04	0.08	0.82	0.83	0.46
9	0.05	0.03	0.25	0.33	0.43	0.48
12	0.03	0.39	0.36	0.11	0.36	0.34
14	0.03	0.37	0.52	0.11	0.26	0.22

Appendix 3.11. Analysis of variance (ANOVA) for the change in DO concentrations (mg L^{-1}) in the lab-scale treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	189.90	8	23.74	9.962	.05
Treatments	224.73	5	44.95	18.864	.05
Error	95.31	40	2.38		

Appendix 3.12. Post hoc multiple comparison (Turkey's HSD test) of DO concentration (mg L^{-1}) among treatments subjected to different amount of mat areas (4, 8, 12, 16, and 20 cm^2, for T_1, T_2, T_3, T_4, T_5, respectively). No mat material was introduced to control.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
T1-Control	-1.3174074	3.494718	-0.8599030	0.4708080
T2-Control	-1.8985185	-4.075829	0.27879195	0.1185712
T3-Control	-3.2729629	-5.450273	-1.0956525	0.0007711
T4-Control	-5.4044444	-7.581755	-3.2271339	0.0000001
T5-Control	-5.4770370	-7.654348	-3.2997265	0.0000001
T2-T1	-0.5811111	-2.758422	1.59619935	0.9661129
T3-T1	-1.9555555	-4.132866	0.22175491	0.1002826
T4-T1	-4.0870370	-6.264348	-1.9097265	0.0000231
T5-T1	-4.1596296	-6.336940	-1.9823191	0.0000168
T3-T2	-1.3744444	-3.551755	0.80286602	0.4235925
T4-T2	-3.5059259	-5.683236	-1.3286154	0.0002881
T5-T2	-3.5785185	-5.755829	-1.4012080	0.0002112
T4-T3	-2.1314814	-4.308792	0.04582898	0.0580523
T5-T3	-2.2040740	-4.381385	-0.0267636	0.0457679
T5-T4	-0.0725925	-2.249903	2.10471787	0.9999985

Appendix 3.13. Mean± Standard deviation of electrical conductivity (mS cm^{-1}) in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average EC (mS cm-1)					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	0.95	0.95	0.95	0.96	0.95	0.96
1	0.95	0.95	0.95	0.95	0.95	0.96
2	0.94	0.94	0.95	0.96	0.95	0.95
3	0.94	0.95	0.96	0.96	0.98	0.99
4	0.95	0.95	0.96	0.96	1.01	1.02
6	0.95	0.96	0.97	0.99	1.05	1.07
9	0.96	0.97	0.98	1.01	1.08	1.10
12	0.96	0.99	1.02	1.09	1.14	1.15
14	0.96	1.02	1.06	1.12	1.17	1.16
Time (day)	Standard deviation					
0	0.005	0.00	0.00	0.005	0.00	0.00
1	0.005	0.00	0.00	0.005	0.00	0.005
2	0.00	0.005	0.00	0.005	0.00	0.00
3	0.00	0.00	0.00	0.00	0.005	0.00
4	0.005	0.00	0.00	0.005	0.005	0.01
6	0.005	0.00	0.00	0.01	0.01	0.01
9	0.00	0.00	0.005	0.02	0.005	0.02
12	0.01	0.005	0.005	0.01	0.005	0.02
14	0.00	0.005	0.01	0.01	0.005	0.01

Appendix 3.14. Analysis of variance (ANOVA) for the change in EC (mS cm^{-1}) in the lab-scale treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.11426	8	0.014283	13.31	.05
Treatments	0.05738	5	0.011477	10.70	.05
Error	0.04292	40	0.001073		

Appendix 3.15. Post hoc multiple comparison (Turkey's HSD test) of EC concentration (mS cm^{-1}) among treatments subjected to different amount of mat areas (4, 8, 12, 16, and 20 cm^2, for T_1, T_2, T_3, T_4, T_5, respectively). No mat material was introduced to control.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
T1-Control	0.014444444	-0.0317600	0.06064896	0.9348250
T2-Control	0.028518518	-0.0176859	0.07472303	0.4486611
T3-Control	0.048888889	0.002684377	0.09509340	0.0326658
T4-Control	0.080370370	0.034165859	0.12657488	0.0000857
T5-Control	0.088518519	0.042314007	0.13472303	0.0000160
T2-T1	0.014074074	-0.03213043	0.06027859	0.9412995
T3-T1	0.034444445	-0.01176006	0.08064896	0.2468360
T4-T1	0.065925926	0.019721414	0.11213044	0.0015348
T5-T1	0.074074074	0.027869563	0.12027859	0.0003076
T3-T2	0.020370371	-0.02583414	0.06657488	0.7727989
T4-T2	0.051851852	0.005647340	0.09805636	0.0199903
T5-T2	0.060000000	0.013795489	0.10620451	0.0047075
T4-T3	0.031481481	-0.01472303	0.07768599	0.3394661
T5-T3	0.039629630	-0.00657488	0.08583414	0.1296119
T5-T4	0.008148148	-0.03805636	0.05435266	0.9947153

Appendix 3.16. Mean± Standard deviation of pH in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average pH					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	7.43	7.40	7.40	7.40	7.40	7.37
1	7.40	7.40	7.40	7.33	7.33	7.33
2	7.40	7.40	7.30	7.30	7.37	7.27
3	7.33	7.30	7.30	7.30	7.37	7.37
4	7.30	7.30	7.30	7.27	7.40	7.33
6	7.40	7.40	7.30	7.27	7.27	7.30
9	7.43	7.30	7.37	7.37	7.27	7.37
12	7.40	7.33	7.27	7.40	7.33	7.37
14	7.40	7.37	7.37	7.33	7.37	7.37
Time (day)	Standard deviation					
0	0.05	0.00	0.00	0.00	0.00	0.05
1	0.00	0.00	0.00	0.05	0.05	0.05
2	0.00	0.00	0.00	0.00	0.05	0.05
3	0.05	0.00	0.00	0.00	0.05	0.05
4	0.00	0.00	0.00	0.05	0.00	0.05
6	0.00	0.00	0.00	0.19	0.05	0.00
9	0.05	0.00	0.05	0.05	0.05	0.05
12	0.00	0.05	0.05	0.00	0.05	0.05
14	0.00	0.05	0.05	0.05	0.05	0.05

Appendix 3.17. Mean± Standard deviation of temperature in the lab-scale treatments subjected to different area of mat materials (4, 8, 12, 16, and 20 cm^2 for T_1, T_2, T_3, T_4, and T_5, respectively). No mat material was introduced to control.

	Average temperature (°C)					
Time (day)	Control	T_1	T_2	T_3	T_4	T_5
0	21.03	20.93	20.93	20.83	20.87	20.80
1	21.10	21.10	21.03	20.97	20.90	20.93
2	21.07	20.93	20.73	20.87	20.87	20.97
3	20.90	20.90	20.93	20.87	20.87	20.87
4	20.83	20.87	20.90	20.87	20.90	20.87
6	20.80	20.87	20.93	20.97	20.87	21.00
9	20.87	20.73	21.00	20.87	20.83	20.97
12	20.73	20.80	20.90	20.73	20.60	20.67
14	20.77	20.83	20.77	20.83	20.83	20.67
Time (day)	Standard deviation					
0	0.05	0.05	0.05	0.05	0.05	0.00
1	0.00	0.00	0.05	0.05	0.00	0.05
2	0.05	0.05	0.05	0.05	0.05	0.05
3	0.08	0.00	0.05	0.09	0.05	0.05
4	0.05	0.05	0.00	0.05	0.00	0.05
6	0.08	0.05	0.05	0.09	0.05	0.14
9	0.05	0.09	0.08	0.05	0.05	0.09
12	0.05	0.00	0.00	0.05	0.00	0.05
14	0.05	0.09	0.05	0.09	0.05	0.48

Effect of ammonia concentrations and aeration (Experiment 2)

Appendix 3.18. Mean± Standard deviation of ammonia concentration in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16 mg N L^{-1}) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	Average NH_x concentration (mg N L^{-1})					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	0.35	0.34	0.43	0.42	0.67	0.66
1	0.38	0.38	0.52	0.52	0.80	0.81
3	0.35	0.34	0.47	0.45	0.71	0.71
5	0.36	0.36	0.49	0.48	0.73	0.72
7	0.38	0.37	0.51	0.49	0.75	0.73
9	0.41	0.40	0.54	0.51	0.77	0.77
11	0.45	0.43	0.58	0.54	0.80	0.80
13	0.46	0.43	0.60	0.55	0.82	0.82
15	0.47	0.43	0.62	0.58	0.85	0.85
Time (day)	Standard deviation					
0	0.005	0.005	0.008	0.017	0.016	0.017
1	0.005	0.005	0.005	0.005	0.000	0.000
3	0.005	0.005	0.005	0.005	0.008	0.005
5	0.005	0.005	0.005	0.005	0.005	0.005
7	0.005	0.005	0.000	0.005	0.000	0.008
9	0.005	0.009	0.000	0.005	0.005	0.012
11	0.005	0.005	0.005	0.005	0.009	0.012
13	0.005	0.000	0.008	0.005	0.005	0.012
15	0.009	0.000	0.005	0.005	0.008	0.014

Appendix 3.19. Analysis of variance (ANOVA) for the change in ammonia concentrations in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16 mg N L^{-1}) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration). Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	4485	8	561	46.77	.05
Treatments	16047	5	3209	267.77	.05
Error	479	40	12		

Appendix 3.20. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), removal rate (RR, mg N day^{-1}), and removal efficiency (RE, %) of ammonia-N in treatments with different ammonia concentrations (15, 30, 60, 16 mg N L^{-1}) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

Parameters	**Treatments**					
	T_1	**T_2**	**T_3**	**T_4**	**T_5**	**T_6**
k_{nit}	0.537	0.57	0.168	0.17	0.052	0.067
$T_{1/2}$	1.3	1.2	4.1	4.1	13.3	10.3
RR	1.05	1.94	2.12	1.04	1.87	2.46
RE	99.80	99.88	93.01	91.59	52.44	60.23

Appendix 3.21. Volatilization rate (day^{-1}) in the treatments subjected to different ammonia concentrations (15, 30, 60, 16 mg N L^{-1}) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	0.001164636	0.001203514	0.001164636	0.001203514	0.001126996	0.001126996
1	0.001164636	0.00124367	0.001203514	0.001164636	0.001126996	0.001164636
3	0.001126996	0.001203514	0.001203514	0.001203514	0.001126996	0.001126996
5	0.00124367	0.001285143	0.001164636	0.001203514	0.001203514	0.001164636
7	0.001164636	0.00124367	0.001126996	0.001203514	0.001203514	0.001164636
9	0.001203514	0.001164636	0.001164636	0.001126996	0.001126996	0.001164636
11	0.001164636	0.001203514	0.001126996	0.001203514	0.001126996	0.001126996
13	0.001164636	0.001164636	0.001126996	0.001126996	0.001126996	0.001126996
15	0.001126996	0.001126996	0.001126996	0.00124367	0.001164636	0.001126996

Appendix 3.22. Mean± Standard deviation of nitrite concentration in the lab-scale treat ments subjected to different nitrite concentrations (15, 30, 60, 16) with and without aera tion. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	Average NO_2^- concentration (mg N L^{-1})					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	3.24	2.73	4.82	4.16	5.41	5.52
1	7.50	8.66	8.60	8.38	8.08	8.77
3	10.34	10.17	10.48	11.34	11.11	11.52
5	12.63	12.54	13.52	11.41	13.38	13.90
7	14.20	15.97	15.63	14.93	15.68	17.02
9	19.36	19.54	21.07	20.71	21.53	23.23
11	20.41	20.38	23.07	23.19	24.22	27.47
13	23.49	24.91	29.60	29.30	30.10	34.22
15	21.72	20.27	32.65	32.75	33.83	35.48
Time (day)	Standard deviation					
0	0.12	0.26	0.39	0.19	0.25	0.23
1	0.37	0.57	0.35	0.05	0.33	0.36
3	0.51	0.14	1.33	1.24	1.05	0.88
5	0.56	0.70	0.25	1.23	0.92	0.96
7	0.52	1.33	0.29	0.52	0.67	0.72
9	0.32	0.47	0.57	0.72	1.41	1.16
11	0.25	0.45	0.42	1.36	0.83	1.42
13	0.99	5.26	0.21	0.88	0.85	1.61
15	0.25	0.52	1.00	1.78	1.50	2.34

Appendix 3.23. Analysis of variance (ANOVA) for the change in nitrite concentrations in the lab-scale treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	3812	8	476.5	96.790	.05
Treatments	162	5	32.4	6.576	.05
Error	197	40	4.9		

Appendix 3.24. Mean± Standard deviation of total oxidized N concentration in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	Average NO_x concentration (mg N L^{-1})					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	4.92	6.26	6.11	5.52	5.34	6.42
1	5.94	6.61	6.41	6.99	6.55	6.76
3	7.29	7.95	8.60	7.94	9.02	8.13
5	10.26	11.77	11.35	11.66	10.87	11.22
7	12.56	13.62	14.17	15.52	14.62	17.09
9	16.87	18.40	18.10	16.88	18.11	19.91
11	17.42	20.19	21.47	20.50	21.09	25.46
13	18.29	23.11	27.20	21.80	26.67	29.35
15	18.70	32.93	31.42	19.02	31.26	35.92
Time (day)	Standard deviation					
0	0.10	0.54	0.60	0.22	0.40	0.14
1	0.20	0.45	0.73	0.18	0.15	0.39
3	0.73	0.23	0.77	0.11	0.62	0.20
5	0.98	0.18	0.89	0.84	0.92	0.11
7	0.63	0.42	0.83	0.49	1.08	0.66
9	0.97	1.14	2.14	1.31	0.91	0.86
11	0.82	0.84	1.05	1.37	1.25	2.27
13	0.18	3.82	1.94	5.62	1.24	3.35
15	1.31	1.90	1.46	0.69	2.06	4.03

Appendix 3.25. Mean± Standard deviation of DO concentration in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	DO (mg L^{-1})					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	7.88	8.48	7.66	8.35	7.88	8.35
1	6.38	8.58	6.59	8.50	6.76	8.34
3	5.04	8.59	4.84	8.16	5.20	8.16
5	4.02	8.72	2.87	8.77	3.68	8.64
7	2.90	8.62	2.40	8.79	2.92	8.72
9	2.29	8.49	1.98	8.42	1.96	8.73
11	2.33	8.43	1.78	8.31	1.73	8.41
13	4.94	8.59	0.65	8.35	0.62	8.39
15	7.78	8.57	1.97	8.58	1.96	8.70
Time (day)	Standard deviation					
0	0.04	0.21	0.02	0.18	0.03	0.16
1	0.10	0.32	0.18	0.12	0.27	0.16
3	0.55	0.13	0.21	0.05	0.17	0.34
5	0.36	0.04	0.32	0.05	0.18	0.04
7	0.55	0.19	0.33	0.08	0.49	0.08
9	0.21	0.19	0.26	0.37	0.20	0.11
11	0.30	0.43	0.31	0.53	0.10	0.08
13	0.72	0.10	0.03	0.26	0.05	0.29
15	0.08	0.12	0.30	0.20	0.24	0.02

