universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Impact of clogging on treatment efficacy and greenhouse gas emissions in treatment wetland“

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science, (MSc)

Wien, 2018 / Vienna 2018

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 299
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Interdisziplinäres Masterstudium Environmental Sciences
Betreut von / Supervisor:	Univ.-Prof. Dipl.-Geogr. Dr. sc. agr. Stephan Glatzel

Abstract

The Schottenhof treatment wetland (TW) system in Vienna, Austria, has been treating the wastewater of 115 Population Equivalents for over 20 years. The nutrient removal efficacy and greenhouse gas emissions were measured for two side-by-side TWs from the system, one clogged and one not significantly affected by clogging (CTW and UTW, respectively), from April to June 2017. Both TWs experienced high total carbon (NPOC) removal efficacies, averaging $79.0 \pm 3.84\%$ and $86.1 \pm 1.9\%$ for the CTW and UTW, respectively, which were significantly influenced by influent NPOC concentrations. Total nitrogen (TN) removal efficacy was low in both TWs, averaging $24.1\% \pm 3.5$ and $34.4\% \pm 9.4$ for the CTW and UTW, respectively. No significant differences in NPOC or TN removal efficacy was observed between the TWs. It is thought that various processes within the CTW compensated to achieve a nutrient removal efficacy similar to that of the UTW, namely increased anaerobic degradation of carbon and enhanced processes within the free water zone, such as sedimentation, nutrient assimilation by microorganisms, and ammonium volatilisation. A combination of the static and floating chamber method was used to measure fluxes of methane (CH_4) and nitrous oxide (N_2O). Emission rates of CH_4 and N_2O varied significantly on both a spatial and temporal scale. For the CTW and UTW, respectively, CH_4 emission rates ranged from 0.79 to 4.46 $\mathrm{mg\,m^{-2}\,h^{-1}}$ and 0.03 to 1.13 $\mathrm{mg\,m^{-2}\,h^{-1}}$, and N_2O emission rates ranged from 0.04 to 29.24 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$ and 0.59 to 9.87 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$. The CTW produced significantly greater emissions of CH_4 (average 2.4 ± 0.55 vs 0.43 ± 0.17 $\mathrm{mg\,m^{-2}\,h^{-1}}$), averaging 5.4 times greater, and emissions of N_2O (average 9.40 ± 3.88 vs 4.42 ± 1.10 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$), averaging 2.1 time greater. CH_4 emission rates were highest in the inlet section of both TWs and were significantly positively influenced by the amount of influent NPOC and TN and by pH. Overall, CH_4 emissions were most significantly influenced by water level, as overland flow of wastewater created anaerobic conditions that favoured CH_4 production. N_2O emission rates were most significantly influenced by water level and the presence of accumulated clog matter on the media surface as a result of clogging. In saturated, anaerobic conditions, complete denitrification was favoured and N_2O emission rates were low. Once drying of clogged sections occurred and the water level receded, the semi-saturated conditions of the clog matter became less anaerobic and favoured incomplete denitrification processes, resulting in high N_2O emission rates. As drying of the clog matter progressed and conditions became aerobic, denitrification processes were inhibited and N_2O emission rates were low. Overall, the emission rates of CH_4 were 3 to 4 magnitudes higher than N_2O emission rates and contributed significantly more to the global warming potential (GWP) (50.41 ± 12.16 and 9.10 ± 2.99 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$, respectively). However, as N_2O emissions are more problematic long-term, strategies to mitigate N_2O emissions should take precedence and are outlined in this study. Despite the relatively high contribution of the TWs to GWP - especially the CTW - when compared with conventional wastewater treatment plants, the contribution is 3 to 4 magnitudes less and has less overall negative impact on the environment.

Keywords: horizontal subsurface flow treatment wetland, clogging, clog matter, nutrient removal efficacy, greenhouse gas emissions

Abstrakt

Das Schottenhof pflanzenkläranlage (TW) in Wien, Österreich, reinigt seit mehr als 20 Jahren das Abwasser von 115 Einwohnergleichwerten. Die Nährstoffentfernungswirksamkeit und die Treibhausgasemissionen wurden von April bis Juni 2017 für zwei nebeneinander liegende TWs aus dem System gemessen, eines verstopft und eines nicht verstopft (CTW bzw. UTW). Beide TW wiesen hohe Entfernungseffizienzen des Gesamtkohlenstoffgehalts (NPOC) auf, durchschnittlich 79.0 $\pm$ 3.84% und 86.1 $\pm$ 1.9 % für die CTW bzw. UTW, die signifikant durch einströmende NPOC Konzentrationen beeinflusst wurden. Die Gesamtwirksamkeit des Entfernens von Stickstoff (TN) war in beiden TWs niedrig und betrug im Durchschnitt 24.1% $\pm$ 3.5 und 34.4% $\pm$ 9.4 für die CTW bzw. UTW. Zwischen den TWs wurde kein signifikanter Unterschied in der Wirksamkeit der NPOC oder TN Entfernung beobachtet. Es wird angenommen, dass verschiedene Prozesse innerhalb des CTW kompensiert werden, um eine Nährstoffentfernungswirksamkeit ähnlich der des UTW zu erreichen, nämlich verstärkter anaerober Abbau von Kohlenstoff und verstärkter Prozesse innerhalb der freien Wasserzone, wie Sedimentation, Nährstoffassimilation durch Mikroorganismen und Ammoniumverflüchtigung. Eine Kombination der statischen und der Floating-Chamber-Methode wird verwendet, um die Flüsse von Methan (CH_4) und Lachgas (N_2O) zu messen. Die Emissionsraten von CH_4 und N_2O unterscheiden sich räumlich und zeitlich signifikant. Für CTW bzw. UTW, betrugen die Emissionsraten von CH_4 zwischen 0,79 und 4,46 $\mathrm{mg\,m^{-2}\,h^{-1}}$ und zwischen 0,03 und 1,13 $\mathrm{mg\,m^{-2}\,h^{-1}}$, und die N_2O Emissionsraten lagen im Bereich von 0,04 bis 29,24 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$ und 0,59 bis 9,87 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$. Die CTW hat signifikant höhere Emissionen von CH_4 (durchschnittlich 2,4 $\pm$ 0,55 vs 0,43 $\pm$ 0,17 $\mathrm{mg\,m^{-2}\,h^{-1}}$), durchschnittlich produziert 5,4 Mal größer, und Emissionen von N_2O (durchschnittlich 9,40 $\pm$ 3,88 vs 4,42 $\pm$ 1,10 $\mu\mathrm{g\,m^{-2}\,h^{-1}}$), durchschnittlich 2,1 mal größer. Die Emissionsraten von CH_4 waren im Einlassbereich beider TW am höchsten und wurden signifikant durch die Menge an zufließendem NPOC und TN und durch den pH beeinflusst. Insgesamt wurden die CH_4 Emissionen vor allem durch den Wasserstand beeinflusst, da der Überlauf von Abwasser anaerobe Bedingungen erzeugte, die die Produktion von CH_4 förderten. Die Emissionsraten von N_2O wurden am stärksten durch den Wasserstand und das Vorhandensein von angesammeltem verstopften Material auf der Medienoberfläche infolge von Verstopfung beeinflusst. In gesättigten, anaeroben Bedingungen wurde die vollständige Denitrifikation begünstigt und die Emissionsraten waren niedrig. Sobald die verstopften Abschnitte getrocknet waren und der Wasserspiegel zurückging, wurden die halbgesättigten Bedingungen des verstopften Materials weniger anaerob und begünstigten unvollständige Denitrifikationsvorgänge, was zu hohen Emissionsraten führte. Als das Trocknen der verstopften Materie voranschritt und die Bedingungen aerob wurden, wurden die Denitrifikationsvorgänge inhibiert und die Emissionsraten waren niedrig. Insgesamt waren die Emissionsraten von CH_4 um 3 bis 4 Größenordnungen höher als die von N_2O Emissionsraten und signifikant mehr vom Treibhauspotential (GWP) (50,41 $\pm$ 12,16 und 9,10 $\pm$ 2,99 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$. Da N_2O Emissionen langfristig jedoch problematischer sind, sollten Strategien zur Minderung der N_2O Emissionen Vorrang haben und werden in dieser Studie hervorgehoben. Trotz des relativ hohen Beitrags der TW zum GWP - insbesondere des CTW - im Vergleich zu konventionellen Kläranlagen ist der Beitrag um 3 bis 4 Größenordnungen geringer und hat insgesamt weniger negative Auswirkungen auf die Umwelt.

Schlüsselwörter: horizontale Pflanzenkläranlage, Verstopfung, Verstopfungsmaterial, Nährstoffentfernungswirksamkeit, Treibhausgasemissionen

Acknowledgements

From the Department of Geography and Regional Research at University of Vienna, I would firstly like to thank my supervisor, Univ.-Prof. Stephan Glatzel, for giving me the opportunity to pursue a project in line with my interests and passion. I would also like to thank him for providing me with the guidance and support - and the space and time - needed to meet the challenges of this project. I am also thankful to Ass.-Prof. Robert Peticzka and Dipl.-Ing. Andreas Maier for their guidance and assistance with laboratory analyses and Dr. Marco Miani for his help organising field equipment. Lastly, this project would not have been possible without the cooperation of the Municipal Department (MA) 45 – Water Management of the City Government of Vienna, and especially the support of Ing. Raimund Herndl.

Finally, thanks and love to my family and significant other for supporting me always as I explore my intellectual and personal curiosities, even from 14,369 km away, and also to my dear friends, both near and far.

All laboratory analyses for this project were undertaken in the Chemical-Physical Laboratory of the Geography Institute in UZA II at Althanstraße 14, 1090, Vienna.

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CHAPTER 1

Introduction

1.1 Overview and objectives

Treatment wetlands (TWs) are a nature-based technology for water quality improvement. They are engineered systems, designed and constructed to utilise the natural functions of wetland vegetation, soils, and their microbial populations to treat pollutants and contaminants in surface water, groundwater or waste streams (4). In the intricate plant-media-microorganism nexus, a complex combination of physical, chemical, and biological processes work together to transform, degrade, or remove pollutants from wastewater.

Subsurface flow (SSF) TWs are used in Austria and worldwide to treat secondary wastewater, particularly in rural and remote communities (5, 6). This is due to their mechanical simplicity and low operation and maintenance requirements and costs in comparison to conventional wastewater treatment technologies (5). However, despite the many advantages of SSF TWs, they have been identified as net sources of greenhouse gases (GHGs) and are particularly prone to clogging - an inevitable, major, and widespread problem, particularly in HSSF TWs - which negatively impacts on their wastewater treatment efficacy and enhances GHG emissions (7, 8). As TW development and use is increasing globally, it is becoming increasingly important to understand the impacts of clogging on TW systems.

The aim of this study was to elucidate the impact of clogging on the nutrient removal efficacy of two well-established TWs treating secondary wastewater from a small community, and to understand how clogging affects GHG emissions. The specific objectives were to:

- Quantify, examine, and compare the total organic carbon and total nitrogen removal efficacy and methane and nitrous oxide emissions of the clogged and unclogged SSF TWs;
- Understand how clogging impacts nutrient removal and transformation mechanisms within the TWs; and
- Identify strategies that can mitigate the effects of clogging.

As the clogged and unclogged TWs are parallel to one another, this study site provided an excellent opportunity to conduct a side-by-side study as the influent wastewater chemistry was similar and climatological conditions identical. This research will assist TW operators in understanding how clogging impacts on treatment processes and to determine whether a clogged system requires remediation, as remediation of TW media can be costly or difficult for small communities and local governments. Strategies to mitigate the impacts of clogging will also be highlighted.

1.2 Classification and description of treatment wetlands

There are two basic types of TWs: surface flow and subsurface flow. Surface flow TWs are similar to natural wetlands, whereby shallow (<60 cm deep) wastewater flows over saturated media, and are the least common type of TW employed (2). SSF TWs consist of a bed of porous media (filter bed) sealed by an impermeable layer and planted with emergent macrophytes that establish and form an extensive and intricate network of roots within the media (5, 9). The media and plant roots provide a large surface area by which microorganisms can establish, creating a highly biologically active biofilm.

Before being fed into the TW, wastewater is pre-treated in settling and sedimentation tanks (9). The inflowing secondary (pre-treated) wastewater enters at the inlet and flows through the TW - where it is acted on and treated - until it reaches the outlet, where it is collected and discharged (9). Wastewater flow through the SSF TWs can be either horizontal (HSSF) or vertical (VF). The media of HSSF TWs (Fig. 1.1) is permanently saturated, whereas the media of VF TWs cycles through different degrees of saturation due to intermittent dosing of wastewater through the system (5). A combination of HSSF and VF TWs, known as hybrid TWs, can also be employed to treat wastewater. This research project focuses on HSSF TWs, which make up part of a hybrid TW system.

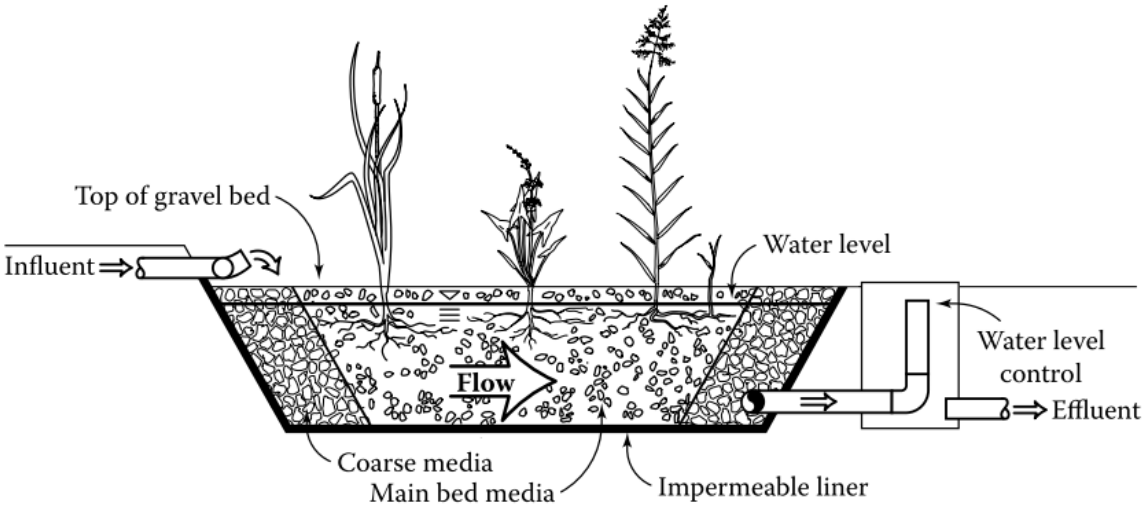

Fig. 1.1: Schematic of horizontal subsurface flow treatment wetland (figure from *Kadlec and Wallace*, 2008)

1.3 Plant-media-microorganism nexus in treatment wetlands

As previously mentioned, TWs utilise the natural functions of wetlands to treat pollutants - in this case excess nutrients - from wastewater through a complex nexus of plants, media, and microorganisms (Fig. 1.2). This section will explore each component of the nexus in detail.

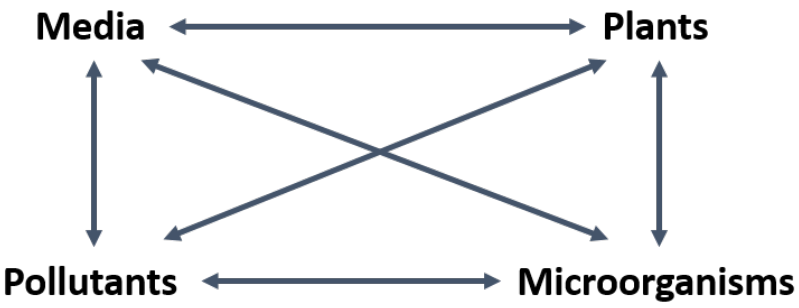

Fig. 1.2: Simplified illustratoin of pollutant interaction with the complex plant-media-microorganism nexus

1.3.1 Plants

Macrophytes are an essential component of the design of a TW; the physical effects of their presence play an important role in the wastewater treatment process (10–12). In SSF TWs, the most important functions of macrophytes are: the provision of surface area (roots and rhizomes) for the attachment and growth of microorganisms and their associated biofilms; radial oxygen loss (oxygen diffusion from the roots to the adjacent media, known as the rhizosphere) and its influence on redox potential; the production of exudates, mainly organic compounds, that can be used as a carbon source for denitrifiers in the rhizosphere; the uptake of nutrients and storage in aboveground biomass; and insulation of the filter beds during winter (if not harvested) (11–13). Macrophytes also have site-specific values by providing habitat for wildlife and enhancing the aesthetics of TW systems (11).

Macrophytes contribute to the removal of nutrients from wastewater by taking up dissolved organic matter, ammonium, and nitrate through their root systems and storing the nutrients as aboveground biomass (11). As macrophytes are very productive, considerable amounts of nutrients can be bound in the biomass and removed from the system, however this is effective only when plants are harvested regularly (9, 11). The presence of macrophytes and their influence on redox conditions also enhances the performance of various nutrient removal mechanisms; the supply of oxygen to the rhizosphere increases aerobic degradation of organic matter and is essential for nitrification and hence nitrogen removal (Fig. 1.3) (11, 14). Studies have also shown that microbial density, activity, and diversity are enhanced in the rhizosphere regions of SSF TWs (15).

The desirable features of a plant used in TWs designed for the treatment of wastewater should therefore: be tolerant of high organic and nutrient loads; have rich belowground organs (i.e. roots and rhizomes) that provide a large surface area for bacterial attachment and growth and oxygenation to the rhizosphere; and have high aboveground biomass for winter insulation in cold and temperate regions and for nutrient storage and removal (via harvesting) (13). The most frequently used plant around the globe is *Phragmites australis* (Common reed) (13).

