Nutrient removal in a slow-flowing constructed wetland treating aquaculture effluent

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ABSTRACT: Danish model trout farms (MTFs) use stream-like constructed wetlands for effluent polishing, and the industry is keen to improve wetland removal efficiency. To facilitate this, we examined longitudinal and seasonal nutrient removals in an MTF wetland with a hydraulic retention time (HRT) of 1.7 d, a free water surface (FWS) area of 7510 m², and a volume of 6008 m³. Biweekly, 24-h composite water samples were obtained for 1 yr at 6 sampling stations along the wetland. Assuming plug flow conditions, reductions in particulate and dissolved nutrient concentrations were modelled as first-order removal processes, and removal rate constants \( k_{1,A} \) were plotted to reveal seasonal fluctuations. Particulate phosphorus and organic matter \( k_{1,A} \) fluctuated more or less randomly through the year, reflecting that particulate nutrient removal predominantly takes place by sedimentation. In contrast, dissolved nitrogen, phosphorus, and organic matter \( k_{1,A} \) fluctuated seasonally, demonstrating that dissolved nutrient removal relies on biologically mediated processes. Temperature oscillations probably governed the observed seasonal fluctuations in nitrate-N \( k_{1,A} \) and could be approximated with an Arrhenius temperature coefficient of 1.07. Furthermore, denitrification appeared to be carbon-limited. Incoming dissolved phosphorous and ammonia became incorporated in the natural wetland growth cycle that included periods of net removal and release, resulting in minimal annual net removal. In summary, this study shows that improving nitrate removal in a slow-flowing MTF wetland would require some kind of carbon dosing, while further improving ammonia and phosphorus removal would require a reduction of the amounts of ammonia and dissolved phosphorus entering the wetland.

KEY WORDS: Recirculating aquaculture system · Effluent treatment · Constructed wetland · Dissolved nutrients · Particulate nutrients · Removal rate constants

1. INTRODUCTION

Within the last 10–15 yr, many Danish fish farmers have reconstructed their land-based flow-through systems into model trout farms (MTFs; Jokumsen & Svendsen 2010), and approximately half of the Danish rainbow trout *Oncorhynchus mykiss* production (15 070 t yr\(^{-1}\)) currently takes place in these systems (Statistics Denmark 2020). MTFs are semi-intensive recirculation systems that, depending on type, typically apply between 2–4 m\(^3\) (type III MTFs) and 5–24 m\(^3\) (type I MTFs) make-up water (MUW, i.e. water exchange) per kg feed (Svendsen et al. 2008a, Danish Ministry of the Environment 2012, Dalsgaard et al. 2018). Type III MTFs are the most common and most intensive MTFs, producing approximately 10 100 t of fish per year (Statistics Denmark 2020). They use ground or surface water as MUW and they are equipped with inline sludge cones, drum filters, and biofilters. Less intensive MTFs (type I) use stream water as MUW and generally do not apply biofiltration (Pedersen et al. 2003). To comply with the EU Water Framework Directive water quality standards (WFD 2000), all Danish MTFs are required to treat system effluent in a free water surface (FWS) constructed wetland or ensure equivalent treatment by other methods (Danish Ministry of the Environment 2016). An MTF wetland is a passively operated
treatment unit that is relatively inexpensive to build, often constructed by interconnecting abolished earthen fish ponds in a stream-like meandering fashion (DCE 2012). System reject water (corresponding in volume to system MUW) and sludge basin runoff run to the wetland, which removes excess nutrients by natural processes prior to final discharge.

An MTF wetland differs from most other constructed wetlands applied for wastewater treatment in that the hydraulic loading rate (HLR) is more or less constant throughout the year, the waste nutrient composition is well known, and nutrient loadings within a MTF are within the same magnitude throughout the year, reflecting that fish are produced year round (Svendsen et al. 2008a, Kadlec & Wallace 2009, Jokumsen & Svendsen 2010). To document their effect, Svendsen et al. (2008a) measured inlet and outlet concentrations and flows for 2 yr in 8 of the first-ever constructed MTF wetlands. FWS areas in the 8 wetlands ranged from 1375 to 14 800 m² and the hydraulic retention time (HRT) ranged from 0.9 to 2.0 d. Mass-balance calculations showed that total nitrogen (TN), total phosphorus (TP), total biochemical oxygen demand (BOD₅_TOT), and total chemical oxygen demand (COD_TOT) were reduced by 50, 76, 93, and 87%, respectively, corresponding to mass removal rates of 2.7 g TN, 0.18 g TP, 4.4 g BOD₅_TOT, and 13.1 g COD_TOT m⁻² d⁻¹. These rates have since been applied for sizing other aquaculture wetlands including faster-flowing wetlands with shorter HRT associated with type I MTFs.