Appendix 3.26. Mean± Standard deviation of EC in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	EC (mS cm^{-1})					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	0.35	0.34	0.43	0.42	0.67	0.66
1	0.38	0.38	0.52	0.52	0.80	0.81
3	0.35	0.34	0.47	0.45	0.71	0.71
5	0.36	0.36	0.49	0.48	0.73	0.72
7	0.38	0.37	0.51	0.49	0.75	0.73
9	0.41	0.40	0.54	0.51	0.77	0.77
11	0.45	0.43	0.58	0.54	0.80	0.80
13	0.46	0.43	0.60	0.55	0.82	0.82
15	0.47	0.43	0.62	0.58	0.85	0.85
Time (day)	Standard deviation					
0	0.005	0.005	0.008	0.017	0.016	0.017
1	0.005	0.005	0.005	0.005	0.000	0.000
3	0.005	0.005	0.005	0.005	0.008	0.005
5	0.005	0.005	0.005	0.005	0.005	0.005
7	0.005	0.005	0.000	0.005	0.000	0.008
9	0.005	0.009	0.000	0.005	0.005	0.012
11	0.005	0.005	0.005	0.005	0.009	0.012
13	0.005	0.000	0.008	0.005	0.005	0.012
15	0.009	0.000	0.005	0.005	0.008	0.014

Appendix 3.27. Mean± Standard deviation of pH in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	pH					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	7.33	7.37	7.33	7.37	7.30	7.30
1	7.33	7.40	7.37	7.33	7.30	7.33
3	7.30	7.37	7.37	7.37	7.30	7.30
5	7.40	7.43	7.33	7.37	7.37	7.33
7	7.33	7.40	7.30	7.37	7.37	7.33
9	7.37	7.33	7.33	7.30	7.30	7.33
11	7.33	7.37	7.30	7.37	7.30	7.30
13	7.33	7.33	7.30	7.30	7.30	7.30
15	7.30	7.30	7.30	7.40	7.33	7.30
Time (day)	Standard deviation					
0	0.047	0.047	0.047	0.047	0.000	0.000
1	0.047	0.000	0.047	0.047	0.000	0.047
3	0.000	0.047	0.047	0.047	0.000	0.000
5	0.000	0.047	0.047	0.047	0.047	0.047
7	0.047	0.000	0.000	0.047	0.047	0.047
9	0.047	0.047	0.047	0.000	0.000	0.047
11	0.047	0.047	0.000	0.047	0.000	0.000
13	0.047	0.047	0.000	0.000	0.000	0.000
15	0.000	0.000	0.000	0.000	0.047	0.000

Appendix 3.28. Mean± Standard deviation of temperature in the lab-scale treatments subjected to different ammonia concentrations (15, 30, 60, 16) with and without aeration. T_1 (15 mg N L^{-1}); (15 mg N L^{-1}, with aeration), (30 mg N L^{-1}); (30 mg N L^{-1}, with aeration), (60 mg N L^{-1}); (60 mg N L^{-1}, with aeration).

	Temperature (°C)					
Time (day)	T_1	T_2	T_3	T_4	T_5	T_6
0	20.07	20.03	20.03	20.01	20.07	20.03
1	20.33	20.13	20.40	20.03	20.33	20.07
3	20.17	20.10	20.17	20.13	20.23	20.13
5	20.13	20.00	20.07	19.90	20.10	19.73
7	20.10	19.93	20.00	19.90	20.03	20.10
9	20.13	20.07	20.10	19.93	20.07	20.03
11	20.20	20.07	20.20	20.23	20.13	20.20
13	20.27	19.97	20.13	19.93	20.17	19.97
15	20.20	20.03	20.10	20.00	20.10	20.00
Time (day)	Standard deviation					
0	0.12	0.05	0.05	0.01	0.17	0.19
1	0.05	0.05	0.00	0.05	0.09	0.05
3	0.05	0.08	0.09	0.12	0.19	0.17
5	0.50	0.00	0.05	0.08	0.14	0.05
7	0.00	0.05	0.00	0.14	0.17	0.00
9	0.05	0.09	0.00	0.12	0.09	0.05
11	0.08	0.19	0.08	0.05	0.12	0.00
13	0.05	0.05	0.05	0.05	0.09	0.05
15	0.00	0.09	0.08	0.08	0.00	0.00

Chapter Four: Mesocosm Experiment
Water balance

Appendix 4.1. Estimated evapotranspiration rates (L m^{-2} day^{-1}) of the treatments over experimental time.

Time (day)	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
1	5.71	5.71	1.43	1.43	0.29	0.29	2.65	2.65	1.83	1.83
2	4.81	4.81	1.20	1.20	0.24	0.24	2.23	2.23	1.55	1.55
3	1.23	1.23	0.31	0.31	0.06	0.06	0.57	0.57	0.39	0.39
4	5.01	5.01	1.25	1.25	0.25	0.25	2.33	2.33	1.61	1.61
5	1.50	1.50	0.38	0.38	0.08	0.08	0.70	0.70	0.48	0.48
6	2.66	2.66	0.66	0.66	0.13	0.13	1.23	1.23	0.85	0.85
7	4.29	4.29	1.07	1.07	0.21	0.21	1.99	1.99	1.38	1.38
8	4.98	4.98	1.24	1.24	0.25	0.25	2.31	2.31	1.60	1.60
9	3.53	3.53	0.88	0.88	0.18	0.18	1.64	1.64	1.14	1.14
10	4.22	4.22	1.06	1.06	0.21	0.21	1.96	1.96	1.36	1.36
11	2.27	2.27	0.57	0.57	0.11	0.11	1.05	1.05	0.73	0.73
12	0.99	0.99	0.25	0.25	0.05	0.05	0.46	0.46	0.32	0.32
13	2.27	2.27	0.57	0.57	0.11	0.11	1.05	1.05	0.73	0.73
14	1.78	1.78	0.45	0.45	0.09	0.09	0.83	0.83	0.57	0.57
15	2.93	2.93	0.73	0.73	0.15	0.15	1.36	1.36	0.94	0.94
16	2.24	2.24	0.56	0.56	0.11	0.11	1.04	1.04	0.72	0.72
17	3.63	3.63	0.91	0.91	0.18	0.18	1.69	1.69	1.17	1.17
18	3.00	3.00	0.75	0.75	0.15	0.15	1.39	1.39	0.96	0.96
19	0.64	0.64	0.16	0.16	0.03	0.03	0.30	0.30	0.21	0.21
20	2.70	2.70	0.68	0.68	0.14	0.14	1.25	1.25	0.87	0.87
21	2.47	2.47	0.62	0.62	0.12	0.12	1.15	1.15	0.79	0.79
22	3.00	3.00	0.75	0.75	0.15	0.15	1.39	1.39	0.97	0.97
23	3.14	3.14	0.79	0.79	0.16	0.16	1.46	1.46	1.01	1.01
24	2.10	2.10	0.52	0.52	0.10	0.10	0.97	0.97	0.67	0.67
25	1.65	1.65	0.41	0.41	0.08	0.08	0.77	0.77	0.53	0.53
26	0.92	0.92	0.23	0.23	0.05	0.05	0.43	0.43	0.30	0.30
27	1.98	1.98	0.50	0.50	0.10	0.10	0.92	0.92	0.64	0.64
28	0.46	0.46	0.12	0.12	0.02	0.02	0.21	0.21	0.15	0.15
29	1.35	1.35	0.34	0.34	0.07	0.07	0.63	0.63	0.43	0.43
30	1.48	1.48	0.37	0.37	0.07	0.07	0.69	0.69	0.48	0.48
31	2.08	2.08	0.52	0.52	0.10	0.10	0.96	0.96	0.67	0.67
32	2.28	2.28	0.57	0.57	0.11	0.11	1.06	1.06	0.73	0.73
33	2.00	2.00	0.50	0.50	0.10	0.10	0.93	0.93	0.64	0.64
34	2.55	2.55	0.64	0.64	0.13	0.13	1.18	1.18	0.82	0.82
35	0.76	0.76	0.19	0.19	0.04	0.04	0.35	0.35	0.24	0.24
36	0.95	0.95	0.24	0.24	0.05	0.05	0.44	0.44	0.31	0.31
37	1.86	1.86	0.46	0.46	0.09	0.09	0.86	0.86	0.60	0.60
38	2.83	2.83	0.71	0.71	0.14	0.14	1.32	1.32	0.91	0.91
39	2.03	2.03	0.51	0.51	0.10	0.10	0.94	0.94	0.65	0.65
40	0.47	0.47	0.12	0.12	0.02	0.02	0.22	0.22	0.15	0.15
41	1.90	1.90	0.48	0.48	0.10	0.10	0.88	0.88	0.61	0.61

Appendix 4.2. Outflow rate (Q_{out}, L day^{-1}) of the treatments during experimental time.

Time (day)	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
1	0.57	4.56	3.71	8.84	4.85	9.99	2.49	7.62	3.30	8.44
2	0.33	5.46	3.93	9.07	4.90	10.03	2.90	8.04	3.59	8.73
3	3.91	9.05	4.83	9.97	5.07	10.21	4.57	9.70	4.74	9.88
4	0.13	5.26	3.88	9.02	4.89	10.02	2.81	7.95	3.53	8.66
5	3.63	8.77	4.76	9.90	5.06	10.20	4.44	9.57	4.65	9.79
6	2.48	7.61	4.47	9.61	5.00	10.14	3.90	9.04	4.28	9.42
7	0.85	5.99	4.06	9.20	4.92	10.06	3.15	8.28	3.76	8.89
8	0.16	5.29	3.89	9.03	4.89	10.02	2.82	7.96	3.54	8.67
9	1.60	6.74	4.25	9.39	4.96	10.10	3.50	8.63	4.00	9.14
10	0.92	6.05	4.08	9.22	4.92	10.06	3.18	8.31	3.78	8.92
11	2.87	8.01	4.57	9.71	5.02	10.16	4.08	9.22	4.41	9.54
12	4.15	9.28	4.89	10.02	5.09	10.22	4.68	9.81	4.82	9.95
13	2.87	8.00	4.57	9.70	5.02	10.16	4.08	9.22	4.41	9.54
14	3.36	8.49	4.69	9.83	5.05	10.18	4.31	9.45	4.56	9.70
15	2.20	7.34	4.40	9.54	4.99	10.13	3.77	8.91	4.19	9.33
16	2.89	8.03	4.57	9.71	5.02	10.16	4.09	9.23	4.41	9.55
17	1.51	6.64	4.23	9.36	4.95	10.09	3.45	8.59	3.97	9.11
18	2.14	7.28	4.39	9.52	4.99	10.12	3.74	8.88	4.17	9.31
19	4.50	9.63	4.98	10.11	5.10	10.24	4.84	9.97	4.93	10.07
20	2.43	7.57	4.46	9.60	5.00	10.14	3.88	9.02	4.27	9.40
21	2.67	7.80	4.52	9.65	5.01	10.15	3.99	9.13	4.34	9.48
22	2.13	7.27	4.39	9.52	4.99	10.12	3.74	8.88	4.17	9.31
23	1.99	7.13	4.35	9.49	4.98	10.11	3.68	8.81	4.13	9.26
24	3.04	8.17	4.61	9.75	5.03	10.17	4.16	9.30	4.46	9.60
25	3.48	8.62	4.72	9.86	5.05	10.19	4.37	9.50	4.60	9.74
26	4.22	9.35	4.91	10.04	5.09	10.23	4.71	9.85	4.84	9.98
27	3.15	8.29	4.64	9.78	5.04	10.17	4.22	9.35	4.50	9.63
28	4.68	9.81	5.02	10.16	5.11	10.25	4.92	10.06	4.99	10.12
29	3.79	8.92	4.80	9.93	5.07	10.20	4.51	9.65	4.70	9.84
30	3.65	8.79	4.77	9.90	5.06	10.20	4.45	9.58	4.66	9.80
31	3.06	8.20	4.62	9.75	5.03	10.17	4.17	9.31	4.47	9.60
32	2.85	7.99	4.57	9.70	5.02	10.16	4.08	9.21	4.40	9.54
33	3.14	8.28	4.64	9.77	5.04	10.17	4.21	9.35	4.49	9.63
34	2.59	7.73	4.50	9.64	5.01	10.14	3.95	9.09	4.32	9.45
35	4.38	9.51	4.95	10.08	5.10	10.23	4.78	9.92	4.89	10.03
36	4.18	9.32	4.90	10.03	5.09	10.22	4.69	9.83	4.83	9.97
37	3.28	8.42	4.67	9.81	5.04	10.18	4.27	9.41	4.54	9.68
38	2.30	7.44	4.43	9.56	4.99	10.13	3.82	8.96	4.23	9.36
39	3.11	8.24	4.63	9.76	5.03	10.17	4.19	9.33	4.48	9.62
40	4.66	9.80	5.02	10.15	5.11	10.25	4.92	10.05	4.98	10.12
41	3.24	8.37	4.66	9.80	5.04	10.18	4.25	9.39	4.52	9.66

Appendix 4.3. Mean ± Standard deviation of ammonia concentration in the mesocosm treatments.