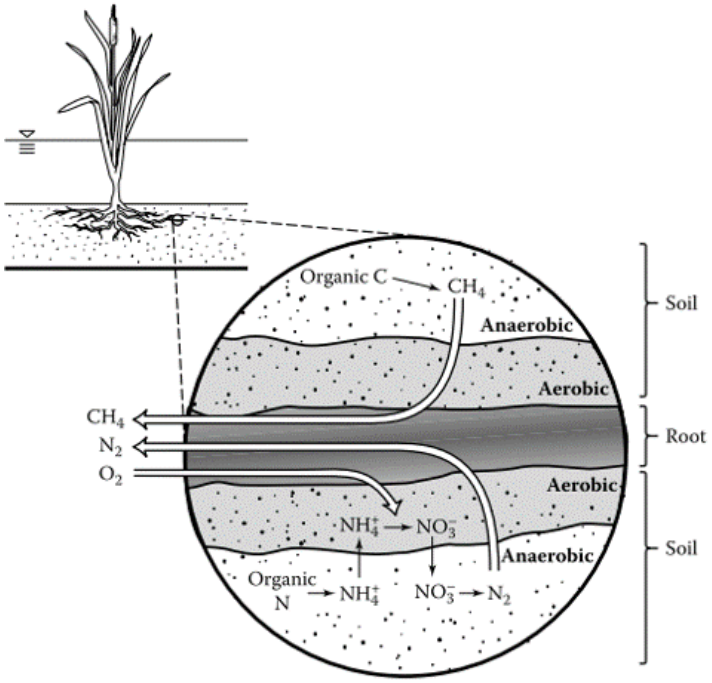

Fig. 1.3: Oxic and anoxic zones and aerobic and anaerobic pathways of nutrient transformations in the rhizosphere (figure from *Kadlec and Wallace*, 2008)

1.3.2 Media

In conventional SSF TWs - that is, TWs that are designed to only treat carbon (C) and nitrogen (N) - the primary role of the filter media is to provide hydraulic conductivity and a surface for adsorption of nutrients and biofilm development (16). It is essential that the hydraulic conductivity is maintained as it governs the hydraulic retention time (HRT), a significant parameter that determines the nutrient removal efficiency of TWs (16, 17). Longer HRTs increase the duration of wastewater contact with the TW and thus increase the time available for transformation, degradation, and removal of nutrients. The main parameter influencing the media hydraulics is the grain-size distribution; systems with fine- and soil-based media have low hydraulic conductivity, and systems with coarse sand- and gravel- based media have higher hydraulic conductivity (18). Long-term studies of TW hydraulics indicate that a mixture of sand and gravel produces the most desirable results in terms of both hydraulic conditions and wastewater treatment (18).

1.3.3 Microorganisms

In SSF TWs, the transformation and mineralisation of nutrients and organic pollutants is played not by plants, but rather by microorganisms (18). Bacteria, fungi, and algae are the common microorganisms found in TWs and form the stable microbiota that comprise biofilm, which is associated with plant roots and/or attached to the surface of the media (2, 19). Bacteria are considered to play the most significant role in the treatment of wastewater (2).

Bacteria are unicellular prokaryotic organisms that can be classified according to their metabolic requirements (1). Heterotrophic bacteria utilise organic C as a source of energy whereas autotrophic bacteria are able to synthesise their own energy from inorganic substances such as carbon dioxide (CO_2). Bacteria that derive their energy from chemical reactions are known as chemotrophs, and can either be

heterotrophic (chemoheterotrophic; most bacteria) or autotrophic (chemoautotrophic; eg. *Nitrosonomas* and *Nitrobacter*) (2).

Bacterial respiration can occur in the presence or absence of oxygen (oxic and anoxic conditions, respectively; aerobic and anaerobic respiration, respectively). In aerobic respiration, oxygen (O_2) is used as the terminal electron acceptor and carbohydrates are transformed to CO_2, water, and energy (2). In anaerobic respiration, inorganic molecules (other than O_2) are used as the final electron acceptor, including iron, sulphate, nitrate, and carbonate (1, 2). These microbially-mediated processes are affected by temperature and subsequently diurnal and annual cycles (20). These processes respond greatest to the lower end of the temperature scale (<15 °C) where they slow down (20).

1.4 Wastewater treatment mechanisms in treatment wetlands

Within the plant-media-microorganism nexus, there are a combination of physical, chemical, and biological processes that work together to treat wastewater - that is, to transform, degrade, and remove nutrients and pollutants. A network of anoxic and oxic zones - restricted to the rhizosphere - provide various strong redox gradients. These gradients enable the formation of many ecological niches that promote a multitude of microbial processes which transform and remove organics and nutrients, primarily in the rhizosphere, known as the active reaction zone of the TW (9, 15, 18). The various mechanisms of wastewater treatment, with regards to C and N, will be discussed in this section in detail.

1.4.1 Suspended solids

Influent suspended solids are mainly retained mechanically by sedimentation, aggregation, and filtration through the media and plant roots (1, 18, 21). Sedimentation of large particles is the major mechanism of solids removal in HSSF TWs and predominantly takes part in the first few metres (the inlet) of the TW (1).

1.4.2 Organic compounds

Organic compounds are fixed or adsorbed by biofilm and undergo mineralisation (18, 21). The mineralisation of organic compounds in TWs depends on the redox chemistry of the media, the bioavailability of organic C and N, and temperature (2, 17).

Anaerobic degradation

Organic compounds are primarily removed from HSSF TWs via anaerobic degradation inside the media pores, as dissolved oxygen is limited in the filter bed (anoxic conditions) (2, 9, 18, 21). In TWs, anaerobic digestion is a two-step process performed by anaerobic heterotrophic bacteria (2). In the first step, fermentation (equation 1, 2, and 3) takes place by acid-forming bacteria, whereby organic matter is converted into new cells, organic acids, alcohols, and CO_2 (2, 22). Acetic acid is the primary acid formed in most flooded soils and sediments (22).

Fermentation

$$C_6H_{12}O_6 \longrightarrow 3\,CH_3COOH\,(\text{acetic acid}) + H_2 \quad \{1\}$$
$$C_6H_{12}O_6 \longrightarrow 2\,CH_3CHOHCOOH\,(\text{lactic}) \quad \{2\}$$
$$C_6H_{12}O_6 \longrightarrow 2\,CH_3CH_2OH\,(\text{ethanol}) + 2\,CO_2 \quad \{3\}$$

In the second step, methanogenesis (equation 4 and 5) - the further oxidation of organic compounds - is performed by methane-forming bacteria, which convert the remaining organic compounds into new bacterial cells, methane (CH_4), and CO_2 (2, 22). The pathways of fermentation and methanogenesis are highly diverse and involve transformations of various compounds (such as iron, sulphate, nitrate) that act as the final electron acceptor (2).

Methanogenesis

$$4\,H_2 + CO_2 \longrightarrow CH_4 + 2\,H_2O \quad \{4\}$$
$$CH_3COOH + 4\,H_2 \longrightarrow 2\,CH_4 + 2\,H_2O \quad \{5\}$$

Aerobic degradation

Aerobic degradation takes place where oxygen is supplied (oxic conditions), namely in the oxic media layer or rhizosphere (2). The aerobic degradation of organic carbon is performed by aerobic chemoheterotrophs (2). These bacteria oxidise organics using oxygen as the final electron acceptor and release CO_2, ammonia, and other stable chemical compounds as by-products (2). Equation 6 provides an example of aerobic microbial degradation of a simple organic pollutant (glucose) (2).

$$C_6H_{12}O_6 + 6\,O_2 \longrightarrow 6\,CO_2 + 6\,H_2O \quad \{6\}$$

1.4.3 Nitrogen

N is one of the principal pollutants in wastewater. Organic N is converted to ammonium N (NH_4-N) via hydrolysis and mineralisation (23). NH_4-N is one of the most important N compounds in receiving water systems and other ecosystems for three reasons: *(i)* it is the preferred nutrient form of N for most plant species and for autotrophic bacteria, and can hence cause eutrophication; *(ii)* it is chemically reduced and can therefore be readily oxidised, consuming and decreasing dissolved oxygen levels in receiving water bodies; and *(iii)* non-ionised ammonia (NH_3) is toxic to many forms of aquatic life, even at low concentrations (>0.2 mg/L) (2, 23, 24).

N exists in both organic and inorganic forms in wastewater. Organic N is present in amino acids, urea, and uric acids, and inorganic N is present as ammonium (NH_4^+), ammonia (NH_3), nitrite (NO_2^-), nitrate (NO_3^-), dissolved elemental N, or gaseous N (2, 24). Gaseous N includes nitrogen gas (N_2), nitrous oxide (N_2O), nitric oxide (NO), and free ammonia (NH_3) (2). N has a complex biogeochemical cycle involving multiple biotic and abiotic transformations (25).

In SSF TWs, the transformation and degradation of N is complex and dynamic, with many variables (24). It is known to involve biological pathways - including ammonification, nitrification-denitrification,

ANAMMOX, plant uptake, and biomass assimilation - and physico-chemical mechanisms such as ammonia adsorption (2, 24). Fig. 1.4 illustrates N conversion in TWs. Not all the transformations that take place in a TW result in the removal of N. Processes that ultimately remove N from wastewater include denitrification, plant uptake (with biomass harvesting), ammonia adsorption, and ANAMMOX, as well as ammonia volatilisation and organic N burial, however these processes are not significant in HSSF TWs (25). Other processes, such as ammonification or nitrification, merely convert N among various N forms, making it available for other processes and facilitating the removal of N from the system (25).

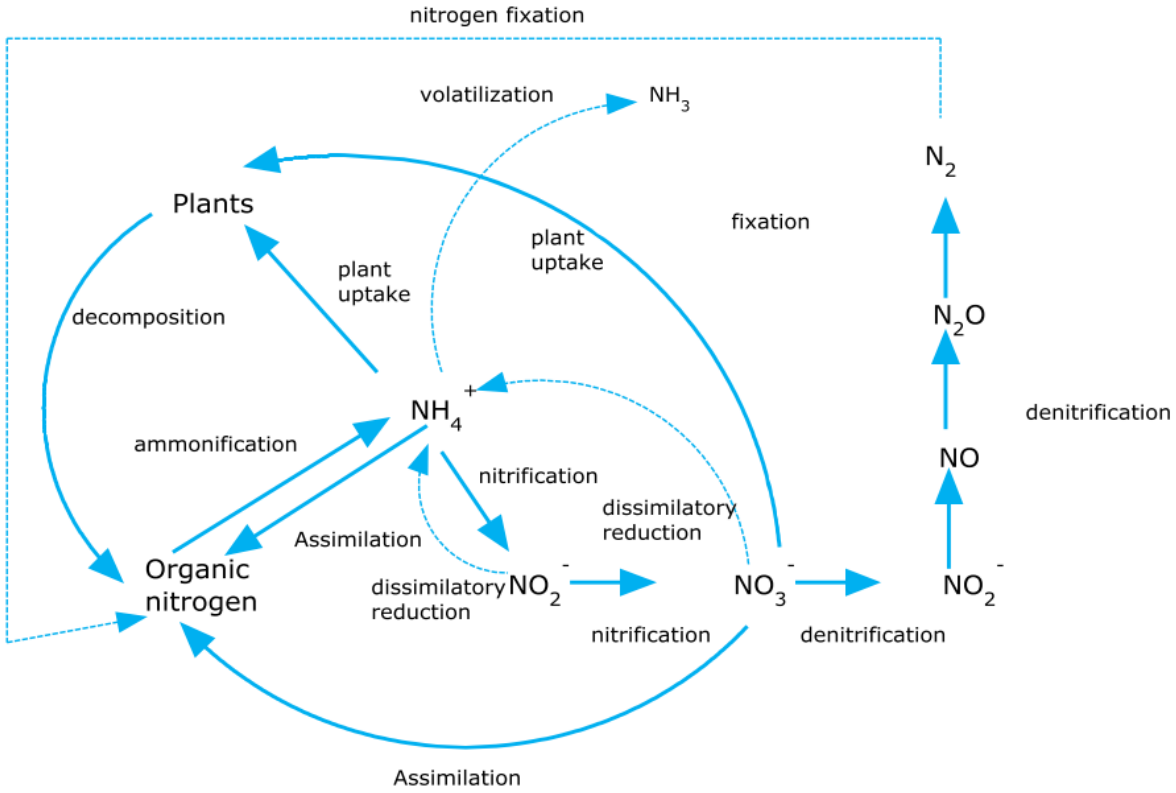

Fig. 1.4: Major nitrogen removal routes in subsurface flow treatment wetlands (figure from *Saeed and Sun*, 2012)

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Biodegradation and biotransformation processes

Ammonification

In SSF TWs treating organic N-rich wastewater, ammonification initiates the first step of N transformations (2). Ammonification (mineralisation) is the process whereby organic N is aerobically and anaerobically converted to NH_3 or NH_4^+, which predominates in TW systems, via a complex, energy-releasing, multi-step, biochemical process (2, 24, 25). The ammonification process is essentially a catabolism of amino acids. In the oxidised (oxic) media layer, amino acids undergo oxidative deamination and produce NH_3, as shown in equation 7 (26). In the reduced (anoxic) media layer, reductive deamination occurs and NH_3 is produced at a very slow rate, as shown in equation 8 (27). In SSF TWs, the ammonification process decreases with depth, indicating that mineralisation rates are fastest in the upper oxic layer and decrease as mineralisation switches from aerobic to facultative anaerobic and obligate anaerobic microorganisms (28, 29).

$$\text{Oxidised media: Amino acids} \longrightarrow \text{Imino acids} \longrightarrow \text{Keto acids} \longrightarrow NH_3\,/NH_4{}^+ \quad \{7\}$$

$$\text{Reduced media: Amino acids} \longrightarrow \text{Saturated acids} \longrightarrow NH_3\,/NH_4{}^+ \quad \{8\}$$

Ammonification rates are also dependent on temperature, pH, C/N ratio, available nutrients, and media conditions including texture and structure (28). Ammonification proceeds faster at higher temperature, doubling its rate with temperature increase of 10°C (20, 30). The optimal pH range for ammonification is 6.5-8.5 and the optimal temperature range in 20-35°C (20).

The inorganic $NH_4{}^+$ produced via ammonification provides a substrate for nitrification-denitrification processes (24). Kinetically, ammonification proceeds more rapidly than nitrification (1, 24). Denitrification is the main process by which $NH_4{}^+$ is removed from SSF TWs. Other processes, including adsorption, plant uptake, and NH_3 volatilisation, also remove $NH_4{}^+$ in SSF TWs, however their contribution is considered to be limited in contrast with nitrification-denitrification (24).

Nitrification

Nitrification is a two-step process, in which $NH_4{}^+$ is biologically oxidised to $NO_2{}^-$ (equation 9) and then to $NO_3{}^-$ (equation 10) (28). The overall nitrification equation is given in equation 11, as proposed by *Reddy et al.*, 1984.

$$NH_4{}^+ + 1.5\,O_2 \xrightarrow{\textit{Nitroso-genus}} 2\,H^+ + H_2O + NO_2{}^- \quad \{9\}$$

$$NO_2{}^- + 0.5\,O_2 \xrightarrow{\textit{Nitro-genus}} NO_3{}^- \quad \{10\}$$

$$NH_4{}^+ + 2\,O_2 \longrightarrow NO_3{}^- + 2\,H^+ + H_2O \quad \{11\}$$

The first step of nitrification (equation 9) is executed by strictly chemolithotrophic (strictly aerobic) bacteria, such as *Nitrosomonas*, *Nitrosococcus*, and *Nitrosospira*, which are entirely dependent on the oxidation of ammonia for the generation of energy for growth (2, 24, 25). In the second step (equation 10), the oxidation of $NO_2{}^-$ to $NO_3{}^-$ is performed by facultative chemolithotrophic bacteria, such as *Nitrospira* and *Nitrobacter*, which use organic compounds, in addition to $NO_2{}^-$, to generate energy for growth (24, 25).

Nitrification is influenced by concentrations of $NH_4{}^+$ and dissolved O_2, temperature, pH value, alkalinity of the water, inorganic C source, moisture, and microbial population (30). Nitrification is a very O_2 demanding process, consuming 3.16 mg O_2/mg $NH_4{}^+$ oxidised and 1.11 mg O_2/mg $NO_2{}^-$ oxidised (24). The optimum temperature for nitrification has been reported to range from 25 to 35°C, however some species can grow in minimum temperatures of 4 to 5°C (31). Optimal pH values for TWs are reported to be 6.6 to 8.0 (31). The pH value is important in the nitrification reaction as nitrification rates swiftly decline when the pH drops below 7.0 (24). pH reduction in TWs may result from the reduction of alkalinity by the acid produced in the nitrification process (24).

Biological removal mechanisms

Denitrification

Denitrification is a major mechanism of total nitrogen removal in HSSF TWs, typically removing 60-95% of total N, in contrast to 1-34% assimilated by plants and algae (32, 33). In HSSF TWs, denitrification is coupled with nitrification (25). It is believed that nitrification-denitrification processes occur in sequence in close proximity to each other in the active reaction zone due to the varying redox conditions (Fig. 1.3) (24, 30). In the denitrification process, denitrifying bacteria decrease concentrations of the inorganic N produced via the nitrification process ($NO_3{}^-$ and $NO_2{}^-$) by converting it to innocuous N_2 under anoxic conditions, with $NO_2{}^-$, NO, and N_2O produced as intermediates (equation 12) (34). At each reductive step, the gaseous product may either be released into the atmosphere or further reduced, however, under anoxic conditions, the majority of gas undergoes complete denitrification to N_2 (35). The denitrification process is illustrated by equation 13 (34).

$$2\,NO_3{}^- \longrightarrow 2\,NO_2{}^- \longrightarrow 2\,NO \longrightarrow N_2O \longrightarrow N_2 \quad \{12\}$$

$$6\,(CH_2O) + 4\,NO_3{}^- \longrightarrow 6\,CO_2 + 2\,N_2 + 6\,H_2O \quad \{13\}$$

Diverse organisms are capable of denitrification (25). Most denitrifying bacteria are chemoheterotrophs, obtaining their energy from chemical reactions and using organic compounds as electron donors and as a source of cellular carbon (34). Under anoxic conditions, nitrogen oxides serve as a terminal electron acceptor (in place of oxygen) for respiratory electron transport, facilitating complete denitrification to N_2 (24, 25, 30). When oxygen is available (suboxic conditions), the synthesis and activity of denitrifying enzymes are inhibited and oxygen is used as the terminal electron acceptor, causing incomplete denitrification to N_2O(2, 24, 25, 36).

The rate of denitrification is influenced by many factors, including the presence of suboxic conditions, redox potential, pH value, temperature, $NO_3{}^-$ concentration, type and quality of organic C source, hydroperiods, soil moisture, presence of denitrifiers, soil type, water level, and the presence of overlying water (30, 35). The optimal pH range is reported to lie between 6 and 8 (37). Denitrification becomes slow but may remain significant below pH 5 (25). Furthermore, when the pH value is low, N_2O reductase is inhibited, and incomplete denitrification takes place (2, 36). Denitrification is also strongly temperature dependent, with the rate of denitrification very slow, but measurable, at temperatures below 5°C (38). At temperatures below 5°C, higher fractions of N_2O and NO are produced, and at higher temperatures mostly N_2 is produced (25). The rate of denitrification increases rapidly with a rise in temperature from 2°to 25°C, and is hence significantly higher in spring and summer (38–40).

ANAMMOX

ANAMMOX (anaerobic ammonium oxidation) is an alternate process that also contributes to N removal in suboxic and anoxic parts of SSF TWs (2, 24). ANAMMOX is the autotrophic oxidation of $NH_4{}^+$ to N_2 gas, whereby $NO_2{}^-$ is used as a terminal electron acceptor (equation 14) (24, 41). As ANAMMOX bacteria are autotrophic, in contrast to classic heterotrophic denitrifiers, they do not require an organic C source for their C and energy supply (42). The coexistence of heterotrophic denitrifiers and ANAMMOX bacteria has been reported by Dong and Sun (43).

$$NH_4{}^+ + NO_2{}^- \longrightarrow N_2 + 2\,H_2O \quad \{14\}$$

The ANAMMOX process is extremely dependent on various parameters including pH range, temperature, NOH_4^+ to NO_2^- ratio, and the presence of various substrates (eg. sulphide) that inhibit the growth of ANAMMOX bacteria (2). The optimal pH range for ANAMMOX is reported as 6.7-8.3 and the optimal temperature range is 20 to 43°C (44).