All Danish MTFs are subjected to discharge control. This means that there is no upper limit to how many fish MTFs may produce as long as wetland effluent total ammonia N (TAN) and BOD₅_TOT concentrations, and discharged TN and TP masses, stay below certain farm-specific regulatory limits. The industry is therefore keen to improve and ensure constant wetland treatment performance year round. Due to a lack of empirical data, it is assumed that nutrients are removed at a constant rate throughout the year, and a better understanding of internal removal dynamics at the flows and loading conditions characteristic for type III MTF wetlands is needed. To facilitate this, the present study for the first time examined the longitudinal and seasonal removal efficiency of N, P, and organic matter (OM) in a slow-flowing type III MTF wetland for a full year. Furthermore, rather than examining total nutrients as done in most wetland studies (see Kadlec & Wallace 2009), particulate and dissolved nutrient fractions were analysed separately to account for different removal mechanisms. A set of simple first-order removal rate constants were derived for all particulate and dissolved nutrient fractions, and the rate constants were plotted against time to disclose seasonal fluctuations in nutrient removal efficiency. Furthermore, rate constants were applied to assess the specific wetland surface area needed to remove a given fraction of certain nutrients in order to keep effluent nutrient concentrations within given limits.

2. MATERIALS AND METHODS

2.1. Study site

The 1 yr monitoring study was conducted in a 10 yr old, slow-flowing Danish type III MTF wetland with a mean ± SD HLR of 0.49 ± 0.10 m d⁻¹ and an average width and depth of approximately 8.5 and 0.8 m, respectively (Fig. 1a,b). The former flow-through farm was rebuilt in 2007 and produced approximately 525 t market-size rainbow trout Oncorhynchus mykiss per year applying 500 t feed. The fish farm used groundwater as MUW. System reject water (averaging 42 ± 9 l s⁻¹ during monitoring) flowed continuously into the wetland, which was constructed by interconnecting abandoned earthen ponds no longer used for fish production. Cutting off 2 minor wetland sub-sections prior to the study (red dotted lines in Fig. 1b) ensured that total farm effluent volume entered a single well-defined wetland string during monitoring. In addition to system reject water, a sludge basin overflow entered the monitored wetland section immediately upstream of sampling Stn 1 (Fig. 1b). The monitored wetland had an FWS area (A) of 7510 m², a volume (V) of 6008 m³, and a total HRT of 1.7 ± 0.4 d (Table 1). Wetland vegetation was dominated by Glyceria spp. growing along the banks of the open-water, stream-like wetland, while duckweed (Lemna spp.) covered parts of the surface area in the main growing season (Fig. 1).

2.2. Sampling

Six sampling stations were positioned at regular distances along the wetland (Fig. 1b, Table 1), assuming that water moved as a plug flow, i.e. at constant velocity and flow direction throughout. Given the constant daily flow, biweekly, 24 h composite water samples were obtained at each station for a year (21 December 2017–05 December 2018), corresponding to 26 sampling days (n = 26). Water samples were obtained using refrigerated automatic samplers.
Fig. 1. (a,b) Google Maps pictures (https://www.google.ca/maps/@56.9984115,9.6734967,494m/data=!3m1!1e3) of the study site including (a) the model trout farm and associated constructed wetland and (b) a close-up of the monitored wetland section (solid red line) and position of sampling Stns 1 to 6, with dotted red lines indicating wetland sections purposely ligated during the study. Also shown are (c) the inlet, (d) the outlet, (e) Stn 5 in November 2017, and (f) Stn 5 in August 2018.
(Glacier® Portable, Teledyne ISCO) programmed to sample 300 ml from the middle of the water column every hour for 24 h. Temperature (T, °C), dissolved oxygen (mg l−1), and pH were obtained by separate measurements in the water column next to sampler inlet pipes at each sampling station upon sample collection using Hach Lange HQ40D multimeters. Flow rates in and out of the wetland were derived by measuring water heights (cm) across well-defined inlet and outflow weirs (Fig. 1c, d) and applying the fundamental weir equation (Winther et al. 2011).