	Average NH_x concentration (mg N L^{-1})										
Time (day)	Inflow	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
2	10.14	7.81	9.10	0.10	2.60	0.12	2.43	1.99	2.39	0.04	1.10
3	9.87	7.34	8.68	0.79	2.69	0.82	1.44	1.90	1.95	0.74	1.27
7	9.03	6.45	7.73	1.23	1.61	0.86	1.34	1.30	2.03	0.38	1.11
10	8.89	6.92	7.60	0.46	1.31	0.56	0.60	0.99	1.31	0.41	0.59
14	9.81	6.54	6.96	1.81	2.05	1.69	1.17	2.54	1.13	1.57	0.52
17	9.12	4.44	6.12	0.21	1.46	0.42	0.67	0.92	1.46	0.70	0.62
21	9.13	1.65	4.71	0.42	2.20	0.31	1.29	0.67	1.23	0.27	0.92
24	9.18	1.30	4.24	0.52	2.08	0.31	1.11	0.58	1.43	0.34	1.11
28	9.25	1.40	3.44	0.63	2.40	0.66	1.21	0.69	1.33	0.43	0.70
31	9.31	1.20	3.18	0.36	2.06	0.33	0.94	0.64	1.41	0.35	0.43
35	9.46	1.38	2.97	0.66	2.49	0.63	1.78	0.69	1.31	0.39	0.93
40	9.43	1.22	3.04	0.45	2.74	0.43	1.88	0.51	1.36	0.19	0.49
Time (day)	Standard deviation										
2	0.31	0.27	0.16	0.01	0.77	0.02	0.03	0.42	0.34	0.00	0.34
3	0.32	0.36	0.05	0.05	0.11	0.14	0.06	0.57	0.24	0.23	0.17
7	0.32	0.28	0.18	0.16	0.04	0.09	0.02	0.35	0.68	0.08	0.32
10	0.57	0.73	0.29	0.06	0.06	0.05	0.02	0.26	0.20	0.10	0.16
14	0.53	0.18	0.67	0.07	0.62	0.85	0.06	0.56	0.37	0.71	0.12
17	0.40	0.66	0.50	0.05	0.05	0.01	0.01	0.19	0.07	0.20	0.15
21	0.40	0.64	0.64	0.09	0.05	0.04	0.06	0.09	0.11	0.03	0.12
24	0.41	0.37	0.71	0.07	0.14	0.05	0.05	0.07	0.21	0.10	0.35
28	0.42	0.50	0.60	0.04	0.16	0.03	0.05	0.03	0.37	0.09	0.12
31	0.45	0.58	0.65	0.03	0.29	0.07	0.03	0.05	0.29	0.05	0.02
35	0.31	0.64	0.64	0.04	0.17	0.04	0.03	0.08	0.38	0.02	0.18
40	0.38	0.67	0.65	0.04	0.27	0.03	0.08	0.03	0.49	0.01	0.11

Appendix 4.4. Analysis of variance (ANOVA) for the change in ammonia concentrations in the mesocosm treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.590	11	0.0536	4.297	.05
Treatments	4.608	9	0.5120	41.018	.05
Error	1.236	99	0.0125		

Appendix 4.5. Post hoc multiple comparison (Turkey's HSD test) of ammonia concentr -ation (mg N L^{-1}) in the mesocosm treatments.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
C1-Inflow	-0.392791	-0.537529	-2.48e-01	0.000
C2-Inflow	-0.218837830	-0.36357585	-7.409981e-02	0.0001
M1-Inflow	-0.816132930	-0.96087095	-6.713949e-01	0.000
M3-Inflow	-0.824227576	-0.96896560	-6.794896e-01	0.000
M2-Inflow	-0.524053629	-0.66879165	-3.793156e-01	0.000
M4-Inflow	-0.659573723	-0.80431174	-5.148357e-01	0.000
V1-Inflow	-0.707938721	-0.85267674	-5.632007e-01	0.000
V2-Inflow	-0.617356787	-0.76209481	-4.726188e-01	0.000
V3-Inflow	-0.856043858	-1.00078188	-7.113058e-01	0.000
V4-Inflow	-0.762174701	-0.90691272	-6.174367e-01	0.000
C2-C1	0.173953878	0.02921586	3.186919e-01	0.006
M1-C1	-0.423341221	-0.56807924	-2.786032e-01	0.000
M3-C1	-0.431435868	-0.57617389	-2.866978e-01	0.000
M2-C1	-0.131261921	-0.27599994	1.347610e-02	0.112
M4-C1	-0.266782015	-0.41152003	-1.220440e-01	0.000
V1-C1	-0.315147013	-0.45988503	-1.704090e-01	0.000
V2-C1	-0.224565078	-0.36930310	-7.982706e-02	0.000
V3-C1	-0.463252150	-0.60799017	-3.185141e-01	0.000
V4-C1	-0.369382993	-0.51412101	-2.246450e-01	0.000
M1-C2	-0.597295100	-0.74203312	-4.525571e-01	0.000
M3-C2	-0.605389746	-0.75012777	-4.606517e-01	0.000
M2-C2	-0.305215799	-0.44995382	-1.604778e-01	0.000
M4-C2	-0.440735893	-0.58547391	-2.959979e-01	0.000
V1-C2	-0.489100892	-0.63383891	-3.443629e-01	0.000
V2-C2	-0.398518957	-0.54325698	-2.537809e-01	0.000
V3-C2	-0.637206029	-0.78194405	-4.924680e-01	0.000
V4-C2	-0.543336872	-0.68807489	-3.985989e-01	0.000
M3-M1	-0.008094646	-0.15283267	1.366434e-01	1.000
M2-M1	0.292079301	0.14734128	4.368173e-01	0.000
M4-M1	0.156559206	0.01182119	3.012972e-01	0.022
V1-M1	0.108194208	-0.03654381	2.529322e-01	0.341
V2-M1	0.198776143	0.05403812	3.435142e-01	0.000
V3-M1	-0.039910929	-0.18464895	1.048271e-01	0.997
V4-M1	0.053958228	-0.09077979	1.986962e-01	0.978
M2-M3	0.300173947	0.15543593	4.449120e-01	0.000
M4-M3	0.164653853	0.01991583	3.093919e-01	0.012
V1-M3	0.116288854	-0.02844917	2.610269e-01	0.241
V2-M3	0.206870789	0.06213277	3.516088e-01	0.000
V3-M3	-0.031816283	-0.17655430	1.129217e-01	0.999
V4-M3	0.062052874	-0.08268515	2.067909e-01	0.943
M4-M2	-0.135520094	-0.28025811	9.217926e-03	0.087
V1-M2	-0.183885093	-0.32862311	-3.914707e-02	0.003
V2-M2	-0.093303158	-0.23804118	5.143486e-02	0.566
V3-M2	-0.331990229	-0.47672825	-1.872522e-01	0.000
V4-M2	-0.238121073	-0.38285909	-9.338305e-02	0.000
V1-M4	-0.048364998	-0.19310302	9.637302e-02	0.990
V2-M4	0.042216936	-0.10252108	1.869550e-01	0.996
V3-M4	-0.196470135	-0.34120816	-5.173212e-02	0.001
V4-M4	-0.102600978	-0.24733900	4.213704e-02	0.421

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
V2-V1	0.090581935	-0.05415608	2.353200e-01	0.609
V3-V1	-0.148105137	-0.29284316	-3.367117e-03	0.040
V4-V1	-0.054235980	-0.19897400	9.050204e-02	0.977
V3-V2	-0.238687072	-0.38342509	-9.394905e-02	0.000
V4-V2	-0.144817915	-0.28955593	-7.989507e-05	0.049
V4-V3	0.093869157	-0.05086886	2.386072e-01	0.557

Appendix 4.6. Mass removal rate of ammonia (mg N day^{-1}) in the treatments during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	56.54	62.63	51.83	84.82	51.48	79.85	52.07	91.87	52.05	97.94
3	49.75	55.68	48.60	80.47	46.69	86.92	49.49	90.13	49.89	93.46
7	45.38	51.87	43.14	80.27	42.20	79.32	45.55	81.02	45.98	85.88
10	25.80	30.45	43.81	79.37	42.82	85.23	42.59	80.53	44.30	86.28
14	35.96	49.68	43.75	82.74	41.92	88.88	44.04	92.10	46.04	96.61
17	37.33	49.12	46.07	80.81	44.72	86.83	44.58	82.62	44.94	88.78
21	43.40	59.57	45.38	74.50	45.36	80.74	45.29	84.49	46.19	86.62
24	42.64	57.74	44.93	74.65	45.58	83.02	45.04	81.78	45.89	84.43
28	42.21	64.35	44.72	72.05	44.18	82.73	44.81	83.00	45.77	88.63
31	44.41	70.31	46.39	76.88	46.18	86.08	45.88	84.06	46.69	92.08
35	42.84	69.54	45.54	72.84	45.40	78.97	45.65	84.86	46.92	88.43
40	44.49	71.47	46.55	71.30	46.28	77.71	46.70	85.22	47.75	92.57

Appendix 4.7. Analysis of variance (ANOVA) for the mass removal of ammonia concentrations in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.0694	11	0.00631	4.086	.05
Treatments	1.9119	9	0.21244	137.651	.05
Error	0.1528	99	0.00154		

Appendix 4.8. Mean ± Standard deviation of nitrite concentration in the mesocosm treatments.

	Average NO_2^- concentration (mg N L^{-1})									
Time (day)	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
2	0.03	0.04	6.99	3.92	4.11	5.58	1.25	3.46	0.03	1.24
3	0.16	0.04	6.18	5.46	4.51	5.59	1.16	3.70	0.07	1.26
7	0.10	0.08	5.19	5.36	2.24	5.19	0.73	3.28	0.09	1.73
10	0.61	0.33	2.91	6.88	3.16	5.87	0.34	2.68	0.07	0.70
14	1.39	0.96	0.27	6.70	2.74	5.48	0.27	1.05	0.11	0.31
17	2.88	1.73	0.04	5.57	1.45	4.88	0.21	0.36	0.12	0.19
21	5.40	2.82	0.09	4.01	0.45	3.90	0.14	0.25	0.03	0.23
24	5.44	3.61	0.08	3.86	0.19	2.71	0.13	0.19	0.02	0.14
28	4.90	4.35	0.10	3.69	0.19	1.57	0.14	0.14	0.03	0.14
31	3.97	4.69	0.07	3.58	0.08	1.13	0.10	0.12	0.03	0.12
35	1.71	4.73	0.08	2.52	0.10	0.47	0.10	0.10	0.03	0.10
40	1.42	4.12	0.08	1.42	0.10	0.30	0.12	0.11	0.02	0.11
Time (day)	Standard deviation									
2	0.00	0.00	0.01	0.24	1.41	0.03	0.57	0.23	0.02	0.31
3	0.10	0.00	0.05	0.10	1.53	0.06	0.63	0.14	0.03	0.46
7	0.03	0.02	0.03	0.30	1.50	0.38	0.34	0.37	0.04	0.59
10	0.18	0.13	1.17	0.07	1.30	0.20	0.02	0.58	0.03	0.17
14	0.37	0.41	0.01	0.14	1.00	0.02	0.03	0.47	0.05	0.16
17	0.50	0.36	0.00	0.42	0.51	0.17	0.03	0.08	0.04	0.03
21	0.31	0.42	0.00	1.00	0.02	0.06	0.01	0.04	0.01	0.03
24	0.10	0.39	0.00	1.47	0.01	0.03	0.01	0.03	0.01	0.02
28	0.10	0.24	0.00	1.44	0.00	0.05	0.01	0.03	0.01	0.01
31	0.53	0.25	0.00	1.40	0.01	0.00	0.01	0.01	0.01	0.01
35	1.29	0.09	0.00	0.99	0.01	0.00	0.00	0.00	0.00	0.01
40	1.11	0.35	0.00	0.54	0.01	0.00	0.02	0.01	0.00	0.01

Appendix 4.9. Analysis of variance (ANOVA) for the change in nitrite concentrations in the mesocosm treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.0.968	11	0.0.0880	1.510	.05
Treatments	4.854	9	0.5393	9.247	.05
Error	5.773	99	0.0583		

Appendix 4.10. Post hoc multiple comparison (Turkey's HSD test) of nitrite concentrati on (mg N L^{-1}) in the mesocosm treatments.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
C2-C1	-0.0062805	-0.3159897	0.303428693	1.0000000
M1-C1	-0.1432868	-0.4529960	0.166422376	0.9096735
M3-C1	0.27998708	-0.0297221	0.589696279	0.1149293
M2-C1	-0.1026153	-0.4123245	0.207093821	0.9909329
M4-C1	0.16082176	-0.1488874	0.470530966	0.8287647
V1-C1	-0.3048965	-0.6146057	0.004812690	0.0576543
V2-C1	-0.1556455	-0.4653547	0.154063656	0.8557906
V3-C1	-0.4099932	-0.7197024	-0.10028406	0.0014660
V4-C1	-0.2743524	-0.5840616	0.035356774	0.1327901
M1-C2	-0.1370063	-0.4467155	0.172702886	0.9312110
M3-C2	0.28626759	-0.0234416	0.595976789	0.0973322
M2-C2	-0.0963348	-0.4060440	0.213374331	0.9944698
M4-C2	0.16710227	-0.1426069	0.476811476	0.7926959
V1-C2	-0.2986160	-0.6083252	0.011093200	0.0691315
V2-C2	-0.1493650	-0.4590742	0.160344166	0.8850839
V3-C2	-0.4037127	-0.7134219	-0.09400355	0.0018793
V4-C2	-0.2680719	-0.5777811	0.041637284	0.1551617
M3-M1	0.42327390	0.113564701	0.732983106	0.0008583
M2-M1	0.04067145	-0.2690377	0.350380648	0.9999976
M4-M1	0.30410859	-0.00560061	0.613817793	0.0589985
V1-M1	-0.1616096	-0.47131888	0.148099517	0.8244312
V2-M1	-0.0123587	-0.32206792	0.297350483	1.0000000
V3-M1	-0.2667064	-0.57641563	0.043002767	0.1603830
V4-M1	-0.1310656	-0.44077480	0.178643601	0.9480957
M2-M3	-0.3826024	-0.69231166	-0.07289325	0.0042275
M4-M3	-0.1191653	-0.42887451	0.190543890	0.9727095
V1-M3	-0.5848835	-0.89459279	-0.27517438	0.0000005
V2-M3	-0.4356326	-0.74534182	-0.12592342	0.0005153
V3-M3	-0.6899803	-0.99968954	-0.38027113	0.0000000
V4-M3	-0.5543395	-0.86404870	-0.24463030	0.0000024
M4-M2	0.26343715	-0.04627205	0.573146348	0.1734160
V1-M2	-0.2022811	-0.51199033	0.107428072	0.5467696
V2-M2	-0.0530301	-0.36273936	0.256679038	0.9999705
V3-M2	-0.3073778	-0.61708708	0.002331322	0.0535909
V4-M2	-0.1717370	-0.48144625	0.137972156	0.7639803
V1-M4	-0.4657182	-0.77542747	-0.15600907	0.0001424
V2-M4	-0.3164673	-0.62617651	-0.00675810	0.0407420
V3-M4	-0.5708150	-0.88052422	-0.26110582	0.0000011
V4-M4	-0.4351741	-0.74488339	-0.12546498	0.0005253
V2-V1	0.14925097	-0.16045823	0.458960169	0.8855796
V3-V1	-0.1050967	-0.41480595	0.204612453	0.9891137
V4-V1	0.03054408	-0.27916511	0.340253287	0.9999999
V3-V2	-0.2543477	-0.56405691	0.055361487	0.2136875
V4-V2	-0.1187068	-0.42841608	0.191002321	0.9734380
V4-V3	0.13564083	-0.17406836	0.445350037	0.9353850

Table 4.11. Mass removal rate of nitrite (mg N day^{-1}) in the treatments during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	2.17	4.33	1.50	3.13	1.34	2.02	2.17	3.60	2.17	4.05
3	2.11	4.21	1.43	2.46	1.19	1.89	2.08	3.33	2.11	3.90
7	1.93	3.85	1.36	2.13	1.48	1.70	1.91	3.08	1.93	3.42
10	1.83	3.69	1.42	1.18	1.24	1.32	1.86	2.88	1.89	3.56
14	1.97	3.91	2.06	1.74	1.53	1.89	2.07	3.86	2.09	4.10
17	1.69	3.38	1.95	1.87	1.65	1.84	1.93	3.79	1.94	3.84
21	1.48	3.05	1.94	2.45	1.86	2.26	1.94	3.83	1.95	3.84
24	1.18	2.63	1.95	2.41	1.93	2.78	1.95	3.86	1.96	3.88
28	1.21	2.34	1.96	2.49	1.94	3.29	1.96	3.91	1.97	3.91
31	1.52	2.43	1.98	2.62	1.98	3.51	1.98	3.95	1.99	3.95
35	1.73	2.21	2.01	3.02	2.00	3.85	2.01	4.01	2.02	4.01
40	1.83	2.60	2.00	3.49	2.00	3.91	2.00	4.00	2.02	4.00

Appendix 4.12. Mean± Standard deviation of nitrate concentration in the mesocosm treatments.