Physico-chemical removal mechanisms

Many physico-chemical processes can take place in SSF TWs, however the major physico-chemical mechanism for N removal is ammonia adsorption (24). In newly built TWs, the contribution of physico-chemical processes to the overall removal of NH_4^+ is generally high but decreases as the TW ages (24).

Ammonia adsorption
Ionised ammonia (NH_4^+) can be adsorbed from solution through a cation exchange reaction with detritus, inorganic sediments, or media (25). The adsorbed NH_4^+ is bound loosely and can be released easily when water chemistry changes (24). Adsorbed NH_4^+ forms an equilibrium with the sorption sites and the water column. Hence, when NH_4^+ concentrations increase in the water column (eg. ammonification), NH_4^+ adsorbs to sites, and when concentrations decrease (eg. nitrification), NH_4^+ desorbs from sites (25). The choice of filter bed material is important for NH_4^+ adsorption, especially with regards to clay content, which has high adsorption capacity. The media generally used for TWs typically has very low adsorption capacity (25). The rate and extent of NH_4^+ adsorption is influenced by the type and amount of clay within the media, alternating submergence and drying patterns, submergence period, characteristics of media organic matter, and the presence of vegetation (45, 46).

1.5 Greenhouse gas emissions from treatment wetlands

Despite the many advantages of TWs, by-products of wastewater treatment processes result in TWs being a net source of gaseous compounds such as CH_4 and N_2O, both of which are problematic due to their global warming potential (GWP) - the heat-trapping capacity of 1 ton of a gas relative to the emissions of 1 ton of CO_2 - and role in climate change (7, 8). Since pre-industrial times, non-CO_2 GHGs account for 28% of the enhanced anthropogenic greenhouse effect and CO_2 accounts for 72%; the two main non-CO_2 GHGs are CH_4 (21%) and N_2O (7%) (47). Over a 100-year time horizon, CH_4 is currently estimated to have a GWP of 21 times the heat-trapping capacity of CO_2 and N_2O has about 310 times (47). Over recent years, the importance of the GHG release from TWs has become increasingly important as their implementation is increasing globally (48, 49).

During the wastewater treatment process, CO_2 and especially CH_4 are produced during the anaerobic degradation of organic compounds (fermentation and methanogenesis, respectively). N_2O is produced during incomplete denitrification when the pH is low or if O_2 is present (2, 36). Among several other environmental factors that control GHG emissions from TWs, the most significant are the availability of C and N which directly depends on wastewater loading, temperature, hydraulic loading rate (HLR; pulsing vs steady-state flow), water table, moisture content of the media, and the presence of plants (48).

Relatively few studies measuring gas fluxes from TWs treating wastewater have been carried out (7, 50–52). However, a comprehensive literature analysis of 158 papers by *Mander et al.*, 2014, has estimated CH_4 emission rates from HSSF TWs to range between 0.048 to 17.5 $\mathrm{mg\,m^{-2}\,h^{-1}}$ and average 7.4 $\mathrm{mg\,m^{-2}\,h^{-1}}$, and N_2O emissions rates to range between 0 and 0.894 $\mathrm{mg\,m^{-2}\,h^{-1}}$ and average 0.24 $\mathrm{mg\,m^{-2}\,h^{-1}}$. Measured rates have shown high spatial and temporal (seasonal and diurnal) variations

resulting from changes in the biogeochemistry of C and N and plant-media-microorganism interactions over time and space (17, 50). In Europe, emissions of CH_4 from SSF TWs are higher during summer than during winter (8, 49). Furthermore, higher emissions of CH_4 have been reported in clogged TWs (8)

1.6 Clogging in subsurface flow treatment wetlands

A major and widespread problem occurring in SSF TWs, particularly in HSSF TWs, is the progressive or sudden clogging of the porous media (Fig. 1.5) (3, 18). Clogging is typically the result of operational and maintenance issues and inappropriate design and has commonly been reported to occur in HSSF TWs whereby soil was used as the filter bed material (1, 3, 6). Clogging can ultimately limit the lifespan of the system - originally estimated at 50-100 years but is now estimated to be between 15 and eight years - as it requires intervention, typically necessitating remediation (removal and replacement) of the clogged media (5). For this reason, VF TWs or hybrid TWs are now the preferred TW design over HSSF TWs.

The material that accumulates to eventuate clogging - organic solids in the case of wastewater treatment - will henceforth be referred to as *clog matter*. Clog matter can accumulate in the subsurface of the SSF TW, reducing hydraulic conductivity, or it can accumulate on the surface, preventing infiltration of surface flow into the media (5). Other factors known to attribute clogging in TWs treating wastewater include solid entrapment, biofilm clogging, and vegetation contributions (Fig. 1.5) (5). Solid entrapment is whereby particles accumulate within the media, causing it to clog (5). Biofilm clogging results when pores plug with impermeable slime, secreted by most biofilms, or when biofilms on separate media particles bridge, causing the hydraulic conductivity of the bulk porous media to tend towards that of the biofilm (5). Biofilm clogging has been reported to commonly occur in the inlet region of a TW where the concentration of organic matter in the wastewater is greatest and biofilm growth and development is maximal (5, 53). Lastly, vegetation can contribute to subsurface clogging by occluding pore volume with subsurface roots, or can contribute to surface clogging by litter accumulation on the surface of the media, especially when winter die-back occurs and if macrophytes are not harvested (5). However, some studies have found that plant evapotranspiration and water retention in the litter layer may counteract loss of hydraulic conductivity caused by plant-mediated clogging (5). As a consequence of the aforementioned factors that eventuate clogging, the media of HSSF TWs is believed to inevitably become clogged over time (1).

Clogging may be accompanied by a decrease in treatment performance, primarily due to decreased HRT in the media, or hydraulic malfunctions such as ponding of wastewater on the surface of the system or horizontal (overland) flow (5, 54). Ponding of wastewater is usually confined to and most sever in the inlet region of the media (1, 54). Overland flow, on the other hand, carries the excess wastewater until the hydraulic conductivity of the media and flow gradient over the remaining distance are sufficient to permit the flow to be carried below the surface (1). If the depth of the overland flow is as much as a few centimetres, most of the wastewater is said to be carried to the outlet by overland flow, bypassing treatment by the media (1).

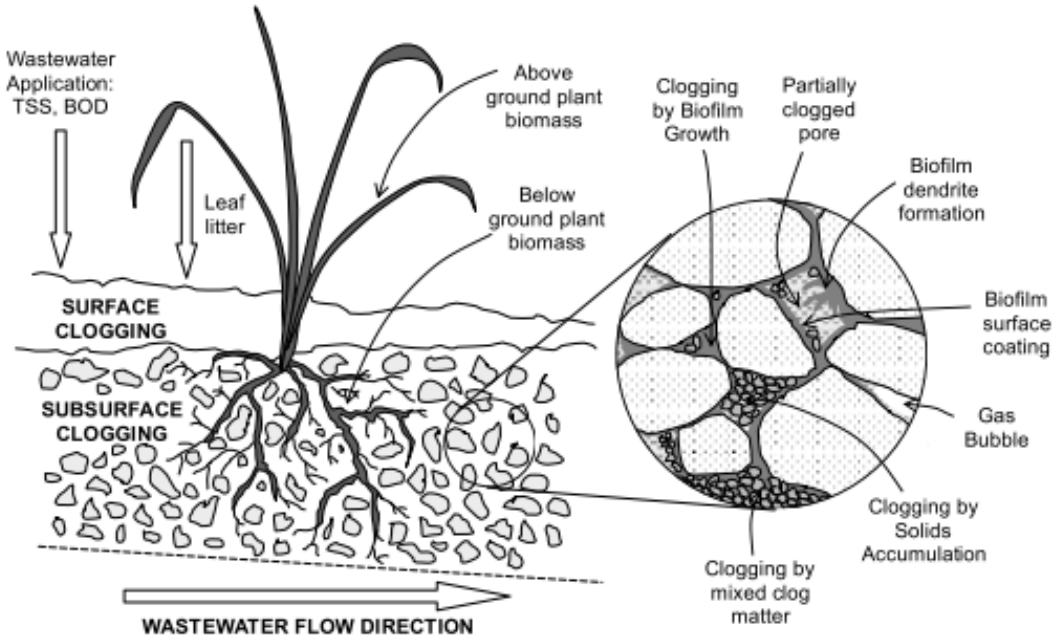

Fig. 1.5: Clogging processes that occur at the surface and in the subsurface of horizontal subsurface flow treatment wetlands (figure from *Knowles*, 2010)

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1.7 Summary

TWs are a common nature-based technology used worldwide to manage and treat secondary wastewater in remote and rural communities. The treatment of wastewater using TWs occurs via a complex combination of physical, chemical, and biological processes that work together to transform, degrade, or remove nutrients and pollutants from wastewater within the intricate plant-media-microorganism nexus. The mechanisms that ultimately remove C and N from wastewater include anaerobic and aerobic degradation of organic compounds, plant uptake (with biomass harvesting), denitrification, NH_4^+ adsorption, and ANAMMOX. As the development and use of TWs is increasing globally, it is important to understand how clogging - an inevitable, major, and widespread problem, particularly in HSSF TWs - impacts on the wastewater treatment efficacy and GHG emissions of well-established SSF TWs. Understanding the impacts of clogging will also assist in identifying possible mitigation strategies or determining whether remediation of the system is required.

CHAPTER 2

Study site and materials and methods

2.1 Site description

Schottenhof (48.23 °N, 16.35 °E) is a restaurant, animal park, and a centre for horse riding situated at 5 Amundsenstraße, 1140, Vienna, Austria. As Schottenhof was disconnected from Vienna's sewerage system, the Municipal Department (MA) 45 – Water Management of the City Government of Vienna built an onsite treatment system at Schottenhof in July 1997, which has been in operation ever since.

Wastewater is pre-treated in a three-chamber septic tank, where coarse material is allowed to settle, leaving only the watery component which is fed into the onsite treatment system. The onsite treatment system consists of five TWs (Fig. 2.1). Four TWs are HSSF and are arranged as a 2 x 2 rectangular grid. The inflowing secondary wastewater enters the top two TWs - TW 1 and TW 3 - and flows through the TW where it is collected at the outlet. The wastewater then enters the bottom two (TW 2 and TW 4) where it again flows through the TW and is collected at the outlet. The collective wastewater then feeds into the fifth TW, which is a vertical subsurface flow TW and acts as the final polishing step before the treated water is discharged into a trench in the nearby forest.

The right side of the grid (TW 1 and TW 2) was clogged and TW 1 was completely inundated with wastewater and experienced overland flow at the onset of sampling, while the left side (TW 3 and TW 4) was unclogged, except for wastewater that ponded in a section of the inlet of TW 3 (Fig. 2.1). The top two TWs, TW 1 and TW 3, are the focus of this study, as the contrast between clogged and unclogged conditions was most pronounced in these two TWs. TW 1 will henceforth be referred to as the clogged TW (CTW) and TW 3 as the unclogged TW (UTW).

2.2 Treatment wetland system design

The system was designed to treat the household and domestic wastewater of 115 Population Equivalents (PE), estimated to produce 26.4 m^3 of sewerage inflow per day. The CTW is 13 m x 7.8 m and the UTW is 13 m x 8.3 m and both have a maximum depth of 0.75 m (Fig. 2.1). The TWs are lined with a 1mm water-tight, UV-resistant lining to prevent seepage into the underlying groundwater. The UTW (TW 3) and TW 4 are filled with the surrounding soil, whereas the CTW (TW 1) and TW 2 are filled with fine-grained sediment (2 - 6.3 mm) from the Vienna River and underlaid with 10 cm of gravel (4 - 8 mm) (55, 56). The River sediment was selected after positive results were obtained with regards to nutrient removal from another TW site in Vienna (55). The influent and effluent drainage area was filled with 16-32 mm gravel (55). The influent wastewater is dispensed through a surface inlet for the CTW and a subsurface inlet for UTW. Both TWs are planted with macrophytes (*Phragmites australis*).

At the time of construction, wastewater biological oxygen demand (BOD), chemical oxygen demand

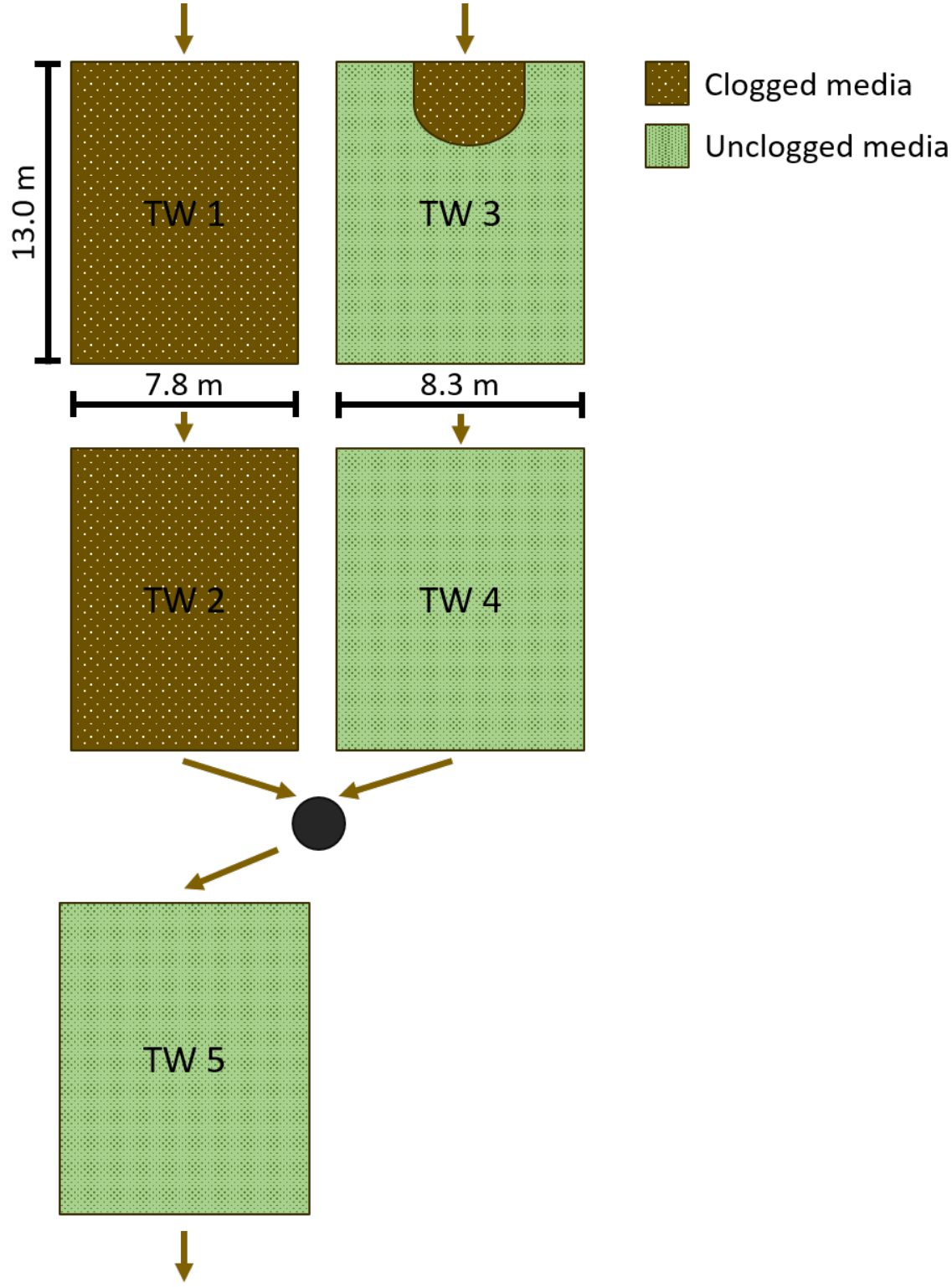

Fig. 2.1: Basic site plan of the treatment wetlands at Schottenhof

(COD), and NH_4-N could not exceed set values (25, 90, and 10 mg/L, respectively) and had to be periodically checked (6, 12, and 52 times per year, respectively), as outlined in the technical certificate produced by MA 58 - Water Authority (57). Regular maintenance of the TWs is undertaken by a reliable and competent person (57).

2.3 Austrian treatment wetland design regulations and effluent regulations

SSF TWs constructed and operated in Austria must meet the requirements of the Austrian Standards. According to the current Austrian design standards for HSSF TWs (*ÖNORM B 2505*, 2009), filter bed surface area must be at least 4 - 6 m^2 per PE, bed depth at least 0.6 m, the filtration material should consist of a mixture of sand and gravel 4/8 mm and 16/32 mm at the inflow zone (54). The hydraulic loading rate should be 5 cm/d and the organic load <112 kg BOD/ha/day (54). The Austrian design standards are updated regularly - 1996, 1997, 2003, 2005, 2008, and recently 2009.

The effluent from the TW must meet the Austrian standards of maximum effluent concentrations (*AEVkA*, 1996): maximum NH_4-N effluent concentration of 10 mg/L for wastewater treatment plants serving less than 500 PE is allowed (this has to be met for effluent water temperatures higher than 12°C only); organic matter maximum effluent concentrations are 30 mg total organic carbon/L, 90 mg COD/L and 25 mg BOD5/L; and for treatment plants with a capacity of less than 500 PE, there are no legal requirements regarding nutrient removal, such as phosphorus. However, in the case of small and sensible receiving waters, additional requirements for nutrient removal can be set by the authorities (6).

2.4 Materials and methods

2.4.1 Wastewater sampling and analysis

Wastewater from the inlet and outlet was collected weekly from 24 April until 26 June 2017. For the most part, wastewater samples were taken from the storage and distribution tanks using a 60 mL syringe (B. Braun Omnifix) and an extension tube whenever necessary. When wastewater from the CTW was overflowing directly into TW 3 (until 14 May), wastewater samples were mostly taken from the overland flow at the outlet. Samples were stored in labelled 100 mL plastic bottles. Within 10 minutes of taking the sample, the time of collection, temperature, pH, and dissolved oxygen (DO) were measured onsite using a digital multi-parameter portable meter (WTW Multi 3420) and the values recorded.