### 2.3. Chemical analysis

Upon return (within 2 h) to DTU Aqua’s Section for Aquaculture in Hirthshals, Denmark, sampling containers were gently shaken to obtain homogeneous subsamples for total (TOT) and dissolved (DISS) nutrient analysis, while particulate (PART) nutrient concentrations were determined as the difference between the 2 measurements. Subsamples were filtered through 0.2 μm sterile syringe filters (Filtropur S 0.2, Sarstedt) prior to analysis of NDISS compounds, while other subsamples were filtered through 1.6 μm glass microfiber filters (Whatman® GF/A, GE Healthcare) prior to analysis of dissolved organic compounds and PDISS compounds. TN was analysed according to Danish Standards Foundation (1975), International Organization for Standardization (1986, 1997), while TAN, nitrate-N (NO₃-N), and nitrite (NO₂-N) were analysed following Danish Standards Foundation (1975), International Organization for Standardization (1986), and Danish Standards Foundation (1991; detection limit 1 μg l⁻¹), respectively.

For total and dissolved OM, carbonaceous BOD₅ was analysed as described in International Organization for Standardization (2003b) modified by adding allylthiourea to inhibit oxygen consumption due to nitrification (International Organization for Standardization 2003a, Tchobanoglous et al. 2003), while COD was analysed as described in International Organization for Standardization (1989) using LCK 114 digestion vials (Hach Lange). TP and PDISS (PO₄-P) were analysed as described in International Organization for Standardization (2004; detection limit 0.01 mg l⁻¹). All analyses were performed in duplicate.

### 2.4. Modelling and statistics

Many biologically mediated wetland processes exhibit Monod kinetics, i.e. first-order reactions at limiting concentrations of a key substrate and zero-order reactions at non-limiting concentrations (Mitchell & McNevin 2001). Consistent with this, many wetland removal processes, including sedimentation and sorption, behave as first-order processes within observed concentration ranges (Kadlec & Wallace 2009). Given measured inflow concentrations in the present study, and assuming plug flow conditions (long and relatively narrow channel), nutrient removal along the wetland was therefore modelled as a first-order process applying the equation (Kadlec & Knight 1996):

\[
C - C^* = [C_0 \cdot e^{(-k_{1,A}/q)}] - [C^* \cdot e^{(-k_{1,A}/q)}]
\]

where \( C \) is the nutrient concentration (mg l⁻¹) at a specific sampling station along the wetland, \( C_0 \) is the nutrient concentration (mg l⁻¹) at inflow Stn 1 (see Fig. 1b), \( C^* \) is the nutrient background concentration (mg l⁻¹), \( k_{1,A} \) is the area-based first-order rate constant (m d⁻¹), and \( q \) is the hydraulic loading rate (HLR; m d⁻¹) at a specific wetland station relative to Stn 1.

According to this model, nutrient concentrations decline asymptotically towards a residual background concentration (\( C^* \)) characterized by no net nutrient uptake or conversion. The same background concentrations as applied by Dalsgaard et al. (2018) for a faster-flowing type I MTF wetland (HLR of 2.23 versus 0.49 m d⁻¹ in the present study) were used (Table 2). Values for \( k_{1,A} \) were derived for each sampling day by fitting Eq. (1) to measured concentrations at each station along the wetland that day, and minimizing the sum of squared residuals between observed and predicted nutrient concentrations using the Solver GRG non-linear function in Microsoft Excel®. Rate constants were subsequently plotted against sampling date to disclose seasonal fluctuations.