	Average NO_3^- concentration (mg N L^{-1})									
Time (day)	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
2	1.54	1.46	3.78	2.54	5.88	2.77	3.12	2.65	0.30	2.47
3	1.38	1.42	2.40	2.99	4.76	2.55	3.48	2.72	0.55	1.88
7	1.24	1.30	3.29	3.07	5.74	2.18	3.83	3.18	0.64	3.37
10	1.32	1.26	3.90	3.11	4.09	2.44	4.27	3.78	0.76	4.30
14	1.39	1.41	6.41	3.51	5.54	2.83	3.97	4.68	1.15	4.45
17	1.93	1.72	7.73	3.62	6.35	3.46	5.43	4.89	1.47	4.71
21	2.85	2.08	7.67	3.86	6.88	4.20	5.34	5.09	1.30	4.73
24	3.04	2.30	7.47	4.34	7.58	4.92	5.32	5.20	0.99	4.93
28	3.25	2.67	7.42	4.32	7.64	5.68	5.50	5.45	1.04	5.29
31	4.15	2.92	8.26	4.84	7.77	6.36	5.72	5.62	1.28	5.73
35	5.45	2.90	9.12	4.64	8.74	6.41	5.62	5.78	1.31	5.74
40	5.79	3.67	9.12	5.96	8.27	6.58	5.75	5.95	1.45	6.29
Time (day)	Standard deviation									
2	0.01	0.02	0.07	0.07	0.90	0.01	1.12	0.04	0.02	0.13
3	0.09	0.01	0.95	0.02	1.15	0.04	1.13	0.20	0.09	0.76
7	0.01	0.02	0.01	0.16	1.06	0.06	0.79	0.22	0.17	0.17
10	0.04	0.03	1.03	0.06	1.33	0.06	0.77	0.23	0.21	0.26
14	0.16	0.12	0.21	0.06	0.81	0.01	0.94	0.32	0.37	0.33
17	0.16	0.11	0.00	0.33	0.03	0.04	0.61	0.17	0.51	0.47
21	0.17	0.13	0.02	0.77	0.24	0.04	0.65	0.15	0.47	0.34
24	0.05	0.12	0.03	0.86	0.06	0.05	0.73	0.12	0.34	0.33
28	0.18	0.16	0.01	0.86	0.07	0.03	0.80	0.16	0.41	0.36
31	0.51	0.19	0.49	0.75	0.19	0.01	0.93	0.08	0.49	0.29
35	0.98	0.12	0.56	1.11	0.53	0.03	1.07	0.31	0.46	0.19
40	0.82	0.61	0.53	0.19	0.08	0.02	1.09	0.36	0.54	0.12

Appendix 4.13. Analysis of variance (ANOVA) for the change in nitrate concentrations in the mesocosm treatments. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1.261	11	0.1146	35.48	.05
Treatments	3.335	9	0.3706	114.71	.05
Error	0.320	99	0.0032		

Appendix 4.14. Post hoc multiple comparison (Turkey's HSD test) of nitrate concentrat ion (mg N L^{-1}) in the mesocosm treatments.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
C2-C1	-0.065393	-0.150862	0.020075799	0.3074182
M1-C1	0.29942779	0.21395887	0.384896719	0.0000000
M3-C1	0.13973720	0.05426828	0.225206128	0.0000229
M2-C1	0.33018402	0.24471510	0.415652946	0.0000000
M4-C1	0.15073126	0.06526234	0.236200186	0.0000035
V1-C1	0.21258695	0.12711803	0.298055873	0.0000000
V2-C1	0.19329522	0.10782630	0.278764147	0.0000000
V3-C1	-0.2455778	-0.3310468	-0.16010894	0.0000000
V4-C1	0.18235698	0.09688805	0.267825901	0.0000000
M1-C2	0.36482092	0.27935200	0.450289844	0.0000000
M3-C2	0.20513033	0.11966140	0.290599253	0.0000000
M2-C2	0.39557715	0.31010822	0.481046071	0.0000000
M4-C2	0.21612439	0.13065546	0.301593311	0.0000000
V1-C2	0.27798007	0.19251115	0.363448999	0.0000000
V2-C2	0.25868835	0.17321942	0.344157273	0.0000000
V3-C2	-0.1801847	-0.2656536	-0.09471582	0.0000000
V4-C2	0.24775010	0.16228118	0.333219026	0.0000000
M3-M1	-0.1596905	-0.2451595	-0.07422166	0.0000007
M2-M1	0.03075623	-0.0547127	0.116225151	0.9831207
M4-M1	-0.1486965	-0.2341654	-0.06322760	0.0000050
V1-M1	-0.0868408	-0.1723097	-0.00137192	0.0430313
V2-M1	-0.1061325	-0.1916015	-0.02066364	0.0039237
V3-M1	-0.5450056	-0.6304745	-0.45953674	0.0000000
V4-M1	-0.1170708	-0.2025397	-0.03160189	0.0008257
M2-M3	0.19044682	0.10497789	0.275915742	0.0000000
M4-M3	0.01099406	-0.0744748	0.096462982	0.9999980
V1-M3	0.07284975	-0.0126191	0.158318670	0.1711924
V2-M3	0.05355802	-0.0319109	0.139026944	0.6077830
V3-M3	-0.3853150	-0.4707840	-0.29984615	0.0000000
V4-M3	0.04261977	-0.0428491	0.128088697	0.00000
M4-M2	-0.1794527	-0.2649216	-0.09398383	0.0000000
V1-M2	-0.1175970	-0.2030660	-0.03212814	0.0007636
V2-M2	-0.1368888	-0.2223577	-0.05141987	0.0000367
V3-M2	-0.5757618	-0.6612308	-0.49029296	0.00000
V4-M2	-0.1478270	-0.2332959	-0.06235812	0.0000058
V1-M4	0.06185569	-0.0236132	0.147324612	0.3895142
V2-M4	0.04256396	-0.0429049	0.128032886	0.8627007
V3-M4	-0.3963091	-0.4817780	-0.31084020	0.0000000
V4-M4	0.03162572	-0.0538432	0.117094639	0.9793265
V2-V1	-0.0192917	-0.1047606	0.066177198	0.9996324
V3-V1	-0.4581648	-0.5436337	-0.37269589	0.0000000
V4-V1	-0.0302299	-0.1156989	0.055238952	0.9851391
V3-V2	-0.4388730	-0.5243420	-0.35340417	0.0000000
V4-V2	-0.0109382	-0.0964071	0.074530678	0.9999981
V4-V3	0.52793485	0.342465592	0.513403772	0.000000

Appendix 4.15. Mean± Standard deviation of total oxidized N concentration in the mesocosm treatments.

	Average NO_x concentration (mg N L^{-1})									
Time (day)	C_1	C_2	M_1	M_2	M_3	M_4	V_1	V_2	V_3	V_4
2	1.58	1.51	10.77	6.47	9.98	8.35	4.36	6.11	0.33	3.71
3	1.55	1.46	8.58	8.45	9.27	8.14	4.65	6.42	0.62	3.14
7	1.34	1.38	8.48	8.43	7.97	7.38	4.56	6.46	0.73	5.11
10	1.93	1.60	6.81	10.00	7.25	8.31	4.60	6.46	0.83	5.00
14	2.78	2.37	6.68	10.22	8.28	8.31	4.24	5.73	1.26	4.76
17	4.81	3.45	7.77	9.19	7.80	8.33	5.65	5.24	1.58	4.90
21	8.25	4.90	7.76	7.87	7.33	8.10	5.48	5.34	1.33	4.96
24	8.48	5.91	7.55	8.20	7.77	7.63	5.44	5.39	1.01	5.07
28	8.16	7.02	7.52	8.01	7.83	7.25	5.65	5.59	1.07	5.42
31	8.11	7.60	8.33	8.43	7.85	7.49	5.82	5.74	1.31	5.85
35	7.17	7.63	9.20	7.17	8.84	6.88	5.72	5.88	1.34	5.84
40	7.21	7.79	9.20	7.38	8.37	6.88	5.87	6.06	1.47	6.39
Time (day)	Standard deviation									
2	0.01	0.02	0.07	0.31	2.01	0.04	0.94	0.26	0.01	0.42
3	0.17	0.01	0.99	0.12	2.40	0.02	1.76	0.14	0.07	1.00
7	0.04	0.01	0.03	0.30	2.26	0.37	0.54	0.27	0.17	0.68
10	0.22	0.14	2.18	0.11	1.09	0.25	0.78	0.67	0.24	0.09
14	0.36	0.37	0.15	0.06	0.20	0.02	0.69	0.21	0.29	0.32
17	0.45	0.46	0.01	0.17	0.53	0.20	0.62	0.11	0.49	0.46
21	0.48	0.37	0.02	1.42	0.25	0.04	0.66	0.14	0.48	0.31
24	0.15	0.32	0.04	2.05	0.06	0.07	0.74	0.10	0.34	0.31
28	0.25	0.40	0.01	2.01	0.07	0.03	0.81	0.13	0.41	0.36
31	0.02	0.20	0.49	1.84	0.18	0.01	0.92	0.08	0.49	0.30
35	1.99	0.09	0.56	1.41	0.53	0.03	1.08	0.30	0.46	0.18
40	1.68	0.26	0.54	0.67	0.09	0.01	1.10	0.36	0.54	0.12

Kinetics and performance assessment

Appendix 4.16. Estimated removal rate constants (k, day^{-1}) of ammonia in the effluent of treatments during experimental time.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	0.04	0.01	13.35	0.40	11.41	0.44	0.57	0.45	32.54	1.14
3	0.03	0.01	1.49	0.34	1.44	0.75	0.54	0.52	1.61	0.87
7	0.05	0.02	0.89	0.65	1.34	0.80	0.83	0.48	3.18	1.00
10	0.06	0.04	2.91	0.93	2.35	2.20	1.28	0.93	3.29	2.21
14	0.06	0.04	0.58	0.49	0.63	0.97	0.37	1.01	0.69	2.34
17	0.15	0.07	6.10	0.75	2.95	1.79	1.27	0.75	1.73	1.96
21	0.65	0.14	2.97	0.45	4.05	0.87	1.82	0.92	4.73	1.28
24	0.88	0.17	2.41	0.49	4.08	1.05	2.13	0.78	3.79	1.05
28	0.81	0.24	1.98	0.41	1.88	0.96	1.78	0.86	2.92	1.76
31	0.98	0.28	3.58	0.51	3.96	1.29	1.98	0.81	3.73	3.02
35	0.83	0.31	1.90	0.40	1.99	0.61	1.81	0.88	3.34	1.31
40	0.96	0.30	2.84	0.35	3.00	0.57	2.51	0.85	6.84	2.58

Appendix 4.17. Analysis of variance (ANOVA) for the rate constants of ammonia removal in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.766	11	0.0697	2.974	.05
Treatments	4.529	9	0.5032	21.491	.05
Error	2.318	99	0.0234		

Appendix 4.18. Estimated removal half-life ($T_{1/2}$, day) of ammonia in the effluent of mesocosms during experimental time.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	18.45	57.12	0.05	1.74	0.06	1.59	1.22	1.55	0.02	0.61
3	20.97	118.87	0.47	2.06	0.48	0.92	1.29	1.33	0.43	0.80
7	12.82	32.46	0.78	1.07	0.52	0.87	0.83	1.44	0.22	0.69
10	11.60	16.69	0.24	0.74	0.30	0.31	0.54	0.75	0.21	0.31
14	12.31	15.64	1.20	1.40	1.11	0.72	1.87	0.69	1.01	0.30
17	4.59	9.85	0.11	0.93	0.23	0.39	0.54	0.92	0.40	0.35
21	1.06	5.12	0.23	1.53	0.17	0.80	0.38	0.75	0.15	0.54
24	0.79	4.11	0.29	1.41	0.17	0.66	0.32	0.89	0.18	0.66
28	0.86	2.84	0.35	1.68	0.37	0.72	0.39	0.81	0.24	0.39
31	0.71	2.45	0.19	1.35	0.18	0.54	0.35	0.85	0.19	0.23
35	0.83	2.23	0.36	1.74	0.35	1.13	0.38	0.78	0.21	0.53
40	0.72	2.30	0.24	1.99	0.23	1.21	0.28	0.82	0.10	0.27

Appendix 4.19. Analysis of variance (ANOVA) for the degradation half-life of ammonia concentrations in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1.750	11	0.1591	3.596	.05
Treatments	9.913	9	1.1015	24.900	.05
Error	4.379	99	0.0442		

Appendix 4.20. Removal efficiency of ammonia concentration (%) in the mesocosms during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	20.83	7.83	98.94	73.62	98.76	75.36	79.86	75.79	99.56	88.89
3	18.79	3.92	91.25	70.22	90.95	84.05	79.02	78.43	91.84	85.91
7	27.46	13.00	86.17	81.89	90.34	84.87	85.38	77.12	95.70	87.53
10	29.49	22.52	95.32	86.69	94.26	93.91	89.93	86.67	95.84	93.94
14	28.28	23.67	80.19	77.57	81.41	87.13	72.16	87.56	82.77	94.24
17	51.38	33.00	97.71	83.97	95.38	92.62	89.90	84.05	92.37	93.20
21	82.06	48.66	95.41	75.99	96.59	85.91	92.73	86.56	97.07	89.98
24	85.97	54.13	94.40	77.50	96.61	88.01	93.73	84.57	96.37	88.03
28	84.93	63.07	93.27	74.28	92.93	87.05	92.56	85.73	95.34	92.50
31	87.28	66.45	96.16	78.24	96.51	90.02	93.28	85.06	96.31	95.49
35	85.37	68.52	93.02	73.62	93.31	81.08	92.68	86.08	95.90	90.19
40	87.05	67.81	95.20	70.90	95.45	80.02	94.61	85.56	97.95	94.75