From the wastewater samples, 10 mL was taken using a 5 mL syringe (Sigma-Aldrich syringe PP/PE without needle) and filtered onsite using disposable syringe filters (Chromafil Xtra PET-45/25). Syringe filters were rinsed with water between samples and replaced regularly. The filtered samples were stored in a cool, shaded place in labelled 100 mL plastic bottles. Proceeding sampling, the samples were transported to the laboratory where they were further analysed for total organic carbon (non-purgeable organic C; NPOC) and total nitrogen (sum of ammonia, organic and reduced N, and NO_3^- - NO_2^-; TN) using a Shimadzu TOC-L Series Analyser. If necessary, samples were filtered a second time before analysis as growth of microorganisms in the bottle was not uncommon.

2.4.2 Gas sampling and analysis

Sampling design

Systemic two-dimensional, unaligned, rectangular grid sampling was the sampling method of choice to ensure a comprehensive assessment of the CTW and UTW (60). Sampling sites were systemically spaced; a 3 x 3 rectangular grid was measured out for each TW, ensuring that the grid represented the inlet, middle, and outlet sections (Fig. 2.2). Sampling locations were chosen in each grid based on desirable conditions required to set up a gas chamber (minimal vegetative cover, accessibility, etc.).

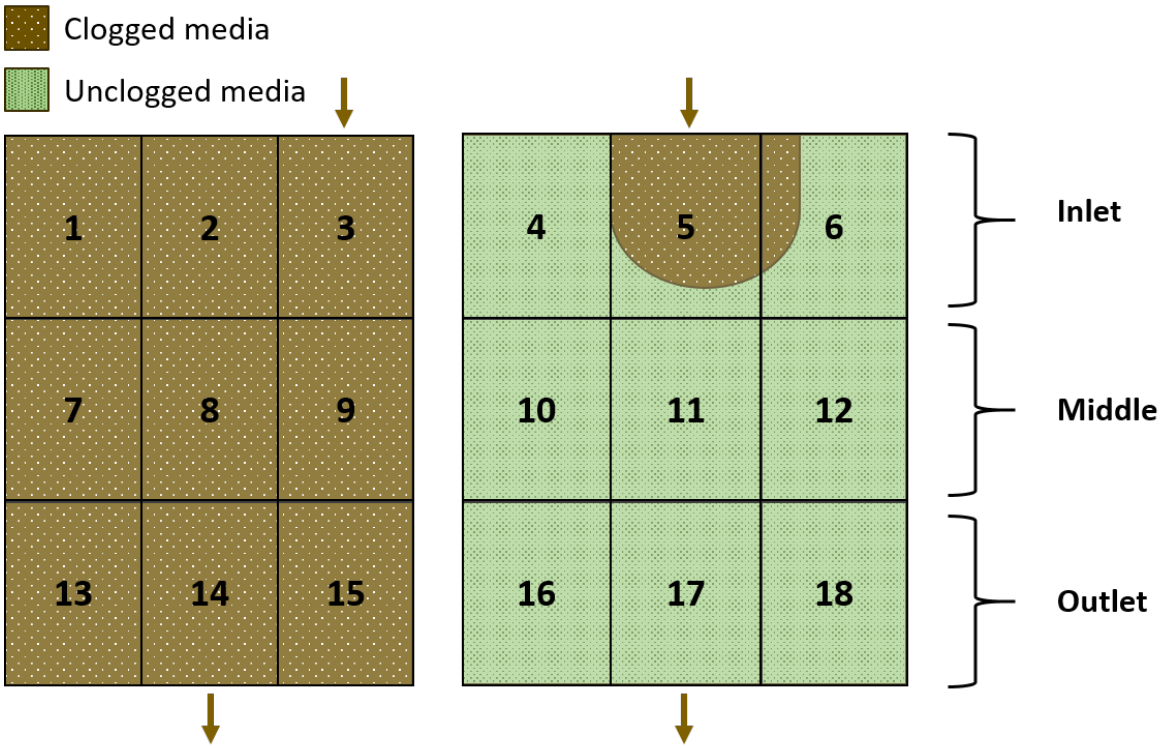

Fig. 2.2: Sampling scheme for the clogged (left) and unclogged (right) treatment wetland

Gas chamber design and construction

Two different chamber methods were used to estimate gas emissions *in situ*. This was the result of different filter bed conditions; the CTW consisted of inundated media and overland flow, whereas the UTW consisted of dry media, except for a section at the inlet where ponding of wastewater occurred (position 5) (Fig. 2.2). For both methods, a lid was used. A total of six lids were constructed, consisting of 110 mm wide PVC piping that was fitted with a PVC cap, having a combined height of ~150 mm (Fig. 2.3A). To enable gas sampling in the headspace by use of a syringe, a synthetic resealable membrane (Macherey-Nagel stopper N20) was fitted into the cap by drilling a ~20 mm hole (Fig. 2.3B). The membranes were replaced regularly to ensure gas did not escape the chamber during sampling due to overuse of the membrane.

For the inundated media (the CTW and position 5), the floating chamber method was used (61). For this method, the aforementioned chamber lid was modified to float using a foam pool tube. The tube was cut open and attached to the rim of the lid using water-tight tape (Fig. 2.3C). The tube added 20 - 25 mm height to the chamber, giving it an estimated to have had a volume of ~0.00122 m^3. To allow

undisturbed access to the middle section of the CTW for sampling, a platform was made using a plank of wood supported by a soft drink crate and was installed one week prior to the onset of sampling to allow time for the media to normalise.

For the dry media (the UTW), the static chamber method was used (62). The chamber consisted of the aforementioned lid fitted onto an anchor. A total of nine anchors were made and consisted of 110 mm wide PVC piping cut to ~50 mm in height. Plastic tubing built into the rim of the lid acted as a seal, creating an air-tight chamber. The anchors were also inserted into the sampling points one week prior to the onset of sampling. The anchors were installed at a depth of 25 - 30 mm, leaving 20 - 25 mm to protrude above the media surface (Fig. 2.3A and D). The static chambers are estimated to have had a volume of ~0.00122 m^3.

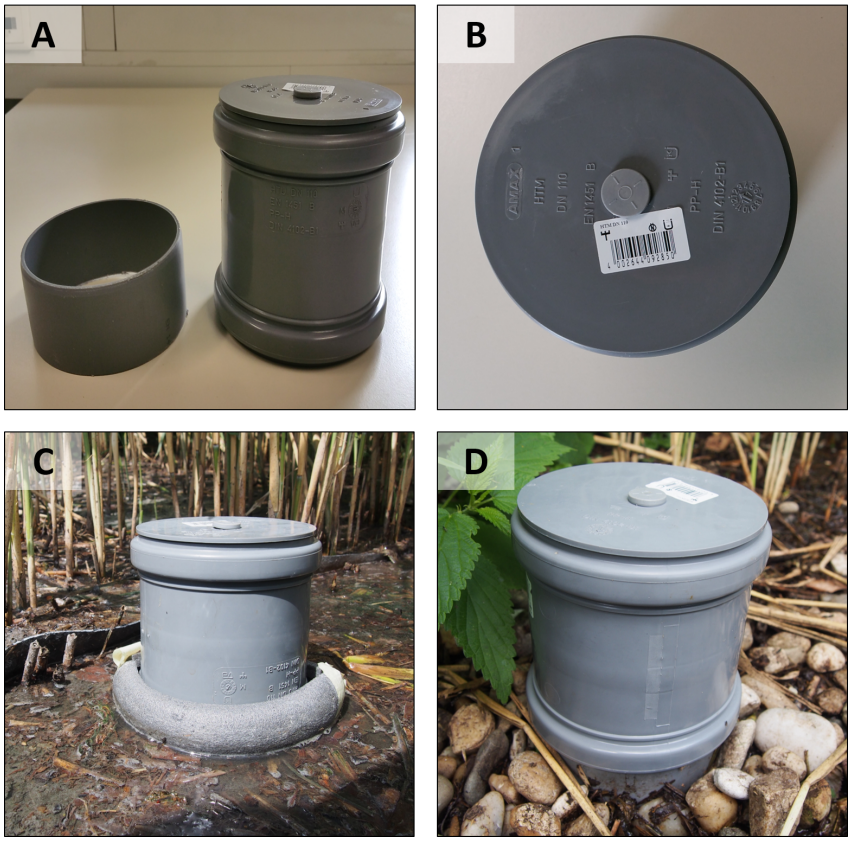

Fig. 2.3: Gas chambers and their components (A) PVC lid with cap and anchor; (B) modified cap with resealable membrane; (C) floating gas chamber; (D) static gas chamber

Gas sampling and analysis

Gas emissions were estimated *in situ* weekly from 28 April until 26 June 2017. Gas sampling took place at regular intervals during two specified windows of time; when the TWs experienced maximum light intensity (1-2 pm) and in the late afternoon, generally once the TWs were shaded (4-6 pm). Initially, the gas samples were collected from each grid point at 0, 10, and 30 minutes, however, after calibration, were collected at 0, 10, and 20 minutes due to high rates of CH_4 emissions by both TWs. At each interval, 21 mL of gas was collected from the headspace of the chamber using a 60 mL syringe (B. Braun Omnifix) with a disposable hypodermic needle (B. Braun Sterican 0.8 x 40 mm) and was stored in

an evacuated 20 mL glass sample bottle. Between sampling, gas chambers were allowed to cool as they became warm in the sun. Glass samples bottles were prepared, labelled, and evacuated no more than 48 hours prior to sampling (Altmann Analytik vial ND20/ND18 20 mL headspace bottles; Macherey-Nagel stopper N20; Macherey-Nagel crimp caps N20; Agilent Technologies 20 mm electronic crimper; Pfeiffer vacuum Adixen). Samples were stored in a cool, shaded place and transported to the laboratory for further analysis, generally within 48 hours of sampling. Samples were analysed for CH_4 and N_2O concentrations using a gas chromatography system (Agilent Technologies 7697A Headspace Sampler; Agilent Technologies 7890B GC System).

2.4.3 Gas flux calculation

The gas fluxes were calculated using Equation 2.1. The change in gas concentration over time (*dc/dt*, ppm h^{-1}) was calculated from the slope of the linear increase or decrease in CH_4 and N_2O concentration during the measurement period using the LINEST function in Microsoft Excel 2016. The fluxes were corrected for the molar mass (*M*, $\mathrm{g\,mol}^{-1}$) of CH_4 and N_2O, atmospheric pressure (*p*, Pa), the chamber volume (*V*, m^3) to ground surface area (*A*, m^2) ratio, and air temperature (*T*, K) (63). The Pearson's correlation coefficient (r^2) for the linear regressions were calculated using the RSQ function in Excel 2016 and were used as a quality check the fluxes. For CH_4, fluxes with a r^2 of <0.80 were discarded. 28 fluxes were discarded for the CTW and 45 for the UTW. For N_2O, this approach was not applicable as (as confirmed using a scatter plot) the fluxes were generally very low and fluxes of <0.3 $\mu\mathrm{g\,m}^2\,\mathrm{h}^{-1}$ produced a r^2 of <0.80 (see Appendix 1). Hence, these fluxes were included to ensure the data was not skewed towards higher fluxes of N_2O.

$$f = \frac{MpV}{RTA} \times \frac{dc}{dt} 10^3 \tag{2.1}$$

2.5 Data analysis and interpretation

All statistical evaluations were performed using Microsoft Excel 2016. The normality of variables was checked using QQ plots and Shapiro-Wilk's tests using the 'Real Statistics'resource pack add-in for Excel. If the data were normally distributed, Student's T-test, a parametric test, was performed to determine the significance (α = 0.05; $p < 0.05$) of differences between the inlet, middle, and outlet section (paired T-test), as well as the differences between the CTW and the UTW (two sample T-test). The variance (equal or unequal) of the data were determined using a two-sample F-test. Pearson's correlation (*r*) was used to determine the strength and direction of relationships between the variables with normal distribution and Pearson's critical values (α = 0.05) were used to determine the significance of the relationship. For non-normally distributed data, the significance between data were analysed using non-parametric tests, namely Wilcoxon Signed Ranks test and Wilcoxon Rank-Sum test for paired and two independent samples, respectively. Spearman's Rank Order Correlation was performed to analyse the strength and direction of relationships between gas fluxes and different environmental parameters at the inlet and outlet. The significance of the correlations were determined from Spearman's critical values (α = 0.05).

CHAPTER 3

Results

3.1 Treatment wetland physical evaluation

Both TWs experienced clogging, but to different extents (Fig. 2.1 and 3.1). The conditions in the CTW progressively changed over the sampling months. The media - which was overlaid with up to 0.2 m of accumulated clog matter - was mostly inundated from ponded wastewater, with patches of semi-saturated areas in the middle section (Fig. 3.2B). At the beginning of the study, the ponded wastewater measured up to 30 cm in depth and experienced overland flow that overflowed directly into TW 2 by flowing over the soil that separates the two TWs (Fig. 3.2A). In the ponded wastewater, there was extensive growth of microorganisms, most notably blue-green algae in the free water zone, which bloomed mostly in early Spring, and periphytic algae, which grew on the surface of the submerged media and plant debris (Fig. 3.2A, B, C and D). In the early weeks of Spring, extensive gas bubbles were observed on the surface of the clog matter, likely resulting from algal growth and aerobic above-water degradation of organic matter (Fig. 3.2D). From 14th May, the middle and outlet section began to dry and had dried substantially by the end of the study. The overlaying clog matter however appeared to remain semi-saturated for the most part (Fig. 3.2E). As the TW dried, the overland flow ceased and wastewater no longer overflowed directly into TW 2 (Fig. 3.2C). For the entirety of the study, whitish-grey microorganisms grew on the surface of the gravel and media and also on the surface of the ponded wastewater at the inlet (Fig. 2.1 and 3.1). Over the course of the study, the wastewater ponded at the inlet became dark brown in colour and the presence of visible microorganisms, especially algae, was significantly reduced, decreasing the turbidity of the ponded wastewater at the inlet (Fig. 3.2F). At the inlet, gas bubbled to the surface from the media below; some of the smaller gas bubbles remained on the wastewater surface and formed clusters (Fig. 3.2F).

The UTW experienced ponding of wastewater at the inlet, mostly at position 5 and to a small extent at position 6, which measured 5 cm in depth (Fig. 2.1 and 3.1). The clogging is believed to be a recent occurrence as no ponded wastewater was observed in Spring 2016. Over the course of the study, the ponded wastewater at position 5 became darker in colour. The clogging appeared to become progressively worse at the inlet of the UTW, encroaching on the gas sampling point of section 6 (Fig. 3.1). Unlike with the CTW, visible microorganisms, especially algae, did not become abundant in the ponded wastewater (Fig. 3.3A).

Prior to the study, the macrophytes were harvested and stood at 0.2-0.3 m tall. By the end of the study, the macrophytes were over 2 m in height. The CTW appeared to have less biodiversity than the UTW. The UTW had a noticeably greater diversity of plants, with a thick undercover consisting initially of Wild Garlic (*Allium ursinum*) which progressed to a think undergrowth of Stinging Nettle (*Urtica dioica*) that grew everywhere except where wastewater ponded (Fig. 3.3B). Apart from the macrophytes, grass was the only other plant that appeared to grow in the CTW and it only began to grow on the accumulated clog matter once the TW began to dry up.

Fig. 3.1: Schottenhof treatment wetlands at beginning of study; the clogged treatment wetland (top) and the unclogged treatment wetland (bottom) with ponding of wastewater at position 5

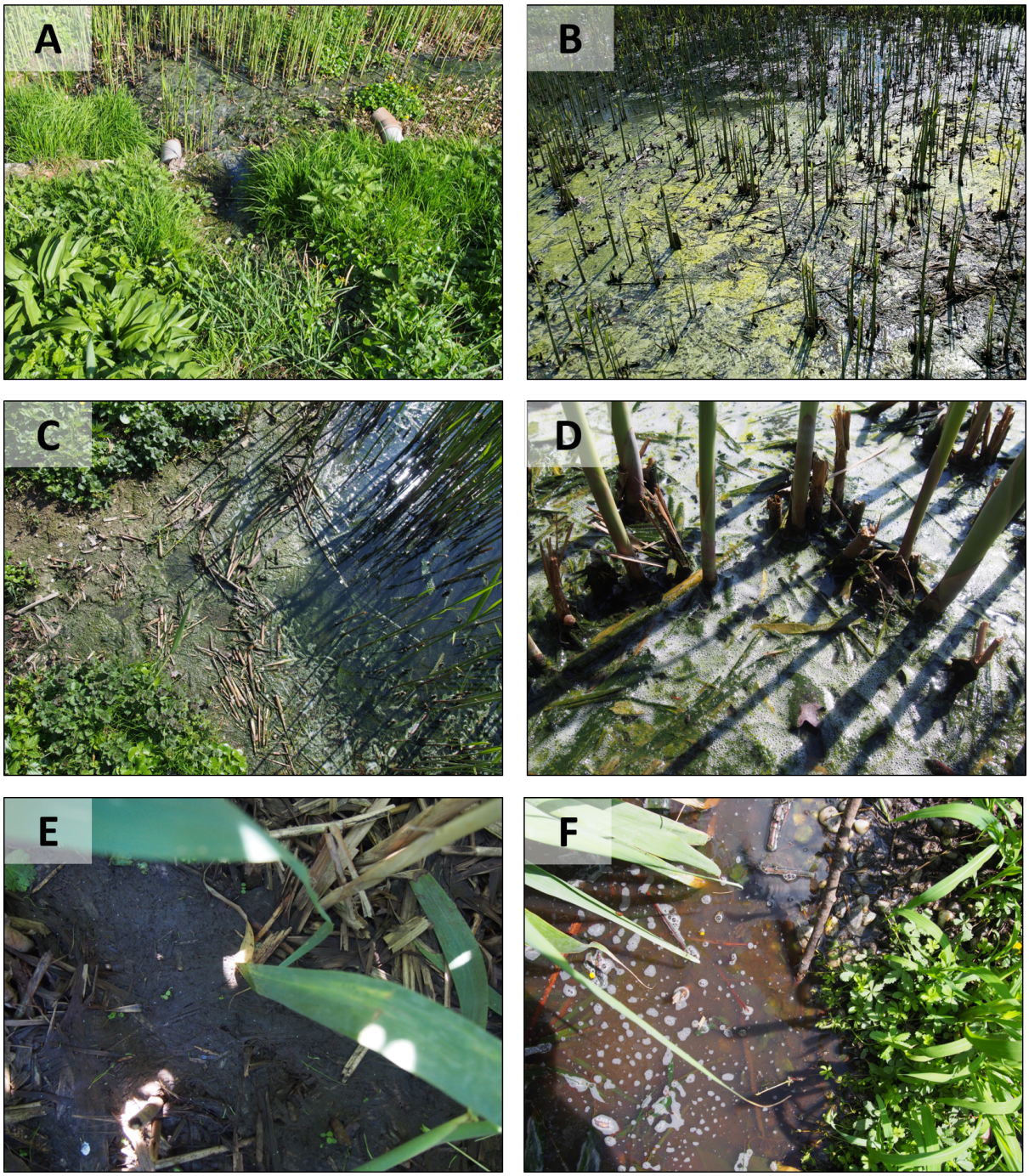

Fig. 3.2: Various conditions of the clogged treatment wetland: (A) wastewater overflow into TW 2; (B) microorganism growth, particularly algae, and various degrees of media saturation; (C) algae growth at the outlet on the surface of media and on plant debris; (D) gas bubbles on surface of decaying debris; (E) semi-saturated media following drying of the treatment wetland; and (F) gas bubble clusters in inlet and brown wastewater with low turbidity

Fig. 3.3: (A) Wastewater ponding at inlet and (B) plant diversity (*Allium ursinum* left, *Urtica dioica* right) in the unclogged treatment wetland

3.2 Wastewater characteristics

3.2.1 Influent data and trends

The descriptive statistics of the influent physico-chemical parameters for the CTW and the UTW are displayed in Table 3.1. From the correlations given in Table 3.1, it can be deduced that the wastewater influent concentrations of NPOC and TN, as well as temperature and pH, were highly correlated (Pearson >0.90) for the CTW and the UTW over the course of the study. The influent concentrations of DO differed between the TWs however not significantly. Over the sampling period, influent NPOC and TN had an large range in both the CTW (129.59 and 124.98 mg/L) and UTW (11.12 and 126.33 mg/L) (Table 3.1). A closer look at these parameters reveals noticeable trends; the concentration of NPOC fluctuated over the sampling period whereas TN increased and plateaued around 150 mg/L (Fig. 3.4).