### Table 1. Wetland sampling stations (see Fig. 1) with corresponding free water surface (FWS) area, volume, and hydraulic retention time (HRT; mean ± SD, n = 26) measured relative to the previous station. HRT = V/Q where V is volume and Q refers to the inflow rate (m³ d⁻¹)

| Sampling station | FWS area (m²) | Volume (m³) | HRT (d) |
|------------------|--------------|-------------|--------|
| 1 (inflow)       | 0            | 0           | 0      |
| 2                | 1616         | 1293        | 0.37 ± 0.08 |
| 3                | 1120         | 896         | 0.26 ± 0.06 |
| 4                | 1838         | 1470        | 0.42 ± 0.09 |
| 5                | 1870         | 1496        | 0.43 ± 0.10 |
| 6 (outflow)      | 1066         | 853         | 0.24 ± 0.05 |
| Total (accumulated) | 7510     | 6008        | 1.72 ± 0.39 |
mediated turnover and physical sedimentation, removal of dissolved nutrients due to downward infiltration.,

\[ \text{removal rate} = k_{\text{LA}} \cdot \text{loading rate} \]

where \( k_{\text{LA}} \) is the nutrient removal rate (kg m\(^{-2}\) d\(^{-1}\)) at the zero-order volumetric rate constant (g m\(^{-2}\) d\(^{-1}\)), and \( \tau \) is hydraulic retention time (d).

In addition to nutrient removal due to biologically mediated turnover and physical sedimentation, removal of dissolved nutrients due to downward infiltration/leakage and transpiration was estimated as:

\[ r = \sum_{i=1}^{n} \left( C_{i} + C_{i+1} / 2 \right) Q_{gw,i} - Q_{gw,i} \]

where \( r \) is the nutrient removal rate (kg d\(^{-1}\)) due to downward infiltration and transpiration, \( C_{i} \) is the nutrient concentration at a given wetland station, \( i \) (mg l\(^{-1}\)), and \( Q_{gw,i} \) is the downward infiltration and transpiration rate (m\(^3\) d\(^{-1}\)) between the inlet station and any given downstream wetland station, calculated as:

\[ Q_{gw,i} = \left[ (Q_{\text{in}} - Q_{\text{gw},i}) / Q_{\text{in}} \right] \cdot Q_{\text{in}} \cdot (A_{i} / A) \]

where \( Q_{\text{in}} \) is the wetland inlet flow rate (m\(^3\) d\(^{-1}\)), \( Q_{\text{gw},i} \) is the wetland outlet flow rate (m\(^3\) d\(^{-1}\)), \( A_{i} \) is total wetland surface area (m\(^2\)), and \( A \) is measured wetland surface area between inlet Stn 1 and a given downstream wetland station (m\(^2\)).

Correlations were examined using Pearson product-moment correlation tests, and a modified Arrhenius temperature coefficient was derived using Eq. (5) to resolve the impact of temperature on NO\(_3\)-N removal (Kadlec & Wallace 2009):

\[ k_{T} = k_{20} \cdot \theta^{(T-20)} \]

where \( k_{T} \) is the rate constant at a given temperature, \( k_{20} \) is the rate constant at 20°C, and \( \theta \) is the dimensionless temperature correction coefficient.

Statistical analyses were performed using GraphPad Prism 8.4.1 (© 2018 GraphPad Software) considering \( p \leq 0.05 \) as statistically significant. Results are shown as means ± SD, and concentration time series are shown as 3-point moving averages with missing data points replaced by arithmetic means of neighbouring data points.

3. RESULTS

3.1. Water measurements and flow rates

Wetland water temperatures oscillated in a sinusoidal manner (Fig. 2a), as also seen in other temperate
climate wetlands (Kadlec & Wallace 2009), including a winter minimum (<0.2°C in February) and a summer high (16.1 ± 1.2°C in May through August). Wetland surface waters froze at one instance (27−28 February 2018), making sampling impossible. Spring and summer 2018 were unusually hot, with air temperatures above 29°C in much of May−August (Cappelen 2019). Drought, rather than altered operation routines, therefore likely explains the fact that inlet flow rates dropped from an average of 50 l s−1 in May to 44 to 25 l s−1 in February−April. The changes corresponded to an average water loss of 12% in December−February, increasing to above 50% in May, and stabilizing at approximately 22% in August−November (Fig. 2c).