Appendix 4.21. Analysis of variance (ANOVA) for the removal efficiency of ammonia concentrations in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.0270	11	0.00245	1.571	.05
Treatments	0.4458	9	0.04953	31.724	.05
Error	0.1546	99	0.00156		

Appendix 4.22. Estimated removal rate constants (k, day^{-1}) of nitrite in the effluent of mesocosms during experimental time.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	43.48	34.18	0.06	0.23	0.21	0.12	1.02	0.28	43.17	1.02
3	8.45	32.07	0.09	0.12	0.17	0.11	1.07	0.24	19.43	0.98
7	12.52	15.63	0.11	0.10	0.43	0.11	1.63	0.25	14.23	0.60
10	1.94	3.69	0.29	0.04	0.26	0.07	3.64	0.33	16.90	1.66
14	0.87	1.32	4.97	0.07	0.37	0.11	5.05	1.19	12.15	4.35
17	0.31	0.61	34.28	0.09	0.75	0.12	5.93	3.52	11.14	6.61
21	0.10	0.32	13.70	0.18	2.79	0.19	9.28	5.13	40.55	5.60
24	0.10	0.22	15.89	0.20	6.87	0.34	10.19	6.88	67.24	8.92
28	0.13	0.16	12.62	0.22	6.94	0.70	9.03	9.03	43.05	9.57
31	0.19	0.14	18.32	0.23	17.56	1.03	12.99	10.92	48.84	11.22
35	0.65	0.14	16.77	0.39	12.78	2.74	13.14	13.48	47.35	13.23
40	0.81	0.18	17.15	0.81	13.95	4.36	11.37	12.19	60.83	12.57

Appendix 4.23. Analysis of variance (ANOVA) for the rate constants of nitrite removal in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	3.155	11	0.2868	1.741	.05
Treatments	16.476	9	1.8306	11.113	.05
Error	16.309	99	0.1647		

Appendix 4.24. Estimated removal half-life ($T_{1/2}$, day) of nitrite in the effluent of mesocosms during experimental time.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	0.02	0.02	10.78	3.06	3.30	5.94	0.68	2.52	0.02	0.68
3	0.08	0.02	8.15	6.02	4.09	6.34	0.65	2.91	0.04	0.71
7	0.06	0.04	6.55	7.06	1.60	6.56	0.43	2.77	0.05	1.15
10	0.36	0.19	2.36	16.66	2.68	9.42	0.19	2.09	0.04	0.42
14	0.80	0.53	0.14	10.48	1.88	6.14	0.14	0.58	0.06	0.16
17	2.25	1.14	0.02	7.62	0.92	5.59	0.12	0.20	0.06	0.10
21	7.01	2.17	0.05	3.79	0.25	3.62	0.07	0.14	0.02	0.12
24	7.05	3.15	0.04	3.52	0.10	2.03	0.07	0.10	0.01	0.08
28	5.47	4.30	0.05	3.21	0.10	0.99	0.08	0.08	0.02	0.07
31	3.60	4.91	0.04	3.03	0.04	0.67	0.05	0.06	0.01	0.06
35	1.07	4.85	0.04	1.76	0.05	0.25	0.05	0.05	0.01	0.05
40	0.86	3.76	0.04	0.86	0.05	0.16	0.06	0.06	0.01	0.06

Appendix 4.25. Analysis of variance (ANOVA) for the degradation half-life of nitrite concentrations in the mesocosm treatments throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1.132	11	0.1029	1.440	.05
Treatments	5.683	9	0.6315	8.842	.05
Error	7.071	99	0.0714		

Appendix 4.26. Removal efficiency of nitrite concentration (%) in the mesocosms during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
1	99.67	99.58	31.03	61.29	59.50	44.95	87.71	65.82	99.67	87.76
3	98.34	99.56	37.33	44.64	54.29	43.36	88.21	62.50	99.27	87.25
7	98.87	99.09	42.54	40.72	75.22	42.50	91.93	63.70	99.01	80.82
10	93.14	96.27	67.28	22.55	64.45	34.00	96.22	69.85	99.16	92.08
14	85.85	90.23	97.21	31.65	72.04	44.13	97.25	89.26	98.84	96.82
17	68.37	80.98	99.59	38.90	84.08	46.49	97.65	96.10	98.73	97.88
21	40.89	69.09	98.97	56.14	95.12	57.29	98.48	97.29	99.65	97.51
24	40.77	60.65	99.11	57.97	97.96	70.54	98.62	97.97	99.79	98.42
28	47.02	53.02	98.88	60.16	97.98	83.01	98.44	98.44	99.67	98.53
31	57.43	49.68	99.23	61.54	99.19	87.86	98.91	98.71	99.71	98.74
35	81.90	50.02	99.16	73.35	98.89	95.04	98.92	98.95	99.70	98.93
40	84.93	56.35	99.17	84.94	98.99	96.83	98.76	98.84	99.77	98.88

Biomass and volatilization

Appendix 4.27. Plant biomass (g) in the vegetated treatments during the study.

Initial biomass (g)		**Mid biomass (g)**				**Final biomass (g)**			
V_1 and V_2 (2 plants)	**V_3 and V_4 (4 plants)**	**V_1**	**V_2**	**V_3**	**V_4**	**V_1**	**V_2**	**V_3**	**V_4**
6.22	12.44	44.29	87.18	102.29	122.56	67.00	111.66	134.80	225.60
5.80	11.60	44.84	88.35	103.59	124.15	54.50	130.66	161.68	131.08
5.04	10.08	45.39	89.51	104.89	125.74	61.06	124.08	127.40	154.08
5.84	11.68								
4.78	9.56								
5.40	10.80								
5.26	10.52								
4.06	8.12								
6.88	13.76								
7.46	14.92								

Appendix 4.28. Analysis of variance (ANOVA) for plant biomass in the vegetated treatments at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	0.3251	3	0.10838	21.13	.05
Error	0.0410	8	0.00513		

Appendix 4.29. Analysis of variance (ANOVA) for plant growth rate in the vegetated treatments at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	0.04011	3	0.01337	0.704	.05
Error	0.15199	8	0.01900		

Appendix 4.30. TN-content (mg N day^{-1}) in plant tissues during the study.

Initial plant N content (mg N)		**Mid plant N content (mg N)**				**Final plant N content (mg N)**			
V_1, V_2 (2 plants)	**V_3, V_4 (4 pants)**	**V_1**	**V_2**	**V_3**	**V_4**	**V_1**	**V_2**	**V_3**	**V_4**
84.83	169.67	609.65	1325.70	1450.34	2001.53	956.49	908.52	1898.65	3740.55
57.27	114.54	615.77	1339.35	1465.31	2022.30	906.81	2397.20	2565.08	2365.08
62.70	125.40	621.89	1353.01	1480.28	2043.07	911.31	2634.55	2232.41	3027.36
38.46	76.92								
32.29	64.59								
65.12	130.25								
84.83	169.67								

Appendix 4.31. Analysis of variance (ANOVA) for assimilated N in the plants at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	0.4208	3	0.14026	7.033	.05
Error	0.1595	8	0.01994		

Appendix 4.32. Microbial biomass in the mesocosms during the study.

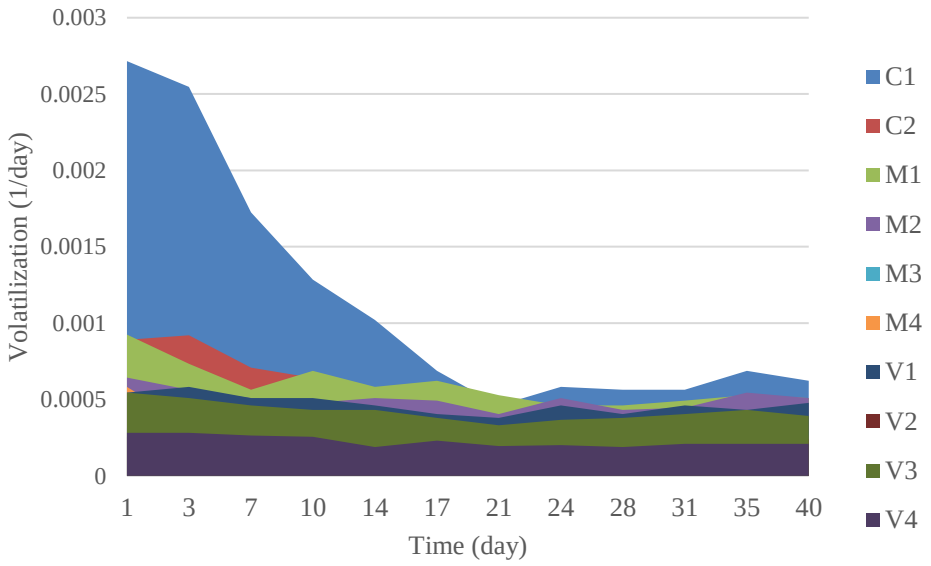
Initial microbiomass (CFU/g or mL)		**Final microbiomass (CFU/g or mL)**									
		C_1	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
Free-float (CFU/mL)	240	2×10^3	480	440	7×10^2	7×10^2	220	420	2×10^3	420	260
	180	2×10^2	640	120	120	440	10^2	60	280	660	700
	160	2×10^3	10^3	100	280	440	220	220	10^3	60	680
Epimatrix* (CFU/g)	2			6×10^4	28×10^4	42×10^3	38×10^4	44×10^3	10^5	8×10^3	16×10^4
	3			66×10^3	84×10^4	8×10^4	18×10^4	3×10^3	24×10^3	18×10^3	22×10^3
	2			6×10^4	9×10^5	9×10^4	18×10^4	16×10^3	32×10^3	22×10^4	28×10^3
Epiphytic (CFU/g)	6×10^4							32×10^4	72×10^3	96×10^4	92×10^3
	4×10^4							8×10^4	14×10^4	28×10^4	36×10^3
	4×10^4							44×10^3	36×10^3	66×10^3	24×10^3

Appendix 4.33. Analysis of variance (ANOVA) for microbial biomass in the mesocosms at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	356.5	9	39.61	161.1	.05
Error	4.9	20	0.25		

Appendix 4.34. Analysis of variance (ANOVA) for microbial growth rate in the mesocosms at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	0.11877	9	0.013196	102.7	.05
Error	0.00257	20	0.000129		



Appendix 4.35. Rate constants of volatilization in the mesocosms during experimental time.

Physicochemical parameters

Appendix 4.36. pH in the mesocosms during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
2	8.20	7.77	7.10	7.33	6.73	6.70	6.57	6.50	6.57	6.60
3	8.13	7.80	6.87	6.77	6.60	6.80	6.63	6.50	6.50	6.60
7	7.73	7.53	6.60	6.47	6.50	6.50	6.50	6.43	6.40	6.53
10	7.43	7.43	6.80	6.33	6.43	6.50	6.50	6.40	6.33	6.50
14	7.20	7.20	6.63	6.40	6.50	6.43	6.40	6.37	6.33	6.20
17	6.80	7.10	6.70	6.53	6.47	6.73	6.27	6.47	6.20	6.40
21	6.40	6.77	6.53	6.40	6.27	6.33	6.20	6.37	6.07	6.23
24	6.63	6.73	6.40	6.50	6.50	6.40	6.40	6.33	6.17	6.27
28	6.60	6.57	6.40	6.43	6.33	6.53	6.27	6.23	6.20	6.20
31	6.60	6.43	6.47	6.43	6.37	6.50	6.40	6.47	6.27	6.30
35	6.80	6.70	6.53	6.50	6.57	6.53	6.33	6.37	6.33	6.30
40	6.70	6.63	6.50	6.53	6.50	6.60	6.43	6.43	6.23	6.30

Appendix 4.37. Analysis of variance (ANOVA) for pH in the mesocosms at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	8.350	9	0.9278	10.98	.05
Error	9.296	110	0.0845		

Appendix 4.38. Dissolved oxygen (mg L^{-1}) in the mesocosms during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
2	9.57	8.28	3.55	3.93	2.84	4.24	4.24	2.03	3.59	1.93
3	9.12	8.28	4.79	1.87	2.84	0.86	4.58	1.89	3.20	2.43
7	8.86	8.11	6.80	1.79	2.70	1.11	3.18	1.60	2.77	1.76
10	8.21	7.53	4.08	1.84	2.84	1.17	2.58	1.43	3.55	1.73
14	7.61	6.51	2.29	2.28	2.81	1.30	2.85	1.22	1.91	1.34
17	5.78	5.43	2.14	3.39	2.87	1.92	1.93	1.70	2.20	1.51
21	5.03	4.67	2.65	3.13	2.37	1.10	2.21	1.41	1.83	1.20
24	6.40	4.01	2.85	2.22	3.83	1.37	2.66	2.03	2.49	1.76
28	6.65	3.46	2.26	1.98	3.04	1.32	2.40	1.86	2.03	2.01
31	6.91	3.45	2.58	1.80	2.01	1.07	2.74	2.33	2.62	1.98
35	6.22	3.77	3.09	1.43	1.88	1.27	2.81	2.31	2.35	2.22
40	5.77	3.53	2.43	1.51	1.49	1.11	2.63	2.24	2.60	1.90

Appendix 4.39. Analysis of variance (ANOVA) for DO in the mesocosms at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	358.4	9	39.82	35.48	.05
Error	123.5	110	1.12		

Appendix 4.40. Electrical conductivity (mS cm^{-2}) in the mesocosms during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
2	0.47	0.48	0.44	0.48	0.48	0.47	0.44	0.43	0.44	0.44
3	0.46	0.50	0.45	0.47	0.47	0.47	0.43	0.45	0.41	0.44
7	0.43	0.46	0.39	0.43	0.40	0.41	0.39	0.41	0.37	0.42
10	0.41	0.45	0.41	0.41	0.39	0.42	0.40	0.41	0.37	0.42
14	0.55	0.58	0.59	0.59	0.58	0.58	0.56	0.55	0.52	0.46
17	0.44	0.47	0.45	0.43	0.41	0.54	0.40	0.42	0.38	0.41
21	0.36	0.41	0.40	0.38	0.36	0.39	0.36	0.39	0.35	0.37
24	0.39	0.42	0.39	0.41	0.38	0.39	0.38	0.39	0.36	0.38
28	0.48	0.54	0.50	0.46	0.49	0.46	0.43	0.45	0.44	0.44
31	0.46	0.51	0.50	0.48	0.48	0.46	0.43	0.43	0.40	0.42
35	0.41	0.45	0.43	0.44	0.43	0.43	0.41	0.42	0.41	0.42
40	0.40	0.45	0.43	0.45	0.44	0.45	0.41	0.43	0.41	0.43

Appendix 4.41. Analysis of variance (ANOVA) for EC in the mesocosms at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	0.04576	9	0.005084	2.011	.05
Error	0.27809	110	0.002528		

Appendix 4.42. Water temperature (ºC) in the treatments during the study.