Table 3.1: Descriptive statistics of influent wastewater physico-chemical parameters for the clogged and unclogged treatment wetland (CTW and UTW, respectively) from 21 April to 26 June 2017 and their correlation

		Temp. (°C)	pH	DO (mg/L)	NPOC (mg/L)	TN (mg/L)
CTW	**Mean**	17.96	7.23	4.80	124.03	117.82
	Standard error	1.06	0.13	0.45	11.38	12.90
	Standard Dev.	3.51	0.42	1.48	37.75	42.78
	Range	12.2	1.41	5.44	129.59	124.98
	Minimum	10.60	6.39	2.64	71.31	26.22
	Maximum	22.80	7.80	8.08	200.90	151.20
UTW	**Mean**	18.95	7.15	3.87	127.72	118.79
	Standard error	1.03	0.08	0.62	11.78	13.42
	Standard Dev.	3.43	0.26	2.07	39.08	44.50
	Range	11.60	0.89	6.99	133.38	126.33
	Minimum	13.20	6.53	1.49	68.12	25.27
	Maximum	24.80	7.42	8.48	201.50	151.60
	Correlation	0.92	0.91	0.48	0.95	0.99

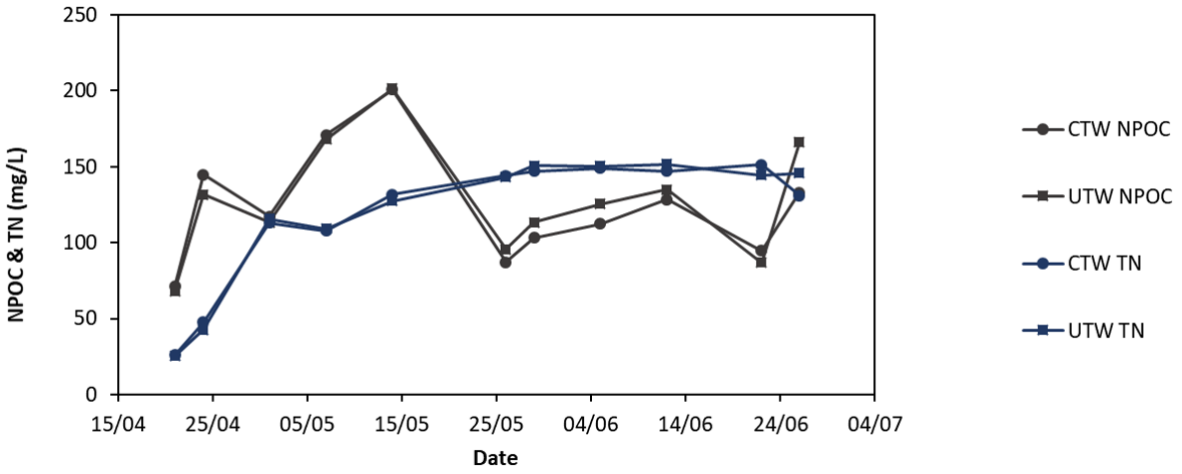

Fig. 3.4: Influent Total Carbon (NPOC) and Total Nitrogen (TN) concentrations of the clogged and unclogged treatment wetland (UTW and CTW, respectively) from 21 April to 26 June 2017

3.2.2 Comparison of influent and effluent nutrient concentrations

Over the study period, significant decreases in NPOC and TN concentrations were observed between the influent and effluent wastewater from both the CTW and UTW (Fig. 3.5 and 3.6). There was a significant positive correlation between influent and effluent concentrations of NPOC (r = 0.77) and TN (r = 0.95). For the UTW, no correlation (r = -0.20) was observed between influent and effluent concentrations of NPOC. A positive correlation (r = 0.47), though not significant, was observed between influent and effluent concentrations of TN, except for two points (5 and 12 June) when effluent concentrations were negatively correlated with influent concentrations.

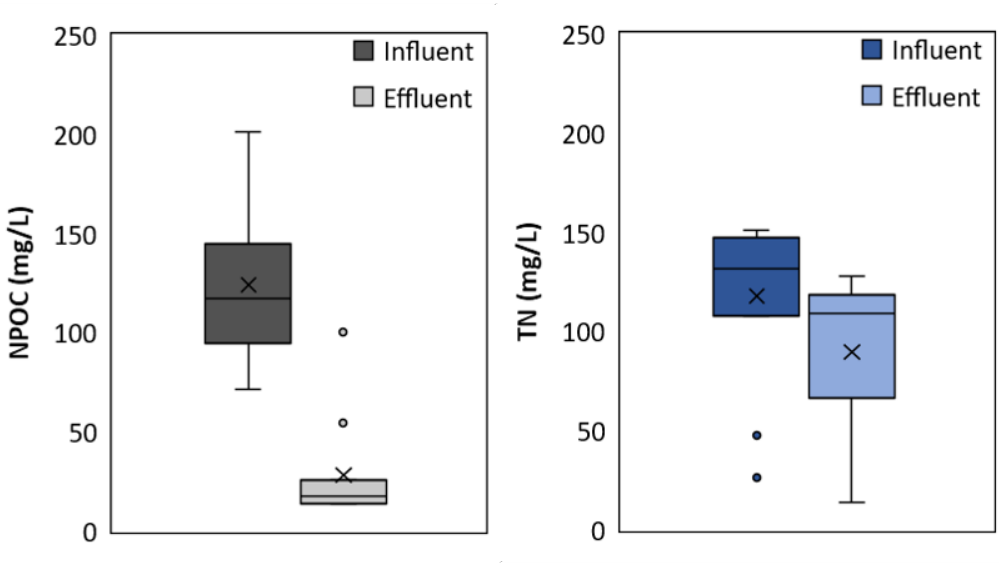

Fig. 3.5: Comparison of influent and effluent concentrations of Total Carbon (NPOC; left) and Total Nitrogen (TN; right) in the clogged treatment wetland (CTW)

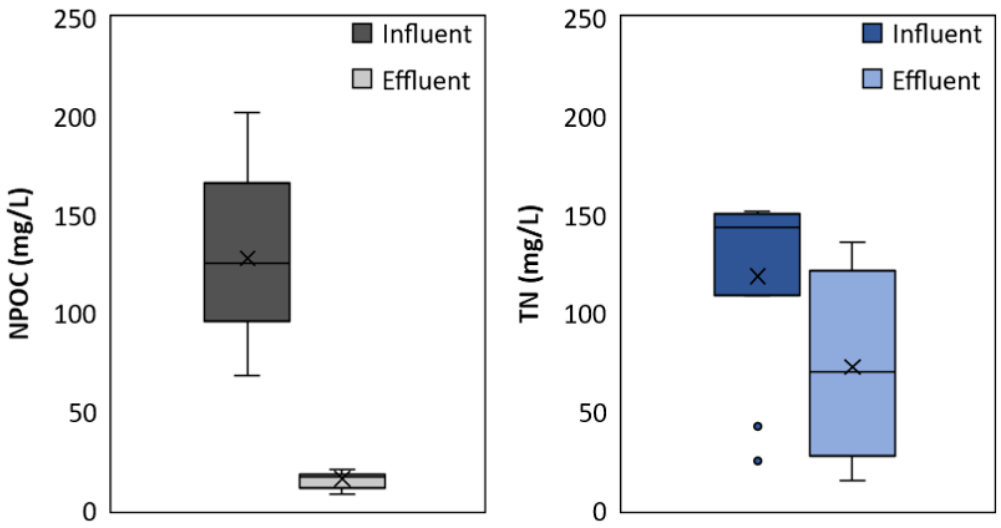

Fig. 3.6: Comparison of influent and effluent concentrations of Total Carbon (NPOC; left) and Total Nitrogen (TN; right) in the unclogged treatment wetland (UTW)

3.2.3 Nutrient removal efficacy

The average influent nutrient concentrations and nutrient removal efficacy for the CTW and the UTW are displayed in Table 3.2. The mean removal efficacy of NPOC for the CTW was 79.0 $\pm$ 3.8% and 86.1 $\pm$ 1.9% for the UTW (Table 3.2). The range of NPOC removal efficacy in the CTW was broad (38.8%) and is negatively skewed, whereas it was narrower and only slightly negatively skewed in the UTW (19.2%) (Fig. 3.7). No significant difference in NPOC removal efficacy was observed between the CTW and the UTW. Over the sampling period, NPOC removal efficacy fluctuated for the CTW and remained consistently above 74% for the UTW (Fig. 3.8). For the CTW, no significant correlations were initially observed between NPOC removal efficacy and the measured physico-chemical parameters (Table 3.3). Upon further investigation, it was found that there were two outlying NPOC removal efficacies on 7 and 14 May. When removed from the data set, a significant positive correlation (Spearman's rank correlation = 0.73) between average NPOC and NPOC removal efficacy was observed, whereas a negative correlation was observed on 7 and 14 May. On 7 and 14 May, the NPOC removal efficacy of the CTW decreased to 68.2% and 50.2%, respectively - a 13.7% and 36.5% decrease in efficacy compared to the average. Also on these days, there was above-average loading of NPOC (169.5 and 201.2 mg/L, respectively; 34.7% and 59.8% larger than average, respectively) - the highest concentrations recorded over the study period. For the UTW, the removal efficacy of NPOC was found to be significantly positively correlated with influent concentrations of NPOC (Spearman's rank correlation = 0.84) (Table 3.2 and 3.3).

For TN, the mean removal efficacy of the CTW was 24.1 $\pm$ 3.5% and 34.4 $\pm$ 9.4% for the UTW (Table 3.2). The range of TN removal efficacy in the CTW was 61.6% and data distribution is slightly positively skewed (Fig. 3.9). For the UTW, the range of removal efficacy was extremely broad, 98.8%, and is positively skewed (Fig. 3.9). No significant difference in TN removal efficacy was observed between the CTW and the UTW. From Fig. 3.9, it can be seen that the TN removal efficacy of the CTW had a negative trend over time. For the UTW, TN removal efficacy progressively increased until 7 May then decreased until 5 June when it spiked before decreasing after 12 June (Fig. 3.9). On 5 and 12 June, the TN removal efficacy of the UTW was substantially higher than average, increasing to around 89.8%, a 345.5% increase in efficacy compared with the average (Table 3.2). No significant correlations

were observed between TN removal efficacy and the measured physico-chemical parameters for both the CTW and UTW (Table 3.3).

Table 3.2: Average influent Total Carbon (NPOC) and Total Nitrogen (TN) concentrations and nutrient removal efficacies of the clogged and unclogged treatment wetland (CTW and UTW, respectively) from 21 April to 26 June 2017

		NPOC removal efficacy (%)			TN removal efficacy (%)	
Date	**Average influent NPOC (mg/L)**	**the CTW removal efficacy**	**the UTW removal efficacy**	**Average influent TN (mg/L)**	**the CTW removal efficacy**	**the UTW removal efficacy**
21/4/2017	69.72	63.88	74.74	25.75	46.19	-8.94
24/4/2017	138.20	88.49	86.65	45.22	19.52	27.47
1/5/2017	115.35	88.14	84.02	114.35	24.92	39.47
7/5/2017	169.50	68.20	93.83	108.40	38.18	45.48
14/5/2017	201.20	50.17	91.57	129.60	17.10	35.86
26/5/2017	91.46	81.62	78.65	143.70	30.31	18.65
29/5/2017	108.30	83.94	84.95	148.90	19.25	9.75
5/6/2017	118.90	87.88	91.00	149.70	26.76	89.88
12/6/2017	131.75	88.99	93.96	149.40	22.49	89.77
22/6/2017	90.73	81.25	79.89	147.90	18.32	15.91
26/6/2017	149.50	86.67	87.32	138.45	2.52	15.42
Average	125.87	79.02	86.05	118.31	24.14	34.43
S.E.	11.43	3.84	1.92	13.13	3.47	9.43

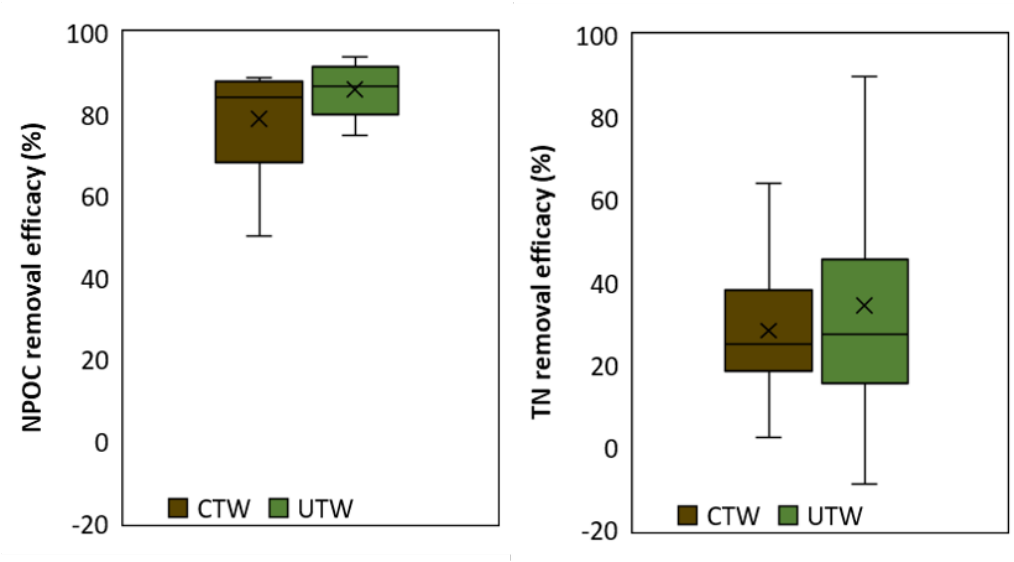

Fig. 3.7: Total Carbon (NPOC) and Total Nitrogen (TN) removal efficacy (left and right, respectively) in the clogged and unclogged treatment wetland (CTW and UTW, respectively)

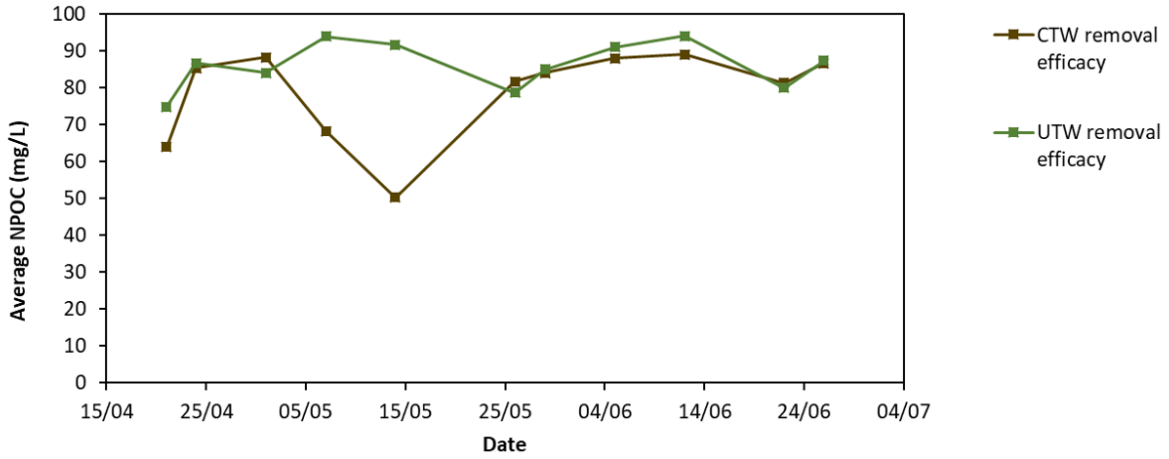

Fig. 3.8: Total Carbon (NPOC) removal efficacy of the clogged and unclogged treatment wetland (CTW and UTW, respectively) over time

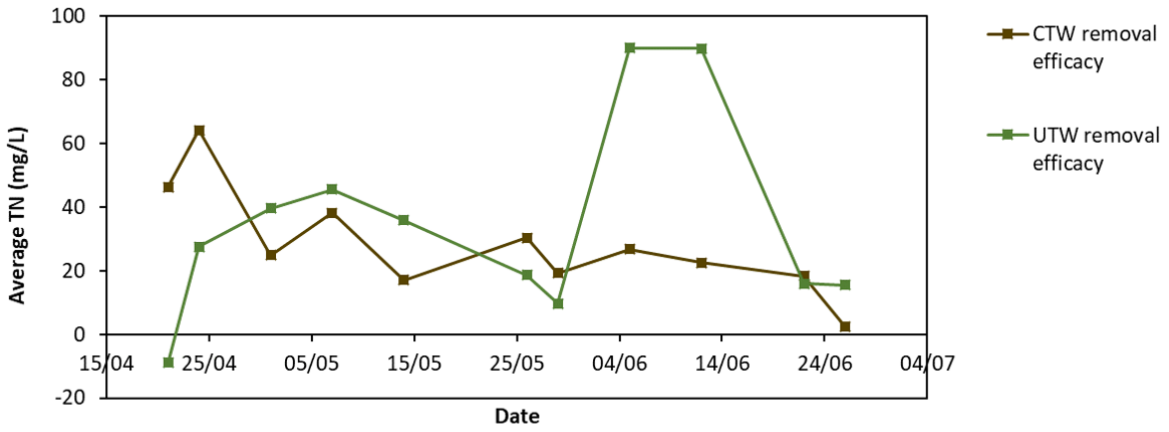

Fig. 3.9: Total Nitrogen (TN) removal efficacy of the clogged and unclogged treatment wetland (CTW and UTW, respectively) over time

Table 3.3: Spearman's correlation between Total Carbon (NPOC) and Total Nitrogen (TN) removal efficacy and physico-chemical parameters for the clogged and unclogged treatment wetland (CTW and UTW, respectively). Green shading indicates significant value

		Environmental parameters			Nutrient concentrations	
		Water Temp	**pH**	**DO**	**Average NPOC**	**Average TN**
NPOC removal efficacy	**CTW**	0.20	0.56	0.10	0.03	0.47
	UTW	0.13	-0.16	-0.06	0.84	0.28
TN removal efficacy	**CTW**	-0.68	-0.19	0.15	-0.22	-0.49
	UTW	-0.19	0.11	0.59	-0.22	0.32

3.3 Gas Fluxes

3.3.1 Temporal variation in gas fluxes

Gas fluxes for both CH_4 and N_2O were calculated for midday and afternoon (Table 3.4). High positive correlations between midday and afternoon CH_4 fluxes were observed for the inlet and outlet of the CTW and the inlet and middle of the UTW (Table 3.4). For N_2O, high positive correlations between midday and afternoon fluxes were observed in the outlet of the CTW and the inlet and outlet of the UTW (Table 3.4). No significant difference in gas fluxes were observed between midday and afternoon for both the CTW and the UTW, hence the midday and afternoon fluxes were averaged and further analysed (Table 3.4).