Oxygen concentrations (measured during daytime) were highest in February−March (>8.6 mg l−1 at all stations) and lowest in August (<1 mg l−1 downstream of Stn 2), and were negatively correlated with wetland temperatures (r = −0.601, p < 0.0001, n = 149). In addition, oxygen concentrations increased in the flow direction during spring and decreased in the flow direction in autumn (Fig. 2b). The wetland was slightly alkaline, with pH values (measured during daytime) averaging 7.2 ± 0.3 (n = 149; Fig. 2d), and there was a strong correlation between pH and oxygen (r = 0.749, p < 0.0001, n = 148).

3.2. Nitrogen

Inflowing TN concentrations were relatively constant through the year (17.2 ± 2.2 mg TN l−1) including 97% dissolved N compounds. NPART was not detected in many instances and results on this fraction are therefore not included. NO3-N constituted approximately 80% of TN DISS, decreasing from 13.2 ± 1.4 mg l−1 at Stn 1 to 11.1 ± 2.3 mg l−1 at Stn 6 (Fig. 3c). TN DISS k1,A fluctuated more or less randomly throughout the year (0.084 ± 0.043 m d−1; Fig. 3b), whereas NO3-N k1,A oscillated between relatively low k1,A values in autumn and winter (0.063 m d−1 in October−February) and high k1,A values in spring and summer (0.183 m d−1 in May−August; Fig. 3d). Furthermore, the k1,A values correlated significantly with average wetland temperatures (r = 0.648, p < 0.001, n = 24), and the oscillations corresponded to a temperature correction coefficient (θ) of 1.07 (p = 0.007, R2 = 0.311, SE = 0.024) and a k20 of 0.20 m d−1.

There was a relatively large input of TAN from the sludge basin, with concentrations increasing from 1.33 ± 0.56 mg l−1 prior to the sludge basin inflow (data not shown) to 2.27 ± 1.23 mg l−1 at Stn 1 (Fig. 3e). Ammonia removal oscillated in a seasonal manner interchanging between net removal in spring and late autumn and net production in early spring and late summer. As Eq. (1) does not apply to negative removal, Fig. 3f instead shows the constant removal rate (g m−2 d−1) based on flow and concentration differences between Stns 1 and 6 divided by the monitored wetland area. NO2-N entered the wetland at 1.33 ± 0.20 m d−1 in May−August; Fig. 3d). Furthermore, the k1,A values were significantly correlated with both NO3-N k1,A (r = 0.527, p = 0.008, n = 24) and average wetland temperatures (r = 0.450, p = 0.024, n = 25).

3.3. Phosphorus

PPART concentrations averaged 0.380 ± 0.475 mg P l−1 at the inlet (Fig. 4a), and there was a strong corre-
Fig. 3. Seasonal and longitudinal changes (d = hydraulic retention time in days) in dissolved nitrogen concentrations (mg l$^{-1}$) shown as 3-point moving averages (only Stn 1 and 6 are shown for simplicity) and associated removal rate constants ($k_{1,A}$, m d$^{-1}$) or removal rates (g m$^{-2}$ d$^{-1}$) of (a,b) total dissolved N (TN$_{DISS}$), (c,d) nitrate-N (NO$_3$-N), (e,f) total ammonia N (TAN), and (g,h) nitrite-N (NO$_2$-N).

As for P$_{DISS}$ and TAN, a large fraction of the OM entering the wetland came with the sludge basin influx, leading to an increase in COD$_{PART}$ concentrations from 4.4 ± 4.2 mg l$^{-1}$ prior to the sludge basin inflow to 10.6 ± 5.3 mg l$^{-1}$ at Stn 1. In comparison, COD$_{DISS}$ increased from 14.9 ± 1.5 to 18.2 ± 1.9 mg l$^{-1}$. COD$_{PART}$ concentrations decreased to 2.6 ± 2.3 mg l$^{-1}$ at the outflow station (Fig. 5a), and $k_{1,A}$ oscillated in a seemingly non-systematic manner, averaging 1.08 ± 0.66 m d$^{-1}$ (Fig. 5b). Decreases in COD$_{DISS}$ concentrations were less pronounced (Fig. 5c) and $k_{1,A}$ values were lower (0.12 ± 0.07 m d$^{-1}$). Furthermore, the $k_{1,A}$ values appeared to decline slightly from December onwards (Fig. 5d).