Time (day)	**C_1**	**C_2**	**M_1**	**M_2**	**M_3**	**M_4**	**V_1**	**V_2**	**V_3**	**V_4**
2	19.8	19.1	20.5	19.7	19.9	19.9	20.6	21.2	21.9	21.0
3	20.1	19.1	22.8	20.6	20.8	22.5	21.1	22.7	22.2	23.0
7	12.8	17.0	16.3	17.0	16.6	17.1	17.1	17.5	17.7	18.3
10	17.6	17.8	18.0	17.6	17.7	17.8	17.9	18.3	18.2	18.2
14	20.1	19.5	20.4	20.1	20.6	19.6	19.9	20.6	20.8	20.9
17	18.6	18.8	19.2	18.8	18.3	19.1	18.6	19.6	19.6	20.0
21	22.1	20.7	23.1	20.6	20.9	19.4	21.0	22.3	22.1	22.2
24	16.8	17.5	16.7	12.0	16.9	17.3	16.9	17.0	16.7	17.3
28	14.2	14.2	14.1	14.3	14.3	14.8	14.6	14.7	14.8	15.1
31	13.7	13.2	13.4	13.6	13.3	13.2	13.6	13.9	14.4	14.4
35	13.6	13.4	13.6	13.6	13.6	13.4	13.7	13.6	14.0	13.8
40	12.3	12.8	11.9	12.9	12.6	12.9	12.8	12.7	13.3	13.4

Appendix 4.43. Analysis of variance (ANOVA) for water temperature in the treatments at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	26.1	9	2.904	0.27	.05
Error	1182.0	110	10.745		

Chapter Five: Pilot-scale study

Water and mass balance

Appendix 5.1. Average water depth of the treatment chambers over four experimental periods.

Batches	Time (day)	Water depth (m)			Water depth (m)	
		FTW	Control		FTW	Control
Batch 1	0	0.38	0.41	Batch 3	0.38	0.40
	1	0.38	0.41		0.37	0.39
	2	0.38	0.41		0.37	0.39
	3	0.38	0.41		0.37	0.39
	4	0.38	0.41		0.37	0.39
	5	0.38	0.41		0.38	0.40
	6	0.37	0.41		0.38	0.40
	7	0.37	0.41		0.38	0.40
	8	0.38	0.41		0.39	0.41
	9	0.38	0.42		0.38	0.41
	10	0.38	0.42		0.38	0.41
	11	0.38	0.42		0.39	0.41
	12	0.38	0.41		0.38	0.41
	13	0.39	0.42		0.38	0.41
	14	0.38	0.41		0.38	0.41
Batch 2	0	0.39	0.40	Batch 4	0.38	0.40
	1	0.38	0.40		0.38	0.39
	2	0.38	0.40		0.37	0.39
	3	0.39	0.40		0.38	0.39
	4	0.38	0.40		0.37	0.39
	5	0.38	0.40		0.37	0.39
	6	0.38	0.39		0.38	0.40
	7	0.38	0.39		0.37	0.39
	8	0.38	0.39		0.38	0.40
	9	0.37	0.39		0.38	0.40
	10	0.37	0.38		0.38	0.40
	11	0.37	0.38		0.38	0.39
	12	0.38	0.39		0.38	0.40
	13	0.38	0.39		0.39	0.41
	14	0.38	0.39		0.38	0.40

Appendix 5.2. Mean± Standard deviation of Organic N concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	3.18	2.94	2.86	3.41	0.39	0.54	0.22	0.13
	3	3.83	1.74	3.33	2.01	0.42	0.43	0.30	0.09
	7	1.76	1.97	2.39	2.31	0.27	0.63	0.24	0.57
	10	1.97	1.60	2.17	1.98	0.12	0.14	0.30	0.37
	14	1.86	1.44	2.25	1.70	0.08	0.28	0.29	0.01
Batch 2	0	4.16	3.75	3.97	4.54	0.22	0.63	0.19	0.75
	3	2.07	3.68	2.39	3.28	0.12	2.00	0.15	1.42
	7	2.18	1.30	3.82	1.49	0.16	0.25	1.81	0.40
	10	3.37	1.38	4.16	1.33	1.49	0.09	2.00	0.04
	14	2.09	2.51	2.20	4.56	0.06	0.38	0.19	0.31
Batch 3	0	3.79	3.91	3.32	3.37	0.54	0.31	0.31	1.55
	3	4.09	5.46	4.08	5.55	0.28	0.80	0.28	1.06
	7	2.73	3.39	2.32	3.66	0.55	0.16	0.04	0.07
	10	2.32	3.06	3.35	3.22	0.35	0.05	1.49	0.15
	14	2.54	1.51	2.19	2.82	0.49	1.27	0.05	0.69
Batch 4	0	3.26	3.13	2.86	3.77	1.42	1.25	0.51	0.82
	3	4.72	3.59	2.24	3.68	2.30	1.73	0.02	1.63
	7	2.29	2.93	3.03	3.05	0.06	0.54	0.98	0.57
	10	2.26	4.82	2.52	3.53	0.62	0.68	0.09	3.42
	14	3.01	3.18	3.22	3.75	0.78	0.79	0.43	0.52

Appendix 5.3. Analysis of variance (ANOVA) for the change in organic N concentrations in the treatment chambers. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	15.95	4	3.987	5.024	.05
Treatments	1.07	3	0.355	0.448	.05
Error	57.15	72	0.794		

Appendix 5.4. Post hoc multiple comparison (Turkey's HSD test) of organic N concent ration (mg N L^{-1}) in the treatment chambers.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
FTW_{368L}-FTW_{560L}	-0.05991	-0.8009018	0.6810651	0.9965701
C_{368L}-FTW_{560L}	-0.06906	-0.8100435	0.6719235	0.9947787
C_{560L}-FTW_{560L}	0.216463	-0.5245201	0.9574468	0.8684499
C_{368L}-FTW_{368L}	-0.00914	-0.7501251	0.7318418	0.9999876
C_{560L}-FTW_{368L}	0.2763816	-0.4646018	1.0173651	0.7606815
C_{560L}-C_{368L}	0.2855233	-0.4554601	1.0265068	0.7421190

Appendix 5.5. Mean± Standard deviation of ammonia concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	2.20	2.52	2.41	2.28	0.07	0.04	0.06	0.06
	3	1.06	2.77	1.54	2.48	0.03	0.14	0.10	0.02
	7	0.43	2.57	1.05	2.76	0.34	0.30	0.01	0.10
	10	0.08	2.21	0.43	2.23	0.01	0.12	0.02	0.05
	14	0.07	2.10	0.38	1.96	0.02	0.26	0.01	0.03
Batch 2	0	1.84	1.88	2.36	1.86	0.11	0.05	0.11	0.14
	3	0.68	1.90	0.99	2.02	0.02	0.25	0.02	0.04
	7	0.31	1.80	0.43	1.73	0.01	0.12	0.01	0.04
	10	0.11	1.61	0.25	1.67	0.00	0.04	0.01	0.03
	14	0.16	1.29	0.30	1.23	0.01	0.12	0.01	0.02
Batch 3	0	1.52	1.57	1.62	1.94	0.04	0.04	0.02	0.01
	3	1.11	1.62	1.28	1.90	0.03	0.05	0.01	0.06
	7	0.37	2.07	0.71	2.02	0.05	0.10	0.01	0.02
	10	0.16	1.80	0.37	2.09	0.01	0.01	0.02	0.03
	14	0.07	1.67	0.24	1.54	0.01	0.22	0.01	0.10
Batch 4	0	2.35	2.14	2.38	2.29	0.05	0.02	0.02	0.04
	3	0.92	2.19	1.14	1.94	0.01	0.04	0.02	0.05
	7	0.14	1.55	0.72	1.51	0.00	0.04	0.02	0.01
	10	0.10	1.53	0.32	1.63	0.01	0.40	0.02	0.68
	14	0.14	1.54	0.28	1.40	0.01	0.02	0.02	0.53

Appendix 5.6. Analysis of variance (ANOVA) for the change in ammonia concentrations in the treatment chambers. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	14.23	4	3.557	19.90	.05
Treatments	24.71	3	8.237	46.08	.05
Error	12.87	72	0.179		

Appendix 5.7. Post hoc multiple comparison (Turkey's HSD test) of ammonia concentr ation (mg N L^{-1}) in the treatment chambers.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
FTW_{368L}-FTW_{560L}	-0.26736	-0.6190046	0.08427461	0.197678
C_{368L}-FTW_{560L}	0.957676667	0.6060371	1.30931628	0.000000
C_{560L}-FTW_{560L}	0.965653333	0.6140137	1.31729295	0.000000
C_{368L}-FTW_{368L}	1.225041667	0.8734021	1.57668128	0.000000
C_{560L}-FTW_{368L}	1.233018333	0.8813787	1.58465795	0.000000
C_{560L}-C_{368L}	0.007976667	-0.3436629	0.35961628	0.999923

Appendix 5.8. Mean± Standard deviation of nitrite concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	1.00	0.51	1.11	0.51	0.03	0.02	0.01	0.02
	3	0.35	0.31	0.38	0.24	0.01	0.13	0.01	0.01
	7	0.04	0.04	0.24	0.04	0.00	0.01	0.02	0.00
	10	0.02	0.04	0.06	0.02	0.02	0.04	0.01	0.01
	14	0.00	0.02	0.02	0.03	0.00	0.01	0.00	0.00
Batch 2	0	0.38	0.35	0.47	0.38	0.05	0.06	0.05	0.05
	3	0.26	0.05	0.23	0.06	0.01	0.02	0.02	0.01
	7	0.08	0.05	0.05	0.02	0.01	0.04	0.01	0.00
	10	0.03	0.02	0.03	0.02	0.00	0.01	0.00	0.00
	14	0.01	0.01	0.01	0.02	0.00	0.01	0.00	0.00
Batch 3	0	0.27	0.28	0.28	0.36	0.01	0.01	0.01	0.03
	3	0.05	0.27	0.04	0.25	0.00	0.00	0.00	0.34
	7	0.04	0.31	0.03	0.22	0.00	0.10	0.00	0.20
	10	0.01	0.08	0.01	0.09	0.00	0.00	0.00	0.02
	14	0.00	0.09	0.02	0.10	0.00	0.00	0.00	0.12
Batch 4	0	1.39	1.55	1.35	1.39	0.08	0.02	0.22	0.21
	3	0.19	1.51	0.10	0.18	0.00	0.02	0.00	0.00
	7	0.01	0.03	0.02	0.22	0.00	0.00	0.00	0.01
	10	0.01	0.31	0.02	0.10	0.00	0.00	0.00	0.00
	14	0.00	0.22	0.01	0.07	0.00	0.00	0.00	0.01

Appendix 5.9. Analysis of variance (ANOVA) for the change in nitrite concentrations in the treatment chambers. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	5.296	4	1.3241	17.683	.05
Treatments	0.118	3	0.0395	0.527	.05
Error	5.391	72	0.0749		

Appendix 5.10. Post hoc multiple comparison (Turkey's HSD test) of nitrite concentrati on (mg N L^{-1}) in the treatment chambers.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
FTW_{368L}-FTW_{560L}	-0.01705	-0.2446380	0.2105347	0.9972649
C_{368L}-FTW_{560L}	0.079618333	-0.1479680	0.3072047	0.7942004
C_{560L}-FTW_{560L}	-0.0072766	-0.2348630	0.2203097	0.9997848
C_{368L}-FTW_{368L}	0.096670000	-0.1309163	0.3242563	0.6802182
C_{560L}-FTW_{368L}	0.009775000	-0.2178113	0.2373613	0.9994795
C_{560L}-C_{368L}	-0.08689500	-0.3144813	0.1406913	0.7474592

Appendix 5.11. Mean± Standard deviation of nitrate concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	1.33	2.08	1.38	1.34	0.29	0.07	0.32	0.34
	3	0.96	2.84	1.04	1.79	0.26	0.02	0.28	0.36
	7	0.46	0.07	0.55	0.09	0.09	0.02	0.08	0.02
	10	0.24	0.07	0.42	0.07	0.02	0.06	0.03	0.01
	14	0.06	0.04	0.21	0.05	0.02	0.02	0.01	0.00
Batch 2	0	1.70	1.80	1.79	1.84	0.32	0.27	0.65	0.35
	3	1.44	0.89	1.26	0.66	0.11	0.05	0.51	0.18
	7	0.83	0.16	0.85	0.15	0.07	0.03	0.13	0.04
	10	0.22	0.15	0.48	0.13	0.15	0.01	0.13	0.03
	14	0.17	0.15	0.37	0.14	0.01	0.02	0.04	0.03
Batch 3	0	1.98	1.97	1.78	2.37	0.61	0.44	0.08	0.39
	3	1.56	1.91	1.31	0.98	0.22	0.43	0.35	0.54
	7	0.62	1.94	0.68	1.48	0.01	0.30	0.07	1.02
	10	0.38	1.19	0.65	1.30	0.05	0.14	0.06	0.21
	14	0.13	0.60	0.57	1.83	0.02	0.56	0.08	1.56
Batch 4	0	1.24	0.96	1.24	1.67	0.49	0.48	0.36	0.29
	3	1.51	1.28	1.55	2.17	0.08	0.27	0.30	0.14
	7	0.22	1.74	0.56	2.32	0.01	0.53	0.08	0.54
	10	0.23	0.93	0.48	0.73	0.06	0.18	0.15	0.13
	14	0.09	1.79	0.42	1.66	0.01	0.50	0.07	0.09

Appendix 5.12. Analysis of variance (ANOVA) for the change in nitrate concentrations in the treatment chambers. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	18.72	4	4.679	16.58	.05
Treatments	2.04	3	0.680	2.41	.05
Error	20.32	72	0.282		

Appendix 5.13. Post hoc multiple comparison (Turkey's HSD test) of nitrate concentrat ion (mg N L^{-1}) in the treatment chambers.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
FTW_{368L}-FTW_{560L}	-0.10996	-0.55184995	0.3319133	0.9136257
C_{368L}-FTW_{560L}	0.249778333	0.19210328	-0.6916599	0.4507756
C_{560L}-FTW_{560L}	0.259700000	-0.18218161	0.7015816	0.4160227
C_{368L}-FTW_{368L}	0.359746667	-0.08213495	0.8016283	0.1500416
C_{560L}-FTW_{368L}	0.369668333	-0.07221328	0.8115499	0.1329497
C_{560L}-C_{368L}	0.009921667	-0.43195995	0.4518033	0.9999254

Appendix 5.14. Mean± Standard deviation of total oxidized N concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	2.33	2.59	2.49	1.85	0.27	0.07	0.31	0.33
	3	1.31	3.15	1.42	2.03	0.25	0.14	0.27	0.37
	7	0.50	0.11	0.79	0.13	0.08	0.02	0.10	0.02
	10	0.26	0.11	0.48	0.09	0.01	0.04	0.03	0.01
	14	0.06	0.07	0.23	0.08	0.02	0.01	0.01	0.00
Batch 2	0	2.07	2.15	2.26	2.22	0.35	0.21	0.61	0.39
	3	1.70	0.94	1.49	0.73	0.12	0.06	0.49	0.18
	7	0.91	0.21	0.91	0.18	0.08	0.06	0.13	0.03
	10	0.25	0.17	0.51	0.15	0.15	0.01	0.13	0.03
	14	0.18	0.02	0.38	0.16	0.01	0.02	0.02	0.04
Batch 3	0	2.25	2.25	2.05	2.73	0.60	0.45	0.07	0.36
	3	1.61	2.18	1.36	1.23	0.22	0.44	0.35	0.27
	7	0.66	2.25	0.70	1.70	0.01	0.26	0.08	1.21
	10	0.39	1.27	0.66	1.39	0.05	0.14	0.06	0.22
	14	0.13	0.69	0.58	1.93	0.02	0.56	0.07	1.63
Batch 4	0	2.63	2.51	2.59	3.06	0.43	0.47	0.15	0.33
	3	1.70	2.78	1.64	2.35	0.08	0.28	0.30	0.14
	7	0.23	1.77	0.59	2.54	0.01	0.53	0.08	0.54
	10	0.24	1.24	0.50	0.83	0.06	0.18	0.15	0.13
	14	0.09	2.01	0.42	1.73	0.01	0.50	0.07	0.09

Appendix 5.15. Mean± Standard deviation of total N concentration in the treatment chambers.