Table 3.4: Comparison of midday and afternoon methane (CH_4) and nitrous oxide (N_2O) fluxes from the clogged and unclogged treatment wetland (CTW and UTW, respectively) between 21 April to 26 June 2017

		CH_4 flux		**N_2O flux**	
		Correlation	***p*-value**	**Correlation**	***p*-value**
CTW	Inlet	0.87	0.43	0.04	0.15
	Middle	0.35	0.24	0.96	0.45
	Outlet	0.97	0.34	0.89	0.15
UTW	Inlet	0.93	0.41	0.96	0.07
	Middle	0.25	0.48	0.54	0.15
	Outlet	0.12	0.11	0.84	0.16

3.3.2 Fluxes of methane

CH_4 emissions in the CTW ranged from 0.79 and 4.46 $\mathrm{mg\,m^{-2}\,h^{-1}}$ and the mean flux was 2.40 $\pm$ 0.55 $\mathrm{mg\,m^{-2}\,h^{-1}}$. Fluxes were significantly higher at the inlet (5.30 $\pm$ 1.09 $\mathrm{mg\,m^{-2}\,h^{-1}}$), the second greatest fluxes were observed at the outlet (1.20 $\pm$ 0.50 $\mathrm{mg\,m^{-2}\,h^{-1}}$), and the lowest fluxes were observed at the middle section (0.70 $\pm$ 0.14 $\mathrm{mg\,m^{-2}\,h^{-1}}$) (Fig. 3.10). The inlet experienced the broadest range of CH_4 fluxes, ranging from 1.20 to 11.25 $\mathrm{mg\,m^{-2}\,h^{-1}}$. On average, CH_4 fluxes at the inlet were 4.4 times greater than at the outlet, and 7.6 times greater than at the middle section. CH_4 emissions initially increased until obtaining a maximum flux of 11.25 $\mathrm{mg\,m^{-2}\,h^{-1}}$ on 5 June and decreasing until 26 June when it increased again (Fig. 3.10). The outlet also experienced progressively greater CH_4 fluxes until reaching a maximum of 5.10 $\mathrm{mg\,m^{-2}\,h^{-1}}$ on 26 May where it then decreased to <1.00 $\mathrm{mg\,m^{-2}\,h^{-1}}$ for the remaining of the sampling period. The middle section also experienced a slight increase in CH_4 fluxes over time, but the fluxes decreased again towards the end of the sampling period.

For the UTW, CH_4 emissions ranged from 0.03 to 1.13 $\mathrm{mg\,m^{-2}\,h^{-1}}$ and the mean CH_4 flux was 0.43 $\pm$ 0.17 $\mathrm{mg\,m^{-2}\,h^{-1}}$. The CH_4 fluxes were significantly higher at the inlet (1.25 $\pm$ 0.41 $\mathrm{mg\,m^{-2}\,h^{-1}}$) and were low at both the middle and outlet section (0.02 $\pm$ 0.01 and 0.03 $\pm$ 0.01 $\mathrm{mg\,m^{-2}\,h^{-1}}$, respectively) (Fig. 3.11). The inlet experienced the broadest range of CH_4 fluxes, ranging from 0.04 to 3.37 $\mathrm{mg\,m^{-2}\,h^{-1}}$. On average, CH_4 fluxes at the inlet were 62.2 times higher than at the middle section and 38.2 times higher than at the outlet. CH_4 fluxes at the inlet were fairly consistent until 29 May when the fluxes increased significantly from 0.17 to 3.23 $\mathrm{mg\,m^{-2}\,h^{-1}}$ (Fig. 3.11). The middle and outlet section had consistently low CH_4 fluxes (Fig. 3.11).

Over the duration of the study, the CTW had significantly higher emissions of CH_4 than the UTW

at the inlet, middle, and outlet sections (Fig. 3.12). Overall, CH_4 fluxes were on average 5.54 times greater in the CTW. Compared with the UTW, the CH_4 fluxes from the CTW were on average 4.3 times greater at the inlet, 34.7 times greater at the middle, and 36.6 times greater at the outlet.

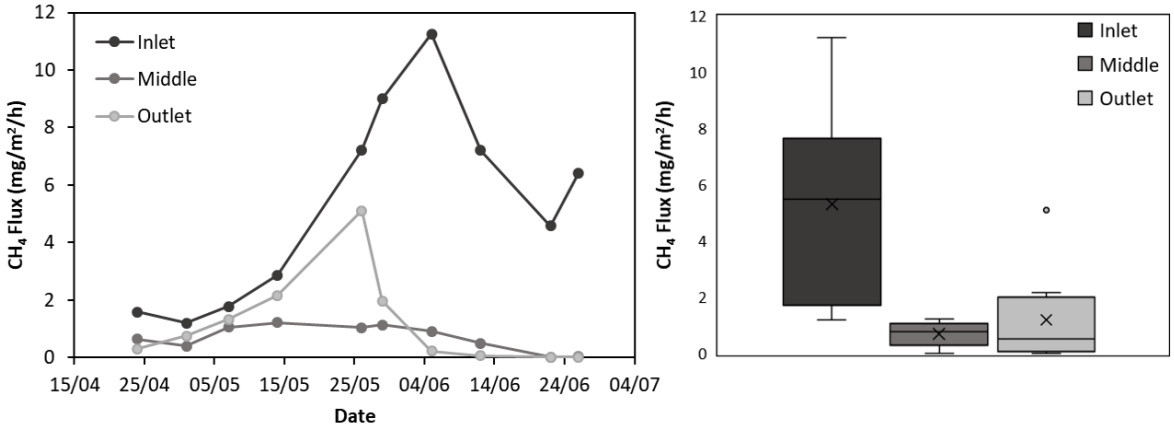

Fig. 3.10: Methane (CH_4) flux from the inlet, middle, and outlet sections of the clogged treatment wetland (CTW) over time (left) and distribution of data (right)

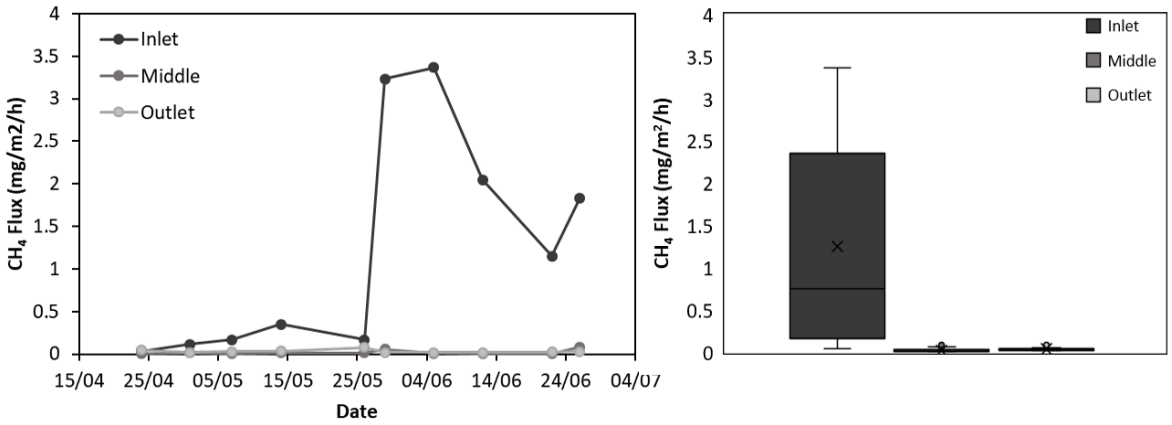

Fig. 3.11: Methane (CH_4) flux at the inlet, middle, and outlet sections of the unclogged treatment wetland (UTW) over time (left) and distribution of data (right)

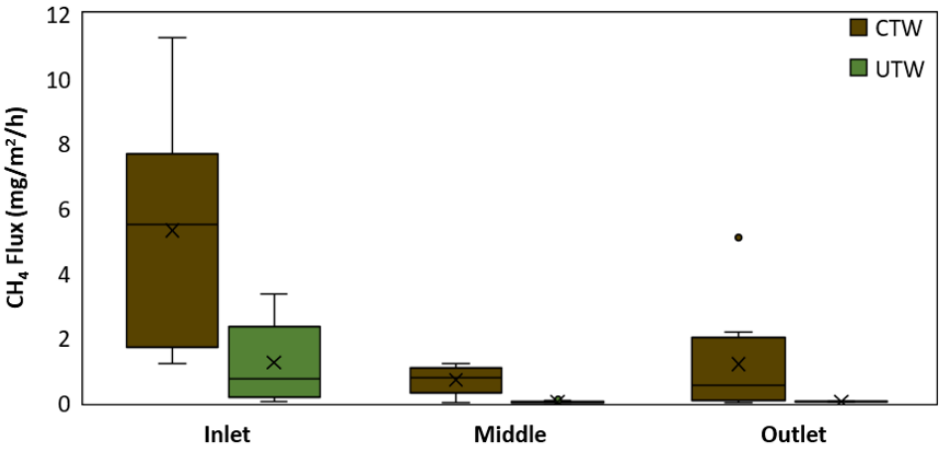

Fig. 3.12: Comparison of methane (CH_4) emissions at the inlet, middle, and outlet sections of the clogged and unclogged treatment wetland (CTW and UTW, respectively)

3.3.3 Fluxes of nitrous oxide

The N_2O fluxes in the CTW ranged from 0.04 to 29.24 $\mu g\,m^{-2}\,h^{-1}$ and the mean flux was 9.40 $\pm$ 3.88 $\mu g\,m^{-2}\,h^{-1}$. In the CTW, N_2O fluxes were highest at the outlet (18.57 $\pm$ 9.48 $\mu g\,m^{-2}\,h^{-1}$), followed by the middle (9.41 $\pm$ 4.88 $\mu g\,m^{-2}\,h^{-1}$), and were lowest at the inlet (0.23 $\pm$ 0.04 $\mu g\,m^{-2}\,h^{-1}$) (Fig. 3.13). On average, N_2O fluxes at the outlet were 2.0 times greater than at the middle and 80.8 times greater than at the inlet. The outlet experienced a broad range of fluxes that were low and stable until 5 June when N_2O flux jumped from 0.5 to 84.6 $\mu g\,m^{-2}\,h^{-1}$, the maximum flux experienced over the study period (Fig. 3.13). After 5 June the N_2O flux steadily decreased (Fig. 3.13). At the middle section, N_2O fluxes were also low and stable until 5 June when the flux increased steadily, experiencing a maximum flux of 38.4 $\mu g\,m^{-2}\,h^{-1}$ on the last day of sampling (26 June) (Fig. 3.13). The inlet experienced low (< 0.4 $\mu g\,m^{-2}\,h^{-1}$) and stable N_2O fluxes for the entirety of the sampling period (Fig. 3.13).

For the UTW, N_2O emissions ranged from 0.59 to 9.87 $\mu g\,m^{-2}\,h^{-1}$ and the average flux was 4.42 $\pm$ 1.10 $\mu g\,m^{-2}\,h^{-1}$. The N_2O fluxes in the UTW were significantly greater at the inlet (12.08 $\pm$ 3.16 $\mu g\,m^{-2}\,h^{-1}$) and low at both the middle and outlet sections (0.80 $\pm$ 0.12 and 0.38 $\pm$ 0.07 $\mu g\,m^{-2}\,h^{-1}$, respectively) (Fig. 3.14). On average, N_2O fluxes at the inlet were 15.1 times higher than at the middle section and 32.1 times higher than at the outlet. The N_2O fluxes at the inlet increased steadily from 7 May, levelled out for 3 sampling days, then peaked to 28.0 $\mu g\,m^{-2}\,h^{-1}$ on 22 June, producing the maximum flux for the sampling period, before decreasing again (Fig. 3.14). Fluxes at the middle section fluctuated between 0.3 and 1.26 $\mu g\,m^{-2}\,h^{-1}$ whereas fluxes remained <0.72 $\mu g\,m^{-2}\,h^{-1}$ at the outlet for the entirety of the study (Fig. 3.14).

Overall, the CTW had greater fluxes of N_2O, though not significant, averaging 2.1 times greater than the UTW (Fig. 3.15). In contrast to the UTW, the CTW experienced 49.3 times greater N_2O fluxes at the outlet and 11.8 time greater fluxes at the middle section, however these differences were not significant (Fig. 3.15). At the inlet, however, the UTW experienced significantly greater N_2O fluxes, averaging 52.6 times greater than at the inlet of the CTW (Fig. 3.15).

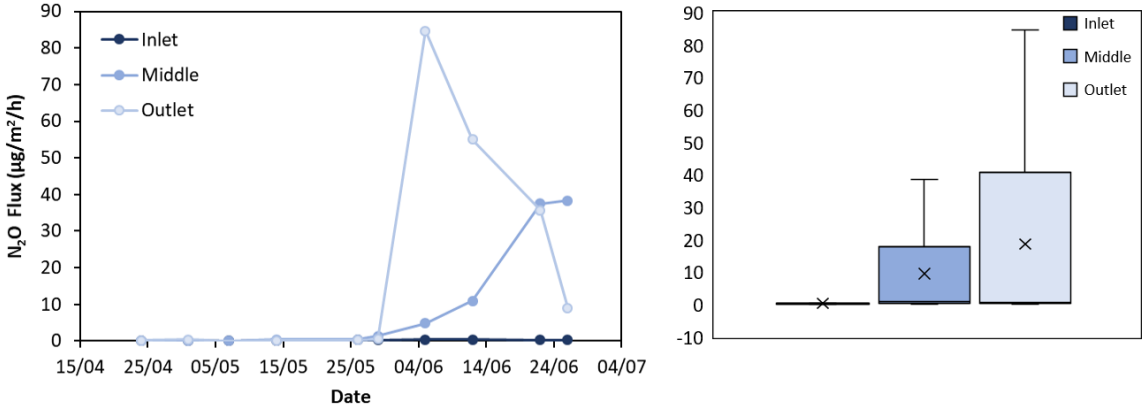

Fig. 3.13: Nitrous oxide (N_2O) flux of the inlet, middle, and outlet sections of the clogged treatment wetland (CTW) over time (left) and distribution of data (right)

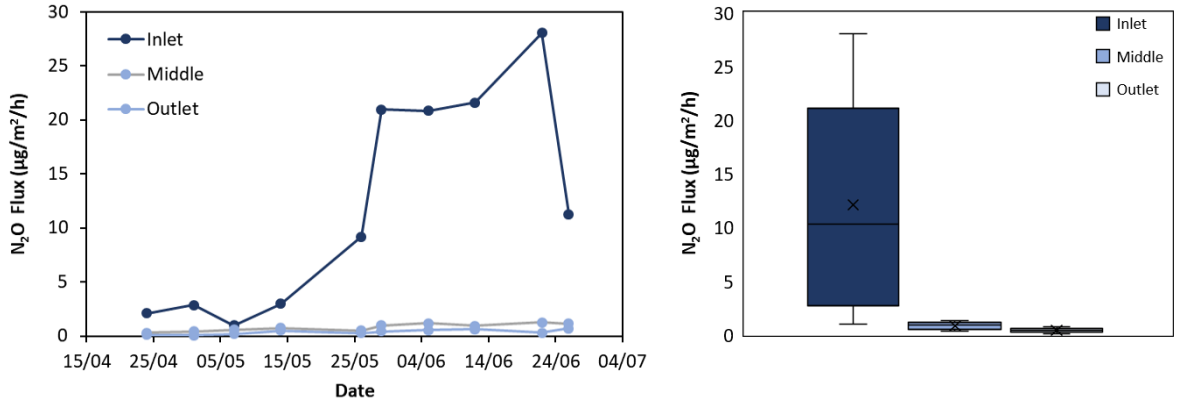

Fig. 3.14: Nitrous oxide (N_2O) flux at the inlet, middle, and outlet sections of the unclogged treatment wetland (UTW) over time (left) and distribution of data (right)

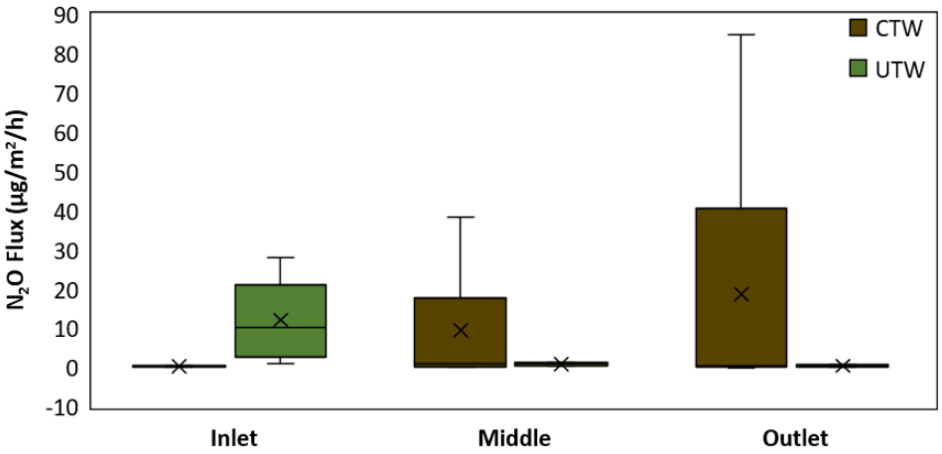

Fig. 3.15: Comparison of nitrous oxide (N_2O) emissions at the inlet, middle, and outlet sections of the clogged and unclogged treatment wetland (CTW and UTW, respectively)

3.3.4 Effect of partial clogging event on greenhouse gas emissions in inlet of unclogged treatment wetland

As the inlet of the UTW recently experienced a clogging event at position 5 (Fig. 2.2), it was therefore hypothesised that this section of the TW would experience similar CH_4 and N_2O emissions to the CTW, particularly at the inlet. To determine if this hypothesis was true, the three positions of the UTWs inlet were compared with one another, with the other sections of the UTW, and to the average emissions at the inlet of the CTW.

From Fig. 3.16, it can be observed that position 5 experienced significantly greater CH_4 emissions than positions 4 and 6 (average of 3.12 $\pm$ 1.02, 0.33 $\pm$ 0.06, and 0.013 $\pm$ 0.01 $\mu g\,m^{-2}\,h^{-1}$, respectively), releasing on average 9.4 times more CH_4 than position 4 and 109.7 times more than position 6. When the emissions from position 5 are ignored, the average CH_4 emissions at positions 4 and 6 (0.18 $\pm$ 0.04 $\mu g\,m^{-2}\,h^{-1}$) were still significantly higher than the average emissions from the middle and outlet of the UTW. Although position 5 experienced significantly greater CH_4 emissions than the rest of the inlet of the UTW, when contrasted with the average emissions from the inlet of the CTW (5.30 $\pm$ 1.09 $mg\,m^{-2}\,h^{-1}$), position 5 had lower emissions.

For N_2O, it can be observed that position 5 had significantly less N_2O emissions than positions 4 and 6 (0.28 $\pm$ 0.11, 20.22 $\pm$ 6.96, and 24.18 $\pm$ 7.56 $\mu g\,m^{-2}\,h^{-1}$, respectively), emitting on average 71.4 times less than position 4 and 24.1 times less than position 6 (Fig. 3.16). When the emissions from position 5 are ignored, the average N_2O emissions from positions 4 and 6 (22.20 $\pm$ 4.94 $\mu g\,m^{-2}\,h^{-1}$) was still significantly greater than the average emissions at the middle and outlet of the UTW. The N_2O emissions at position 5 in the inlet of the UTW were closer to the average emitted at the inlet of the CTW (0.28 $\pm$ 0.11 and 0.23 $\pm$ 0.04 $\mu g\,m^{-2}\,h^{-1}$, respectively).

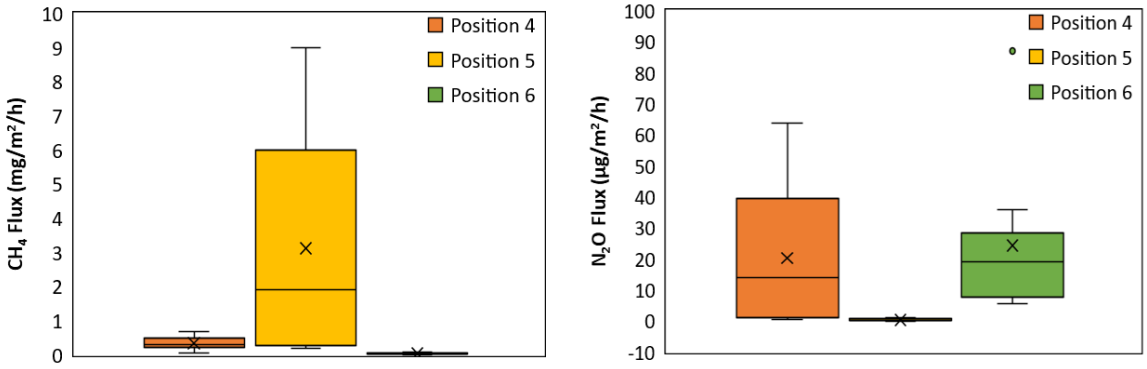

Fig. 3.16: Comparison of methane (CH_4) (left) and nitrous oxide (N_2O) (right) emissions at positions 4, 5, and 6 of the unclogged treatment wetland

3.4 Influence of nutrient concentrations and environmental parameters on gas emissions

From Table 3.5, it can be seen that CH_4 fluxes were significantly positively correlated with NPOC and TN for both TWs, and also with pH for the UTW. N_2O fluxes in the CTW were significantly negatively correlated with NPOC and were significantly positively correlated with water temperature, pH, NPOC, and TN in the UTW (Table 3.5).

Table 3.5: Spearman's correlation between methane (CH_4) and nitrous oxide (N_2O) fluxes and physico-chemical parameters for the clogged and unclogged treatment wetland (CTW and UTW, respectively). Green shading indicates significant values

		Environmental parameters			Nutrient concentrations	
		Water Temp	pH	DO	Average NPOC	Average TN
CH_4 flux	**CTW**	0.21	0.52	0.53	0.50	0.55
	UTW	0.18	0.67	0.11	0.78	0.76
N_2O flux	**CTW**	0.35	-0.26	-0.55	-0.58	0.44
	UTW	0.51	0.57	0.32	0.67	0.72

3.5 Global warming potential

The mean CH_4 and N_2O emissions at the inlet, middle, and outlet of the CTW and the UTW were converted to CO_2 equivalents ($\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$) using the calculation factors given by the *EPA*, 2013 (Table 3.6). The average CH_4 flux for the CTW was 50.41 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ and 9.10 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ for the UTW. For N_2O, the average flux for the CTW was 2.91 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ and 1.37 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ for the UTW (Table 3.6).

Table 3.6: Mean flux ± standard error (S.E) of methane (CH_4) and nitrous oxide (N_2O) at the inlet, middle, and outlet of the clogged and unclogged treatment wetland (CTW and UTW, respectively) and their carbon dioxide (CO_2) equivalentss ($\mathrm{mg\,m^{-2}\,h^{-1}}$)

		CH_4 flux		**N_2O flux**		**CO_2 equivalents**			
		Mean	**S.E**	**Mean**	**S.E**	**CH_4**	**S.E**	**N_2O**	**S.E**
TW1	**Inlet**	5.31	1.09	2.30E-04	3.96E-05	111.51	22.92	0.07	0.01
	Middle	0.70	0.14	9.40E-03	4.88E-03	14.61	2.99	2.92	1.51
	Outlet	1.20	0.50	1.86E-02	9.48E-03	25.11	10.57	5.76	2.94
	Average	2.40	0.58	9.40E-03	4.80E-03	50.41	12.16	2.91	1.49
TW2	**Inlet**	1.25	0.41	1.21E-02	3.16E-03	26.20	8.66	3.75	0.98
	Middle	0.02	0.01	8.00E-04	1.09E-04	0.42	0.18	0.25	0.03
	Outlet	0.03	0.01	3.77E-04	7.07E-05	0.69	0.12	0.12	0.02
	Average	0.43	0.14	4.42E-03	1.11E-03	9.10	2.99	1.37	0.35

The conversion to CO_2 equivalents is given with 310 for N_2O and 21 for CH_4 (47).

CHAPTER 4

Discussion

4.1 Treatment wetland physical evolution throughout study

The most notable physical changes that occurred over the course of the study were to plant biomass and the degree of wastewater pooling and overland flow in the CTW. The macrophytes in both TWs grew significantly over the course of the study, growing from around 0.2 m to 2 m in height and attaining a large amount of aboveground biomass. Though it was not quantified, it is expected that the macrophytes played a significant role in the assimilation of nutrients from wastewater, particularly C and N (32, 33). As the aboveground biomass is harvested yearly, the assimilated nutrients are therefore removed from the system.

The CTW is considered severely clogged and its hydrological conditions changed considerably during sampling period (Fig. 3.2). Initially, the pooled wastewater measured up to 30 cm in depth (Fig. 3.1). Although it was not quantified, until 14 May it is believed that most of the wastewater was transported horizontally via overland flow and hence bypassed the TW media and outlet, rather leaving the system by overflowing directly into TW 2, and caused reduced HRT. From 14 May, the CTW began to dry up and overflow into TW 2 stopped (Fig. 2.1). By the end of sampling, most of the middle and outlet section had dried substantially to the point that there was no overland flow of wastewater (Fig. 3.2). However, the clog matter overlaying the media, which has accumulated from prolonged overland flow of wastewater and mechanical retention processes, remained semi-saturated (Fig. 3.2). The drying event was likely the result of increased temperatures and radiation associated with the progression of Spring, which resulted in a greater amount of water being lost to the atmosphere through evapotranspiration processes (1). Only a small portion of the influent wastewater is believed to have exited the system via the outlet. Hence, it was assumed that, initially, the entire CTW system behaved in a similar manner to a surface flow (SF) TW, and later, following drying, the inlet behaved more like a settling pond. Subsequently, the different media composition between the CTW and UTW -fine-grained sediment and surrounding soil, respectively - is assumed to have had no influence on the results obtained.

The UTW, on the other hand, experienced early stage clogging that resulted in ponding of wastewater at position 5 and in a portion of position 6 at the inlet (Fig. 2.2). This is likely due to a combination of subsurface clogging resulting from the accumulation of clog matter at the subsurface inlet and biofilm clogging, which commonly occurs in the inlet section (5, 53). Overland flow occurred until the middle section where hydrological conductivity and flow gradient were sufficient for the wastewater to flow below the media (Fig. 2.2 and3.3) (1). Ponding of water appeared to become worse as time progressed. Like the CTW, the UTW produced less effluent output towards the end of the sampling period, which was also likely due to increased evapotranspiration processes. The ponded wastewater at the inlet did not appear to be affected by the progression of Spring.

4.2 Wastewater quality and nutrient removal efficacy

Although the amount of wastewater entering the systems was not quantified for this study, it was assumed that both TWs received the same volume of influent wastewater at the same rate. However, the delivery of the wastewater differed, with the CTW receiving wastewater via a surface inlet and the UTW via a subsurface inlet. Influent wastewater parameters (NPOC, TN) and values (temperature and pH) were highly positively correlated for the CTW and the UTW (Table 3.1 and Fig. 3.4), meaning the TWs were receiving homogeneous pre-treated wastewater. DO differed between the two TWs, though not significantly, with a higher average DO measured in the CTW (Table 3.1). This may be explained by differences in sampling points as a result of surface and subsurface delivery of wastewater; for the UTW, samples were taken from the access shaft of the storage tank, whereas for the CTW, samples were taken from the inlet pipe where the wastewater was allowed to flow or drip into the collection bottle, which may have led to increased aeration and hence DO concentration.

After having undergone treatment by the TWs, the effluent concentrations of NPOC and TN from the CTW and the UTW were both significantly less than the influent concentrations (Fig. 3.5 and Fig. 3.6). No significant differences in NPOC removal efficacy were observed between the CTW and the UTW. Both TWs exhibited high overall NPOC removal efficacy (79.0 $\pm$ 3.8% and 86.1 $\pm$ 1.9%, respectively) (Table 3.2). The NPOC removal efficacy in both TWs was higher than the average efficacy of 48.9% reported by *Søvik et al.*, 2006, but was comparable to the 82% recorded by *Jakubaszek and Sadecka*, 2015. A significant positive correlation between NPOC removal efficacy and NPOC loading was observed for both TWs (Spearman's rank correlation = 0.73 and 0.84 for the CTW and UCW, respectively), except for 7 and 14 May when the removal efficacy was negatively correlated in the CTW (Table 3.3 and Fig. 3.8). On 7 and 14 May, there was a noticeable drop in NPOC removal efficacy, which decreased by up to 36.5%, that occurred when influent NPOC concentrations increased substantially above-average (Fig. 3.8). This may indicate that the CTW is not able to adapt its nutrient removal mechanisms to accommodate increased NPOC loading, whereas the UTW is more robust and is able to.

For overall TN removal, no significant difference in TN removal efficacy was observed between the TWs, however the UTW had on average a 10% greater removal efficacy than the CTW (Table 3.2). The average TN removal efficacy of the CTW and the UTW (24.1% $\pm$ 3.5 and 34.4% $\pm$ 9.4, respectively) was less than the average removal efficacy of 42.3% reported by *Vymazal*, 2007 and 40.4% reported by *Jahangir et al.*, 2016 for HSSF TWs. No significant correlations were observed between TN removal efficacy and the measured physico-chemical parameters for both the CTW and UTW (Table 3.3). For the CTW, a negative trend in TN removal efficacy was observed over time and TN removal efficacy was significantly negatively correlated with temperature (Spearman's rank correlation = -0.68) (Fig. 3.9). The UTW had two outlying TN removal efficacy values on 5 and 12 June 2017; on these days, the TN removal efficacy was 345.5% greater than average (Table 3.2 and Fig. 3.9). This significant increase in removal efficacy was likely the result of the samples being taken from stagnant water due to minimal effluent output. It is therefore expected that, in the stagnant sampling point, N was further biologically acted on and removed. Further exposure to biological activity would also explain why, on 12 June, the effluent pH was recorded as 4 - drastically below the average effluent pH of 6.6 - as pH reduction can occur due to acid production from the nitrification process (24). Minimal effluent output was likely due to the warm and dry conditions that preceded those sampling days, which would have resulted in much of the water being lost through evaporation and transpiration by the maturing macrophytes. For this reason, the two outlying data points will henceforth be excluded from further analysis and interpretation. When these two points are excluded, a slight negative correlation between influent TN and TN removal efficacy (Spearman's rank correlation = -0.25) was observed for the UTW, though not significant.

It was originally hypothesised that the CTW would have lower NPOC and TN removal efficacy than the UTW due its differences in water flow regime and changes in hydrology over the course of the study. As previously mentioned, the CTW initially behaved more like a SF TW than a SSF TW until the CTW dried, overflow of wastewater into TW 2 ceased, and wastewater left the system most likely via evapotranspiration processes and to a small extent by drainage, the primary means however is unknown. As no significant differences in nutrient removal efficacy were observed, it is therefore hypothesised that removal mechanisms within the free water zone (of the ponded wastewater) and the accumulated clog matter overlaying the media compensated to achieve similar nutrient removal efficacies as the SSF UTW. For NPOC removal, it is believed that anaerobic degradation of NPOC played a larger role in the CTW than in the UTW. This is investigated in further detail in Section 4.3.2.

For TN removal, in the free water zone, denitrification - the major mechanism of TN removal in HSSF TWs - is expected to have been limited by the availability of NO_2^- due to limited oxygen and hence nitrification in the CTW (26, 32, 64). Hence, a combination of processes are expected to have worked together in the free water zone to removal TN from the CTW, namely assimilation, ammonium volatilisation, and ANAMMOX, though the extent of ANAMMOX processes in the removal of TN are unknown but were likely to have taken place in the sub-oxic and anoxic parts of both TWs (2, 24).

N assimilation, primarily by blue-green algae (cyanobacteria) as well as periphytic algal assemblages and other microorganisms described in Section 1.3.3, is suspected to have played a considerable role in TN removal in the CTW. However, the removal of N via assimilation is only short-term and nutrients are released back into circulation upon decay (ammonification) of algal tissue (54). Blue-green algae and periphytic algae were observed throughout the study and their populations appeared to decrease over time, which may explain the observed decrease in TN removal efficacy over time (Fig. 3.9). Though no samples from the free water zone were taken in the middle of the day, it is well-documented that the presence of algae and microorganisms such as phytoplankton can generate high pH values (> 10) during the day through their photosynthetic activity, providing suitable conditions for ammonia volatilisation to take place (2, 25). Ammonium volatilisation is a physical removal process whereby volatile ammonia gas is removed through mass transfer from the water surface to the atmosphere (called off-gassing) (2). In TWs with an open water surface - like the CTW for a majority of the study - volatilisation may be a significant route of N removal when pH was above 8 (25).

As the CTW progressively dried and ponding became confined to the inlet section, it is believed that incomplete denitrification processes in the middle and outlet sections played a significant role in TN removal. This is thought to result as drying decreased the water table of the TW, aerating the accumulated clog matter overlaying the media and making NH_4^+ available for nitrification and creating substrate for denitrification (17). As the clog matter remained semi-saturated throughout the sampling period, likely the result of the saturated underlying media and rain events, anoxic and oxic pockets likely formed, allowing nitrification-denitrification to occur concurrently. However, as conditions were sub-oxic, denitrificaiton was likely incomplete, emitting higher fluxes of N_2O. N removal by incomplete denitrification is investigated in greater detail in Section 4.3.3.

4.3 Gas fluxes

4.3.1 Temporal and seasonal variation in nutrient removal and gas fluxes

No significant differences in CH_4 and N_2O fluxes were observed between midday and afternoon, indicating that the TW systems are not sensitive to slight daily changes in temperature and radiation (Table 3.4). Although no obvious positive trend in CH_4 was observed as Spring progressed, seasonal variation in CH_4 fluxes are expected to occur; it is well documented that, as water, air, and/or soil temperature increases, CH_4 emissions also increase (8). With regards to N_2O, despite there being definite relationships between temperature and rates of nitrification and denitrification, no clear relationship between temperature and rates of N_2O emissions in SSF TWs has been identified, hence seasonal variation in temperature is not expected to influence N_2O fluxes in this system (8, 25, 30, 31, 35, 38–40, 51).

4.3.2 Fluxes of methane

The range of CH_4 emissions from the CTW (0.79 to 4.46 $\mathrm{mg\,m^{-2}\,h^{-1}}$) fell within the lower end of the range estimated for HSSF TWs (0.048 - 17.5 $\mathrm{mg\,m^{-2}\,h^{-1}}$) (8). For the UTW, the lower end of its range (0.03 to 1.13 $\mathrm{mg\,m^{-2}\,h^{-1}}$) was below the range estimated for HSSF TWs. The average CH_4 emissions for the CTW and UTW (2.4 $\pm$ 0.55 and 0.43 $\pm$ 0.17 $\mathrm{mg\,m^{-2}\,h^{-1}}$, respectively) were less than the estimated average of 7.4 $\mathrm{mg\,m^{-2}\,h^{-1}}$ (8).

CH_4 emissions were significantly higher in the CTW than the UTW, averaging 5.4 times greater (Fig. 3.12). This is indicative of anaerobic activity and is expected to be the result of clogging, as the media was generally inundated with wastewater causing the media to remain anoxic - except for sites adjacent to macrophyte roots - and hence provided an ideal environment for anaerobic degradation of organic compounds to take place (15, 65). Higher CH_4 emissions in the CTW may also be due to decreased CH_4 consumption; a large portion (1 - 90%) of CH_4 produced in wetlands is believed to likely be consumed again under ideal conditions (66). CH_4 consumption involves the oxidisation of CH_4 by methanotrophs at the oxic-anoxic interface of wetlands in the oxic top layer or in the rhizosphere (66). As CH_4 produced in the media and clog matter of the CTW was emitted directly into the atmosphere through off-gassing, a minimal amount of the CH_4 produced was likely to have been consumed, which would result in higher emissions of CH_4 (66). It is expected that when the TW media is exposed to the atmosphere - like with the UTW - CH_4 is consumed and overall CH_4 emissions are reduced. Lastly, as the system was designed as a HSSF TW and not a SF TW, the system is likely subject to high loading that it cannot accommodate, which is known to cause higher CH_4 emissions (8). This is supported by the aforementioned decrease in NPOC removal efficacy that occurred when influent NPOC concentrations were substantially above-average (Section 4.2).