		Average NH_x concentration (mg N L^{-1})				Standard deviation			
Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	7.71	8.05	7.76	7.54	0.40	0.65	0.53	0.28
	3	6.20	7.66	6.30	6.52	0.49	0.44	0.38	0.43
	7	2.70	4.66	4.23	5.20	0.19	0.76	0.17	0.56
	10	2.30	3.93	3.08	4.30	0.11	0.08	0.32	0.32
	14	2.00	3.60	2.86	3.74	0.12	0.04	0.29	0.03
Batch 2	0	8.07	7.78	8.59	8.61	0.42	0.55	0.67	0.92
	3	4.45	6.53	4.87	6.03	0.12	1.83	0.65	1.56
	7	3.40	3.31	5.15	3.40	0.13	0.24	1.80	0.41
	10	3.74	3.16	4.92	3.15	1.42	0.13	2.06	0.01
	14	2.43	3.97	2.88	5.95	0.04	0.37	0.15	0.31
Batch 3	0	7.56	7.72	6.98	8.03	1.09	0.23	0.31	1.65
	3	6.81	9.26	6.72	8.69	0.32	1.01	0.40	0.86
	7	3.76	7.72	3.74	7.38	0.52	0.32	0.10	1.28
	10	2.88	6.14	4.38	6.70	0.29	0.17	1.51	0.35
	14	2.73	3.87	3.01	6.29	0.50	1.30	0.06	2.14
Batch 4	0	8.24	7.78	7.83	9.12	1.12	6.75	0.60	0.76
	3	7.34	8.57	5.02	7.97	2.35	1.96	0.28	1.55
	7	2.66	6.24	4.34	7.10	0.05	0.67	1.02	0.21
	10	2.60	7.59	3.34	5.98	0.66	0.62	0.10	3.11
	14	3.24	6.74	3.92	6.89	0.78	1.26	0.42	0.44

Appendix 5.16. Analysis of variance (ANOVA) for the change in total N concentrations in the treatment chambers. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	194.81	4	48.70	36.03	.05
Treatments	50.80	3	16.93	12.53	.05
Error	97.32	72	1.35		

Appendix 5.17. Post hoc multiple comparison (Turkey's HSD test) of total N concentrat ion (mg N L^{-1}) in the treatment chambers.

		95% Confidence Interval		
Treatments pairs	Mean Difference	Lower Bound	Upper Bound	Sig.
FTW_{368L}-FTW_{560L}	-0.45430	-1.4212365	0.5126298	0.6064266
C_{368L}-FTW_{560L}	1.2180133	0.2510802	2.1849465	0.0077228
C_{560L}-FTW_{560L}	1.4345400	0.4676068	2.4014732	0.0011937
C_{368L}-FTW_{368L}	1.6723167	0.7053835	2.6392498	0.0001237
C_{560L}-FTW_{368L}	1.8888433	0.9219102	2.8557765	0.0000135
C_{560L}-C_{368L}	0.2165267	0.7504065	1.1834598	0.9351119

Kinetics and performance assessment

Appendix 5.18. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), correlation coefficient for first order fits, removal rate (RR, mg m^2 day^{-1}) and removal efficiency (RE, %) of organic-N in the treatment chambers during four experimental batches.

Treatment	Parameters	Experimental batches			
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
FTW_{368L}	$^{*}k_{amo}$ (fitted k_{amo})	0.051 (0.043)	0.025 (0.055)	0.039 (0.035)	0.026 (0.015)
	R^2	0.79	0.51	0.87	0.49
	$T_{1/2}$	13.67	27.51	17.77	26.46
	RR	37.72	59.10	35.58	7.18
	RE	41.52	49.74	32.90	7.70
C_{368L}	k_{amo} (fitted k_{amo})	0.042 (0.063)	0.051 (0.067)	0.073 (0.033)	0.008 (0.008)
	R^2	0.85	0.71	0.79	0.12
	$T_{1/2}$	16.43	13.70	9.56	87.74
	RR	42.92857143	35.2152381	64.09	6.45
	RE	51.12	32.89	78.30	10.00
FTW_{560L}	k_{amo} (fitted k_{amo})	0.026 (0.019)	0.018 (0.023)	0.031 (0.019)	0.011 (0.011)
	R^2	0.78	0.28	0.63	0.12
	$T_{1/2}$	26.36	39.16	22.29	62.45
	RR	37.72	59.10	35.58	7.18
	RE	21.26	44.48	33.95	10.00
C_{560L}	k_{amo} (fitted k_{amo})	0.039 (0.059)	0.023 (0.057)	0.027 (0.004)	0.006 (0.006)
	R^2	0.83	0.27	0.53	0.12
	$T_{1/2}$	17.59	29.62	25.86	119.51
	RR	48.99	0.00	15.48	0.49

$^{*}k_{amo}$: first order mineralisation rate constant; fitted k_{amo} : best-fit mineralisation rate constant which is obtained using root mean squared error (RMSE) between the measured and predicted ON concentrations.

Appendix 5.19. Analysis of variance (ANOVA) for the rate constants of Organic N rem oval in the chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Sq uares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.003148	3	0.0010495	3.033	.05
Treatments	0.002293	3	0.0007645	2.209	.05
Error	0.003115	9	0.0003461		

Appendix 5.20. Analysis of variance (ANOVA) for half-life of Organic N concentration s in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	8546	3	2848.8	6.807	.05
Treatments	1520	3	506.8	1.211	.05
Error	3767	9	418.5		

Appendix 5.21. Analysis of variance (ANOVA) for the removal efficiency of organic N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	4029	3	1343.1	4.069	.05
Treatments	1270	3	423.3	1.282	.05
Error	2971	9	330.1		

Appendix 5.22. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), correlation coefficient for first order fits, removal rate (RR, mg m^2 day^{-1}), and removal efficiency (RE, %) of ammonia-N in the treatment chambers during four experimental batches.

Treatment	**Parameters**	**Experimental batches**			
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
FTW_{368L}	$^{*}k_{nit}$ (fitted k_{nit})	0.266 (0.250)	0.187 (0.291)	0.235 (0.177)	0.225 (0.329)
	R^2	0.99	0.99	0.98	0.99
	$T_{1/2}$	2.61	3.70	2.95	3.08
	RR	60.78	47.95	41.62	63.27
	RE	96.60	91.20	95.68	94.22
C_{368L}	k_{nit} (fitted k_{nit})	0.02 (0.01)	0.03 (0.02)	0.005 (0.005)	0.02(0.03)
	R^2	0.81	0.91	0.32	0.88
	$T_{1/2}$	40.77	26.06	141.46	38.72
	RR	12.07	16.84	5.03	16.91
	RE	16.75	31.41	8.18	27.71
FTW_{560L}	k_{nit} (fitted k_{nit})	0.141 (0.14)	0.156 (0.25)	0.145 (0.12)	0.157 (0.20)
	R^2	0.99	0.99	0.99	0.99
	$T_{1/2}$	4.91	4.45	4.80	4.41
	RR	58.07	58.86	39.32	60.10
	RE	84.26	87.39	85.14	88.34
C_{560L}	k_{nit} (fitted k_{nit})	0.01 (0.001)	0.05 (0.018)	0.01 (0.005)	0.02 (0.040)
	R^2	0.47	0.86	0.46	0.94
	$T_{1/2}$	63.01	14.75	88.87	32.09
	RR	8.96	17.90	11.40	25.42
	RE	13.76	33.77	20.57	38.83

$^{*}k_{nit}$: first order nitrification rate constant; fitted k_{nit} : best-fit nitrification rate constant which is obtained using root mean squared error (RMSE) between the measured and predicted NH_4^+ concentrations.

Appendix 5.23. Analysis of variance (ANOVA) for the rate constants of ammonia N re moval in the chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of S quares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.00024	3	0.00008	0.167	.05
Treatments	0.12776	3	0.04259	89.453	.05
Error	0.00428	9	0.00048		

Appendix 5.24. Analysis of variance (ANOVA) for half-life of ammonia N concentratio ns in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Su m of Squar; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	5200	3	1733	2.349	.05
Treatments	11052	3	3684	4.992	.05
Error	6642	9	738		

Appendix 5.25. Analysis of variance (ANOVA) for the removal efficiency of ammonia N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	417	3	139	2.057	.05
Treatments	18481	3	6160	91.140	.05
Error	608	9	68		

Appendix 5.26. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), correlation coefficient for first order fits, removal rate (RR, mg m^2 day^{-1}), and removal efficiency (%) of nitrite-N in the treatment chambers during four experimental batches.

Treatment	**Parameters**	**Experimental batches**			
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
FTW$_{368L}$	$^{*}k_{nit}$ (fitted k_{nit})	0.412 (0.373)	0.263 (0.191)	0.284 (0.512)	0.484 (0.484)
	R^2	1.00	0.99	0.99	1.00
	$T_{1/2}$	1.68	2.63	2.44	1.43
	RR	28.38	10.40	7.65	39.59
	RE	99.66	97.02	98.44	99.88
C$_{368L}$	k_{nit} (fitted k_{nit})	0.233 (0.235)	0.203 (0.561)	0.097 (0.055)	0.162 (0.160)
	R^2	0.98	0.99	0.75	0.88
	$T_{1/2}$	2.97	3.42	7.15	4.29
	RR	13.90	9.53	5.27	37.94
	RE	95.23	95.72	67.10	85.60
FTW$_{560L}$	k_{nit} (fitted k_{nit})	0.286 (0.301)	0.267 (0.268)	0.189 (0.561)	0.355 (0.867)
	R^2	0.99	0.99	0.99	0.99
	$T_{1/2}$	2.42	2.60	3.68	1.95
	RR	31.12	13.03	7.41	38.32
	RE	98.32	97.19	93.92	99.58
C$_{560L}$	k_{nit} (fitted k_{nit})	0.231 (0.288)	0.198 (0.583)	0.099 (0.099)	0.183 (0.604)
	R^2	0.99	0.99	0.95	0.98
	$T_{1/2}$	3.01	3.49	6.98	3.80
	RR	13.78	10.23	7.31	37.60
	RE	94.54	94.59	71.37	94.78

$^{*}k_{nit}$: first order nitrification rate constant; fitted k_{nit} : best-fit nitrification rate constant which is obtained using root mean squared error (RMSE) between the measured and predicted NO_2^- concentrations.

Appendix 5.27. Analysis of variance (ANOVA) for the rate constants of nitrite N remov al in the chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squar es; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.04335	3	0.01445	5.498	.05
Treatments	0.09548	3	0.03183	12.109	.05
Error	0.02365	9	0.00263		

Appendix 5.28. Analysis of variance (ANOVA) for half-life of nitrite N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	15.771	3	5.257	6.648	.05
Treatments	17.340	3	5.780	7.309	.05
Error	7.117	9	0.791		

Appendix 5.29. Analysis of variance (ANOVA) for the removal efficiency of nitite N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	538.7	3	179.56	3.782	.05
Treatments	473.5	3	157.85	3.325	.05
Error	427.3	9	47.48		

Appendix 5.30. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), correlation coeff icient for first order fits, removal rate (RR, mg m^2 day^{-1}), and removal efficiency (%) of nitrate-N in the treatments chambers during four experimental batches.

Treatment	Parameters	Experimental batches			
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
FTW_{368L}	$^{*}k_{denit}$ (fitted k_{denit})	0.218 (0.153)	0.185 (0.128)	0.199 (0.177)	0.206 (0.129)
	R^2	0.99	0.96	0.98	0.86
	$T_{1/2}$	3.18	3.75	3.48	3.37
	RR	36.33	43.74	52.91	32.85
	RE	95.47	90.18	93.68	92.65
C_{368L}	k_{denit} (fitted k_{denit})	0.328 (0.149)	0.192 (0.263)	0.082 (0.005)	0.028 (0.005)
	R^2	0.82	0.99	0.32	0.15
	$T_{1/2}$	2.11	3.61	8.44	24.93
	RR	58.06	47.13	39.31	6.58
	RE	97.88	91.49	69.69	20.13
FTW_{560L}	k_{denit} (fitted k_{denit})	0.134 (0.122)	0.117 (0.116)	0.086 (0.123)	0.097 (0.075)
	R^2	0.99	0.99	0.98	0.85
	$T_{1/2}$	5.17	5.92	8.11	7.16
	RR	33.37	40.60	34.58	23.49
	RE	84.7	79.4	68.2	66.4
C_{560L}	k_{denit} (fitted k_{denit})	0.283 (0.145)	0.192 (0.330)	0.018 (0.005)	0.029 (0.006)
	R^2	0.82	0.99	0.46	0.35
	$T_{1/2}$	2.45	3.61	38.09	23.58
	RR	36.72	48.52	15.47	0.25
	RE	96.3	92.3	22.90	0.50

$^{*}k_{denit}$: first order denitrification rate constant; fitted k_{denit} : best-fit denitrification rate constant which is obtained using root mean squared error (RMSE) between the measured and predicted NO_3^- concentrations.