Both TWs experienced spatial variation and trends in CH_4 emissions (Fig. 3.10 and 3.11). The inlet section of both TWs experienced the highest emissions of CH_4 (Fig. 3.12). This is consistent with literature and is the result of higher loading rates of suspended solids, nutrients, and importantly BOD, which results in an anoxic environment (51, 67–69). Furthermore, nutrient concentrations are known to decrease with increasing distance from the inlet, reducing the substrate available for degradation and transformation (67). This relationship can further be supported by the overall significant positive correlation observed between CH_4 flux and NPOC and TN concentrations (Table 3.5).

For the CTW, higher emissions at the inlet can be attributed to the anoxic, waterlogged conditions and the higher concentrations of nutrients and BOD in the influent wastewater (Table 3.5). For the UTW, the primary cause of higher CH_4 emissions at the inlet can be attributed to be the clogging at position 5

and to a small extent 6 at the inlet (Fig. 2.2 and 3.16). Examination of position 5 revealed that it emitted significantly greater fluxes of CH_4 than the other two positions at the inlet (Fig. 3.16). It is expected that position 5 become anoxic due to the clogging event, causing a significant increase in CH_4 emissions (Fig. 3.16) (65). The clogging at position 6 did not significantly impact on the gas sampling point and hence no increase in CH_4 emissions were observed (Fig. 3.16). When the CH_4 emissions from position 5 were ignored, CH_4 emissions for the inlet section were still significantly higher than the middle and outlet sections, indicating that higher concentrations of nutrients and BOD in the influent also influenced the inlet of the UTW.

4.3.3 Fluxes of nitrous oxide

Overall, the CTW experienced higher emissions of N_2O than the UTW, though not significant, averaging 2.1 times greater (average 9.40 ± 3.88 and 4.42 ± 1.10 $\mu g\,m^{-2}\,h^{-1}$, respectively). N_2O emissions from the CTW and UTW (0.04 to 29.24 and 0.59 to 9.87 $\mu g\,m^{-2}\,h^{-1}$) fell within the range of N_2O emissions estimated for HSSF TWs (0 - 849 $\mu g\,m^{-2}\,h^{-1}$) but were well below the average of 240 $\mu g\,m^{-2}\,h^{-1}$ (8).

The N_2O emissions from the CTW and the UTW displayed different spatial and temporal variation and trends. In the CTW, N_2O fluxes were low (< 0.5 $\mu g\,m^{-2}\,h^{-1}$) in all sections until 5 June, when emissions spiked suddenly and significantly at the outlet before steadily decreasing, and when emissions increased steadily at the middle (Fig. 3.13). No change in emissions were observed at the inlet (Fig. 3.13). Although soil moisture and the water table depth were not measured in this study, field observations link increases in N_2O emissions with the drying up of the middle and outlet section of the TW. As the outlet is located farthest from the inlet, this section dried before the middle section, which may explain why N_2O emissions at the outlet were higher than at the middle section (Fig. 3.13). Studies have found that N_2O emissions increase with decreases in the height of the water table, likely due to the resulting aeration of the media which hinders complete denitrification, meaning greater amounts of N_2O would result (2, 36, 51, 70). Hence, the opposite should also be true. To test this hypothesis, position 5 in the UTW was again analysed and was found to emit significantly less N_2O emissions than position 4 and 6 (Fig. 3.16). This is likely because conditions were anoxic at position 5, which did not experience drying during the sampling period, and provided ideal conditions for complete denitrification of $NO_3{}^-$ to N_2 to take place, reducing N_2O emissions. After June 6, N_2O emissions gradually decreased. This was likely caused by progressively greater aeration of the media, caused by further drying, which likely inhibited denitrification processes. A significant negative correlation between N_2O emissions and average NPOC concentrations was also observed (Table 3.5). This finding is supported by the findings from other studies that found that N_2O emissions increase when the C/N ratio of TW system is low (8). Analysis of the C/N ratio of the influent wastewater revealed a ratio of 1.03:1, which is lower than the optimal ratio of 5:1 to 10:1 reported by *Yan et al.*, 2012, however it should be noted this optimal ratio was reported for VSSF TWs and the optimal ration for HSSF TWs has not been investigated.

In the UTW, N_2O emissions were significantly greater in the inlet, even when position 5 was excluded, and low in the middle and outlet (Fig. 3.14). The fluxes were significantly and highly positively correlated with TN concentration in the UTW (Table 3.5). This is consistent with field studies, whereby N_2O fluxes were greatest in TWs that received the highest concentrations of TN (49, 51, 72). As nutrient concentrations decrease with increasing distance from the inlet, the lower fluxes at the middle and outlet be explained by substrate limitation (67).

4.3.4 Influence of environmental parameters on greenhouse gas emissions

pH value

pH value was found to be significantly positively correlated with CH_4 emissions in both the CTW and UTW (Table 3.5). These findings are supported by a study by *Wang et al.*, 1993, which demonstrated that, as pH decreases, CH_4 emissions decrease significantly. This may be due to the combined effects of inhibiting the activity of methanogens and by increasing soil redox potential (Eh) chemically to potentially higher values than methanogens' critical Eh value of -150 mV (73, 74). An experiment conducted by *Wang et al.*, 1993, found that, when soil pH was adjusted below 6.4 (0.2 - 0.3 pH unit lower than the natural pH range), the CH_4 production rate was significantly decreased by up to 80% (73). *Wang et al.*, 1993 also found that the highest production rate of CH_4 occurred at a pH between 6.8-7.0 (73). A study by *Dunfield et al.*, 1993 also corroborated that methanogenesis is not adapted to lower pH values. Additionally, it is possible that CH_4 consumption may outweigh production under more acidic conditions, as CH_4 oxidation still occurs in acidic wetlands (75).

In the UTW, pH was also found to be significantly positively correlated with N_2O emissions (Table 3.5). This is in agreement with a comprehensive review on interactions in soils, which concluded that N_2O emissions have been shown to be less in acidic than in neutral or slightly acidic soils (76). As the effluent pH of the UTW (pH 6.6) was on the lower end of the natural pH range for waterlogged soils (pH 6.5 - 7.5), it is therefore plausible that N_2O emissions could rise as pH rises in the UTW (77). This relationship was likely not observed in the CTW as the pH was more stable. Furthermore, the effluent of the UTW was found to be significantly (Wilcoxon Signed-Ranks Test = 7, less than 8 is significant) more acidic than the influent (average 7.1 and 6.6, respectively), whereas no significant difference was observed in the CTW. The significant decrease in pH observed in the UTW may be due to the different flow regimes between the CTW and the UTW, or perhaps due to increased rates of nitrification, which produces acids and lowers pH. Nitrification rates are expected to be higher in the UTW - in contrast to the CTW - due to ideal oxic conditions in the top layer of the media (26, 27). Increased nitrification rates would subsequently provide more substrate for denitrification processes, possibly increasing denitrification rates, which may explain why the UTW had on average a 10% greater TN removal efficacy than the CTW (Fig. 3.6) (24). Substrate availability for denitrification is believed to have been a limiting factor for TN removal in the CTW.

Dissolved oxygen

In the CTW, DO was found to be significantly positively correlated with CH_4 emissions and significantly negatively correlated with N_2O emissions (Table 3.5). As DO in the influent is not expected to have significantly impacted on the redox state of the media or clog matter, these relationships were disregarded.

Water temperature

Water temperature was found to be significantly positively correlated with N_2O emissions in the UTW (Table 3.5). Upon further investigation, significant positive correlations were also observed between temperature and N_2O emissions at the inlet of the CTW (Spearman's rank correlation = 0.71) and the inlet and outlet of the UTW (Spearman's rank correlation = 0.86). From this study, it is difficult to conclude how temperature influences N_2O emissions in the TW systems, as temperature is significantly positively correlated with influent concentrations of TN, which is known to influence N_2O emissions and does so in this study 4.3.3 (49, 51, 72). Influent TN concentrations are likely positively correlated

with temperature as, as the weather warmed, more people visited Schottenhof and presumably used the toilet facilities.

When investigated in greater detail, temperature was also found to be significantly positively correlated with CH_4 emissions in the inlet of the CTW (Spearman's rank correlation = 0.59) and UTW (Spearman's rank correlation = 0.72). As discussed in Section 4.3.1, there is a clear relationship between temperature and CH_4 emissions, so this relationship is likely (8).

4.3.5 Emissions and Global Warming Potential

Emissions of CH_4 ranged from 18.85 to 106.99 and 0.68 to 27.14 $\mathrm{mg\,m^{-2}\,d^{-1}}$ and emissions of N_2O from 0.85 to 718.57 and 14.14 and 236.77 $\mathrm{\mu g\,m^{-2}\,d^{-1}}$ for the CTW and the UTW, respectively. As mentioned in Sections 4.3.2 and 4.3.3, CH_4 and N_2O emissions from the CTW and the UTW were lower than the estimated average. This is most likely due to lower concentrations of influent suspended solids and nutrients in contrast to the HSSF TWs assessed by *Mander et al.*, 2014.

The contribution of CH_4 to the GWP was significantly greater than the contribution of N_2O in both the CTW and the UTW, especially at the inlet section (Table 3.6). The GWP of CH_4 was greatest in the CTW, with an average of 50.41 $\pm$ 12.16 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$, whereas the UTW had an average of 9.10 $\pm$ 2.99 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ (Table 3.6). For the GWP of N_2O, the CTW again had the highest contribution, with an average of 2.91 $\pm$ 1.49 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$, and the UTW had an average of 1.37 $\pm$ 0.35 $\mathrm{mg\ CO_2\ eq\ m^{-2}\,h^{-1}}$ (Table 3.6).

Over a long time scale, the heat-trapping capacity of N_2O is more problematic than CH_4 due to its longer atmospheric lifetime (310 years in contrast to 21 years) (47, 51). Therefore, it can be argued that once clogging of a TW has occurred, minimising N_2O emissions should be prioritised over CH_4 emissions. This will be discussed further in Section 4.4.

To put the GWP of the two TWs at Schottenhof into perspective, it is important to compare TWs with conventional wastewater treatment plants treating the wastewater. *Czepiel et al.* (1995) measured N_2O emissions from wastewater treatment plants and found emissions to be 20 to 1800 $\mathrm{mg\,m^{-2}\,d^{-1}}$ for aerated processes and 10 to 40.8 $\mathrm{mg\,m^{-2}\,d^{-1}}$ for processes without aeration. *Sümer et al.* (1995) found a range of 0 to 77 $\mathrm{mg\,m^{-2}\,d^{-1}}$ N_2O from activated sludge operations, while the range of measurements of *Benckiser et al.* (1996) were much larger, from 53 to 4903 $\mathrm{mg\,m^{-2}\,d^{-1}}$. In general, all the N_2O emissions reported for conventional processes are 3 to 4 magnitudes greater than the emissions from the two TWs studied at Schottenhof, even from the severely clogged TW. It should be noted that the emissions were calculated per $\mathrm{m^2}$, and not per capita, however a Lifecycle Assessment conducted by *Fuchs et al.* (2011) has concluded that overall TWs have less environmental impact, in terms of resource consumption and GHG emissions in contrast to conventional wastewater treatment systems.

4.4 Conclusions and future recommendations

Both the CTW and UTW experienced high NPOC removal efficacy (79.0 $\pm$ 3.84% and 86.1 $\pm$ 1.9%, respectively), which was found to be significantly influenced by influent NPOC concentrations. TN removal efficacy, on the other hand, was lower than the average values reported for other TWs in Europe (24.1% $\pm$ 3.5 and 34.4% $\pm$ 9.4, CTW and UTW respectively). Despite being clogged, the CTW experienced no significant differences in NPOC or TN removal efficacy when compared with the UTW. It is thought that various processes within the CTW compensated to achieve a similar nutrient removal efficacy as the UTW. For NPOC removal, anaerobic degradation is believed to have been enhanced and resulted in the significantly higher emissions of CH_4 by the CTW ($2.4 \pm 0.55\ \mathrm{mg\,m^{-2}\,h^{-1}}$), which were on average 5.4 times higher than the CH_4 emissions from the UTW ($0.43 \pm 0.17\ \mathrm{mg\,m^{-2}\,h^{-1}}$). For TN removal, compensation is thought to have occurred through assimilation processes by microorganisms and by ammonium volatilisation in the free water zone, and by incomplete denitrification in the semi-saturated accumulated clog matter overlaying the media once the CTW had dried. Once drying of the CTW occurred, N_2O emissions from the CTW ($9.40 \pm 3.88\ \mathrm{\mu g\,m^{-2}\,h^{-1}}$), specifically in the middle and outlet section, were significantly higher than from the UTW ($4.42 \pm 1.10\ \mathrm{\mu g\,m^{-2}\,h^{-1}}$).

CH_4 and N_2O emissions from the CTW and the UTW were lower than reported emissions for HSSF TWs in Europe. This is thought to be the case as influent concentrations of suspended solids and nutrients were lower than those reported by *Mander et al.*, 2014. The greatest fluxes of CH_4 were observed at the inlet section and were found to be significantly positively correlated with influent concentrations of NPOC and TN, and pH, which is in agreement with other studies. N_2O emissions, on the other hand, were greatest in sections with larger areas of semi-saturated accumulated clog matter. Fluxes of N_2O in the CTW were significantly negatively correlated with DO and NPOC whereas, in the UTW, fluxes were significantly positively correlated with influent concentrations of NPOC and TN, and pH.

Overall, the TWs at Schottenhof emitted significantly greater fluxes of CH_4 than N_2O, especially in the CTW. To prevent greater emissions of CH_4 in the UTW, clog management strategies, such as more frequent maintenance, should be put in place to ensure overland flow of wastewater does not progressively become more severe. For the CTW, drying out the TW for CH_4 emissions reduction would be outweighed by the negative impact of increased N_2O emissions that would result. Although the contribution of CH_4 to GWP was greater than N_2O, N_2O emissions are more problematic long-term. For this reason, means to mitigate N_2O should be prioritised. Based on the findings of this study, N_2O mitigation strategies will be different for the CTW and UTW. For the CTW, if operation of the TW is still desired, it is important to ensure overland flow of wastewater is maintained. Due to the extent and duration of clogging, clog matter has accumulated on the surface of the media. When the nutrient-rich clog matter dries and becomes aerated, ample substrate becomes available for nitrification processes. If the clog matter remains semi-saturated, either due to the saturated underlying media or rewetting events (eg. rain), this favours incomplete denitrification, resulting in increased emissions of N_2O. Additionally, adjusting the C/N ratio of the influent wastewater to an optimal ratio may also limit N_2O emissions. For the UTW, ensuring that clogging does not progressively become more severe, and that overland flow of wastewater does not occur, is essential to prevent the accumulation of clog matter on the surface of the media and hence mitigate N_2O emissions.

Another interesting finding from this study is that pH manipulation of the media and/or influent wastewater has the potential to reduce N_2O and CH_4 emissions. However, further research needs to be conducted to investigate which pH range is optimal for emissions reduction while maintaining a sufficient nutrient removal efficacy. At this point in time, such a study has not yet been conducted.

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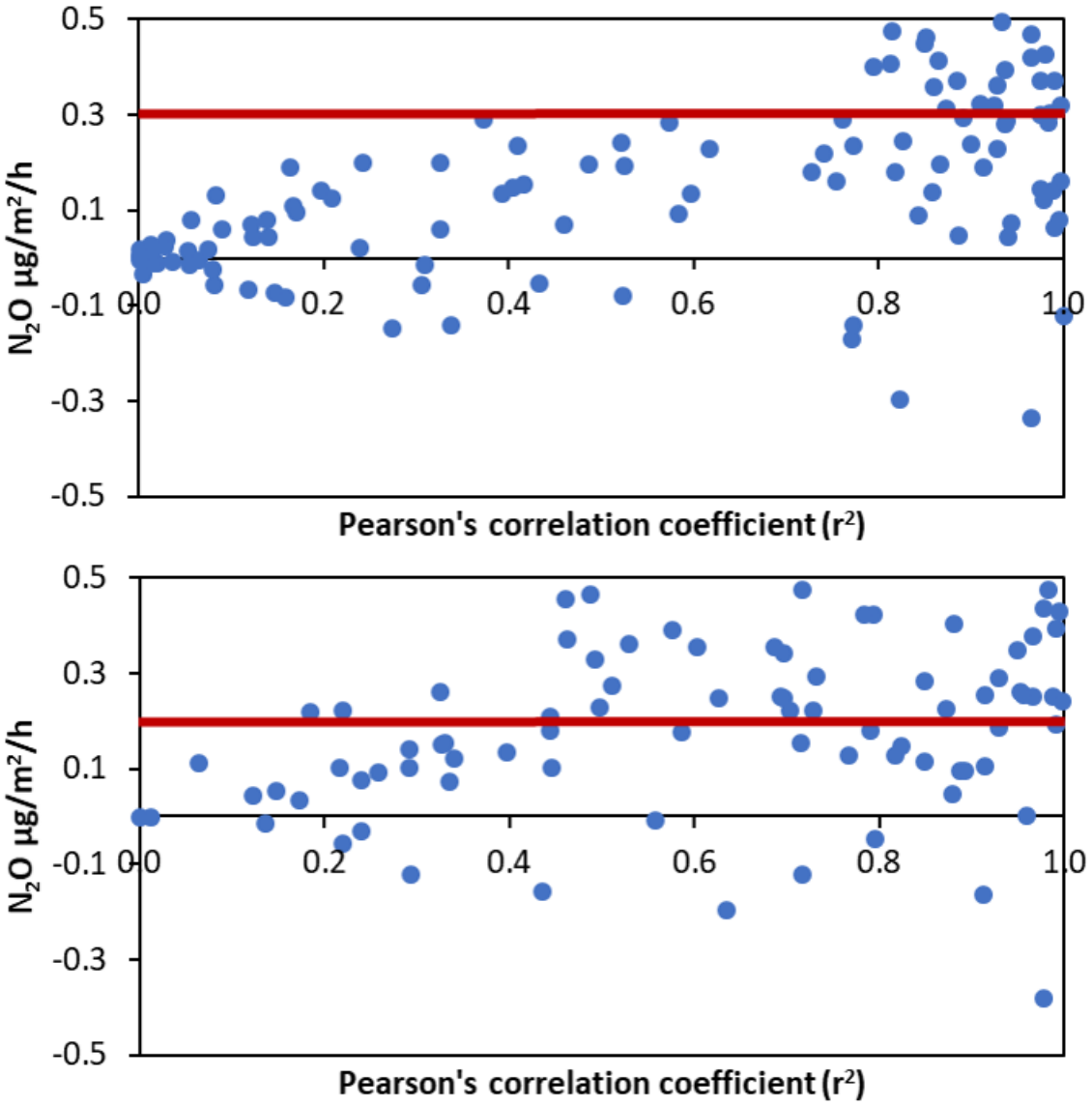
Appendix



Appendix 1: Nitrous oxide (N_2O) flux plotted against Pearson's correlation coefficient (r^2) as quality check