Appendix 5.31. Analysis of variance (ANOVA) for the rate constants of nitrate N remo-val in the chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squa res; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.06073	3	0.020242	4.157	.05
Treatments	0.01944	3	0.006479	1.331	.05
Error	0.04382	9	0.004869		

Appendix 5.32. Analysis of variance (ANOVA) for half-life of nitrate N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum o f Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	478.9	3	159.62	1.959	.05
Treatments	400.1	3	133.35	1.636	.05
Error	733.4	9	81.49		

Appendix 5.33. Analysis of variance (ANOVA) for the removal efficiency of nitrate N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	7333	3	2444	3.654	.05
Treatments	3439	3	1146	1.714	.05
Error	6021	9	669		

Appendix 5.34. Removal rate constants (k, day^{-1}), half-life ($T_{1/2}$, day), correlation coefficient for first order fits, removal rate (RR, mg m^2 day^{-1}), and removal efficiency (%) of total-N in the treatment chambers during four experimental batches.

Treatment	**Parameters**	**Experimental batches**			
		Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
FTW_{368L}	$^{*}k$ (fitted k)	0.106 (0.115)	0.073 (0.104)	0.083 (0.083)	0.083 (0.099)
	R^2	0.98	0.93	0.97	0.91
	$T_{1/2}$	6.56	9.44	8.38	8.34
	RR	163.2	161.2	137.8	142.9
	RE	74.09	69.90	63.81	60.69
C_{368L}	k (fitted k)	0.065 (0.063)	0.059 (0.077)	0.063 (0.026)	0.012 (0.009)
	R^2	0.96	0.89	0.83	0.57
	$T_{1/2}$	10.65	11.79	10.97	55.90
	RR	127.0	108.7	110.1	29.7
	RE	55.21	48.94	58.51	13.36
FTW_{560L}	k (fitted k)	0.077 (0.081)	0.062 (0.077)	0.061 (0.058)	0.050 (0.075)
	R^2	0.99	0.88	0.93	0.91
	$T_{1/2}$	8.98	11.22	11.31	13.84
	RR	139.9	163.0	113.5	111.9
	RE	63.10	66.40	56.90	50.0
C_{560L}	k (fitted k)	0.052 (0.052)	0.038 (0.073)	0.022 (0.015)	0.024 (0.030)
	R^2	0.99	0.67	0.90	0.87
	$T_{1/2}$	13.38	18.10	32.09	29.25
	RR	108.45	76.05	49.67	63.76
	RE	50.37	30.90	21.64	24.48

$^{*}k$: first order reaction rate constant; fitted k : best-fit rate constant which is obtained using root mean squared error (RMSE) between the measured and predicted TN concentrations.

Appendix 5.35. Analysis of variance (ANOVA) for the rate constants of total N remova l in the chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Square s; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.002134	3	0.0007114	4.956	.05
Treatments	0.005868	3	0.0019560	13.627	.05
Error	0.001292	9	0.0001435		

Appendix 5.36. Analysis of variance (ANOVA) for half-life of total N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	663.0	3	221.0	1.817	.05
Treatments	698.2	3	232.7	1.913	.05
Error	1094.7	9	121.6		

Appendix 5.37. Analysis of variance (ANOVA) for the removal efficiency of total N concentrations in the treatment chambers throughout the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	1181.9	3	394.0	4.00	.05
Treatments	2961.8	3	987.3	10.02	.05
Error	886.5	9	98.5		

Biomass and volatilization

Appendix 5.38. Biomass (g dry weight m^{-2}) and growth rate (g dry weight m^{-2} day^{-1}) for *Juncus effusus* and *Phragmites australis* in the FTW cells during the study.

	Initial biomass (g dry weight m^{-2})				**Final biomass (g dry weight m^{-2})**				**Growth rate (g dry weight m^{-2} day^{-1})**	
	J. effuses		***P. australis***		***J. effuses***		***P. australis***		***J. effuses***	***P. australis***
	Root	***Shoot***	***Root***	***Shoot***	***Root***	***Shoot***	***Root***	***Shoot***		
FTW_{368L}	10.22	57.39	32.83	10.00	165.7	189.8	59.35	32.17	1.28	0.18
	10.22	52.83	82.61	11.09	65.0	142.4	46.09	20.00	1.19	0.53
	12.61	42.17	22.61	7.83	92.4	147.8	69.78	42.39	1.77	1.74
	11.52	51.96	31.96	13.48						
	7.17	44.78	18.70	3.91						
	6.52	52.17	21.74	5.65						
	12.83	44.35	6.74	6.09						
	6.30	37.83	25.43	13.04						
	17.83	56.96	9.78	9.13						
	29.78	51.30	49.35	7.39						
FTW_{560L}	6.71	37.71	21.57	6.57	44.71	71.43	24.86	13.57	5.14	0.87
	6.71	34.71	54.29	7.29	36.29	71.86	64.43	26.86	2.58	0.37
	8.29	27.71	14.86	5.14	36.71	98.43	64.00	53.57	3.31	1.46
	7.57	34.14	21.00	8.86						
	4.71	29.43	12.29	2.57						
	4.29	34.29	14.29	3.71						
	8.43	29.14	4.43	4.00						
	4.14	24.86	16.71	8.57						
	11.71	37.43	6.43	6.00						
	19.57	33.71	32.43	4.86						

Appendix 5.39. Analysis of variance (ANOVA) for plant biomass in the vegetated treatments at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	116775	1	116775	31.638	.05
Treatments	25120	1	25120	6.806	.05
Error	33219	9	3691		

Appendix 5.40. Analysis of variance (ANOVA) for plant growth in the treatment chambers during the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	7.39	1	7.390	3.466	.05
Error	8.53	4	2.133		

Appendix 5.41. Microbial biomass in the wetland cells during the study.

Initial microbial biomass (CFU/g or mL)		**Final microbial biomass (CFU/g or mL)**			
		FTW368L	C368L	FTW560L	C560L
Free-float (CFU/mL)	24×10^2	3×10^2	2×10^2	9×10^2	10^3
	12×10^2	2×10^2	12×10^2	4×10^2	7×10^2
	12×10^2	12×10^2	12×10^2	2×10^2	14×10^3
Epimatrix[*] (CFU/g)	2	32×10^5		10^6	
	3	14×10^5		8×10^5	
	2	32×10^5		10^6	
Epiphytic-*Juncus effusus* (CFU/g)	6×10^4	22×10^5		68×10^6	
	4×10^4	2×10^6		78×10^5	
	4×10^4	42×10^5		2×10^7	
Epiphytic-*Phragmites australis* (CFU/g)	10^2	22×10^5		52×10^5	
	140	3×10^6		58×10^5	
	70	24×10^5		5×10^6	

[*] Microbial population developed onto mat material.

Appendix 5.42. Analysis of variance (ANOVA) for microbial biomass in the treatment chambers during the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	1 7.386e+13	1	7.386e+13	2.101	.05
Error	4 1.406e+14	4	3.515e+13		

Appendix 5.43. Total N content (mg N m^2) and uptake rate (mg N m^2 day^{-1}) for *Juncus effusus* and *Phragmites australis* in the FTW cells during the study.

	Initial N content (mg N)				**Final N content (mg N)**				**Uptake rate (mg N m^{-2} day^{-1})**	
	J. effuses		***P. australis***		***J. effuses***		***P. australis***		***J. effuses***	***P. australis***
	Root	***Shoot***	***Root***	***Shoot***	***Root***	***Shoot***	***Root***	***Shoot***		
FTW$_{368L}$	64.64	857.4	21.64	113.1	805.6	1466	668.2	384.6	24.10	12.88
	114.4	508.0	462.3	168.7	287.4	1011	386.6	235.3	12.08	5.36
	94.63	586.8	139.5	98.68	473.4	1200	663.1	679.9	17.72	19.73
	68.76	349.3	207.8	147.1						
	62.10	288.9	164.6	47.96						
	30.46	677.4	162.7	76.98						
FTW$_{560L}$	42.48	563.4	143.6	74.33	205.3	492.6	237.7	196.8	1.64	3.87
	75.23	333.8	303.8	110.9	171.0	574.4	661.2	325.6	6.01	10.22
	62.19	385.6	91.70	64.85	161.8	741.1	778.2	873.8	8.13	26.70
	45.18	229.5	136.5	96.69						
	40.81	189.8	108.2	31.52						
	20.02	445.1	106.9	50.59						

Appendix 5.44. Analysis of variance (ANOVA) for total N content in plant tissue the treatment chambers during the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Treatments	147.9	1	147.9	0.682	.05
Error	866.9	4	216.7		

Appendix 5.45. Volatilization in the treatments during experimental time.

Batches	**FTW$_{368L}$**		**C$_{368L}$**		**FTW$_{560L}$**		**C$_{560L}$**	
	$^{*}f_{FREE}$	$^{**}vol$	f_{FREE}	*vol*	f_{FREE}	*vol*	f_{FREE}	*vol*
Batch 1	0.0133	0.0005	0.0150	0.0005	0.0133	0.0005	0.0153	0.0006
Batch 2	0.0133	0.0005	0.0175	0.0006	0.0130	0.0005	0.0165	0.0006
Batch 3	0.0123	0.0004	0.0156	0.0006	0.0120	0.0004	0.0162	0.0006
Batch 4	0.0130	0.0005	0.0156	0.0006	0.0125	0.0005	0.0165	0.0006

$^{*}f_{Free}$: ration of NH_3:NH_4^+, $^{**}vol$: volatilization rate (day^{-1}).

Physicochemical parameters

Appendix 5.46. pH in the treatments during the four experimental batches.

Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	7.4	7.5	7.4	7.5
	3	7.2	7.2	7.2	7.4
	7	7.2	7.3	7.2	7.4
	10	7.2	7.3	7.2	7.3
	14	7.2	7.5	7.2	7.3
Batch 2	0	7.3	7.5	7.4	7.5
	3	7.2	7.6	7.2	7.5
	7	7.3	7.6	7.2	7.5
	10	7.2	7.4	7.1	7.4
	14	7.2	7.5	7.2	7.4
Batch 3	0	7.4	7.4	7.3	7.4
	3	7.2	7.4	7.2	7.6
	7	7	7.3	7	7.4
	10	7.1	7.4	7.1	7.5
	14	7.1	7.5	7.1	7.3
Batch 4	0	7.4	7.4	7.4	7.4
	3	7.1	7.3	7.1	7.5
	7	7.2	7.5	7.1	7.5
	10	7.1	7.5	7.1	7.5
	14	7.3	7.3	7.2	7.4

Appendix 5.47. Analysis of variance (ANOVA) for pH in the treatment chambers at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.01207	3	0.00402	2.153	.05
Treatments	0.19928	3	0.06643	35.532	.05
Error	0.01683	9	0.00187		

Appendix 5.48. Dissolved oxygen concentration (mg L^{-1}) in the treatments during the four experimental batches.

Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	1.19	1.66	0.93	1.1
	3	1	1.33	0.5	1.49
	7	1.48	1.05	0.97	0.78
	10	2.81	0.7	1.01	0.69
	14	2.86	1.01	1.05	0.92
Batch 2	0	2.02	2.38	2.39	2.52
	3	1.8	1.12	0.84	1.06
	7	2.6	1.01	1.21	0.84
	10	3	0.62	1.44	0.8
	14	2.3	1.08	1.13	0.88
Batch 3	0	0.91	1.05	0.78	0.87
	3	1.06	1.48	0.65	1.77
	7	1.09	1.08	0.8	1
	10	2.35	1.04	1.5	1.13
	14	1.85	1.02	1.7	1.05
Batch 4	0	0.63	0.9	0.67	0.78
	3	0.74	0.84	0.6	0.76
	7	1.18	1.24	0.8	1.35
	10	0.8	1.22	0.67	1.37
	14	1.04	1.81	1.81	2.03

Appendix 5.49. Analysis of variance (ANOVA) for DO in the treatment chambers at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.5127	3	0.17089	1.776	.05
Treatments	0.7693	3	0.25642	2.665	.05
Error	0.8660	9	0.09622		

Appendix 5.50. Electrical conductivity (mS cm^{-1}) in the treatments during the four experimental batches.

Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	1.33	1.32	1.33	1.25
	3	1.22	1.21	1.21	1.28
	7	1.2	1.18	1.19	1.26
	10	1.2	1.17	1.2	1.26
	14	1.19	1.16	1.18	1.25
Batch 2	0	1.35	1.24	1.31	1.22
	3	1.26	1.18	0.86	1.3
	7	1.26	1.24	1.25	1.3
	10	1.25	1.25	1.24	1.29
	14	1.26	1.22	1.24	1.28
Batch 3	0	1.29	1.29	1.28	1.29
	3	1.25	1.23	1.25	1.35
	7	1.12	1.09	1.11	1.3
	10	1.14	1.09	1.11	1.35
	14	1.2	1.17	1.17	1.44
Batch 4	0	1.3	1.28	1.31	0.78
	3	1.32	1.3	1.3	1.33
	7	1.21	1.2	1.2	1.46
	10	1.21	1.19	1.2	1.31
	14	1.21	1.15	1.19	1.29

Appendix 5.51. Analysis of variance (ANOVA) for EC in the treatment chambers at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	0.002805	3	0.000935	0.887	.05
Treatments	0.024199	3	0.008066	7.654	.05
Error	0.009484	9	0.001054		

Appendix 5.52. Water temperature (°C) in the treatments during the four experimental batches.

Batches	Time (day)	FTW_{368L}	C_{368L}	FTW_{560L}	C_{560L}
Batch 1	0	20.4	20.7	20.5	19.6
	3	18.3	17.7	18.2	19.3
	7	17.5	16.7	17.3	17
	10	17.9	17.7	17.6	17.7
	14	18.4	18.1	18.2	18.1
Batch 2	0	19.5	19.2	19.7	19.5
	3	16.7	16.1	17	16.1
	7	19.9	16.5	16.8	16.2
	10	16.7	16.8	16.7	16.5
	14	17.2	17.3	17.1	17.4
Batch 3	0	19.9	20	19.9	19.9
	3	18.6	18.2	18.4	19.9
	7	17.2	16.5	17.2	16.9
	10	17.6	17.6	17.6	17.9
	14	16.6	15.5	16.5	16.3
Batch 4	0	18.6	18.4	18.6	18.9
	3	16.2	15.6	16.5	18.2
	7	15.5	14.4	15.4	14.9
	10	15.7	15.4	15.7	15.8
	14	13	13	13.1	13.7

Appendix 5.53. Analysis of variance (ANOVA) for temperature in the treatment chambers at the end of the study. Df: Degree of Freedom; Sum Sq: Sum of Squares; Mean Sq: Mean Square; Sig: significance level.

Source	Type III Sum of Square	df	Mean Square	F	Sig.
Time	14.475	3	4.825	73.816	.05
Treatments	0.579	3	0.193	2.951	.05
Error	0.588	9	0.065		