BOD$_{5,TOT}$ doubled following the sludge basin inflow (from 5.7 ± 2.5 to 11.2 ± 5.5 mg l$^{-1}$ at Stn 1), and approximately 55% of BOD$_{5,TOT}$ was in particular form, while 45% was in dissolved form (Fig. 5e,g). Removal rate coefficients of BOD$_{5,PART}$ averaged 1.03 ± 0.57 m d$^{-1}$ and were significantly correlated with those for COD$_{DISS}$ (r = 0.768, p < 0.0001, n = 24),
decreasing from 1.30 m d\(^{-1}\) in late December to 0.10 m d\(^{-1}\) in September (Fig. 5h). Furthermore, BOD\(_{5}\)-DISS \(k_{1,A}\) values were negatively correlated with average wetland temperatures (\(r = -0.621, p = 0.001, n = 24\)) and slightly positively correlated with average wetland oxygen concentrations (\(r = 0.430, p = 0.036, n = 24\)).

3.5. Estimated versus observed mass removal

Table 3 summarizes the estimated mass removal of nutrients (kg d\(^{-1}\)) by internal wetland processes (see Eq. 1) and downward infiltration and transpiration (see Eqs. 3 & 4), and compares the sum to the measured mass removal. There was generally a very high consistency between the estimated and measured mass removal. The table also shows that the mass removal of OMPART and PPART was larger than the corresponding mass removal of OMP\(_{\text{Diss}}\) and P\(_{\text{Diss}}\). Furthermore, the estimated mass removal of dissolved nutrients due to downward infiltration and transpiration was in most instances larger than that due to internal wetland processes, while it was opposite for particulate nutrients.

4. DISCUSSION

4.1. Particulate matter removal

The longitudinal concentration profiles of P\(_{\text{PART}}\), BOD\(_{5}\)-PART, and COD\(_{\text{PART}}\) and respective fits of the first-order model support the hypothesis that particulate nutrient removal in this slow-flowing MTF wetland predominantly took place by sedimentation, as also shown by Dalsgaard et al. (2018) in a faster-flowing type I MTF wetland (HLR of 2.23 versus 0.49 m d\(^{-1}\) in the present study). Most of the particulate nutrient removal took place in the first part of the wetland, diminishing in an exponential manner with wetland area/HRT. By inserting the average yearly inflow together with the average P\(_{\text{PART}}\) \(k_{1,A}\) value and inlet concentration in Eq. (1), it can be estimated that 75% of the P\(_{\text{PART}}\) removal took place within the first 3600 m\(^2\) of the wetland, while 95% was removed within 6300 m\(^2\). Along with the results for BOD\(_{5}\)-PART and COD\(_{\text{PART}}\) which rapidly decreased in a similar fashion, these results stress that particulate nutrient treatment efficiency diminishes as the wetland area increases. Hence, rather than improving net particulate matter removal, a larger area/longer HRT may augment the growth of phytoplankton, which may lead to elevated OM and P discharge concentrations, especially in spring and summer when light intensities are high (Kadlec & Wallace 2009). Adding to this, mineralisation of dead plant biomass may seasonally increase the release of OMPART and P\(_{\text{PART}}\) into the water column (Christensen et al. 1990, Kadlec & Wallace 2009), similarly affecting wetland discharge concentrations.

4.2. Nitrate removal

All MTFs with a feed loading above 0.2 kg feed m\(^{-3}\) MUW use nitrifying biofilters, and nitrate is therefore the main nitrogen form in associated treatment wetlands. Nitrogen removal efficiency in existing slow-flowing type III MTF wetlands is typically around 50% (Svendsen et al. 2008a) and the discharge of nitrate is often the ‘first limiting nutrient’ with regards to a production expansion. Denitrification is
ies have shown that denitrification rates in anaerobic FWS sediments rely not only on nitrate but also on readily available carbon. Because of this dependency of a second substrate, first-order kinetics typically better describes the removal of nitrate despite seemingly non-limiting (zero-order) concentrations of nitrate (Reddy et al. 1982, Reddy & Patrick 1984, Kadlec & Wallace 2009). This also applied to the present study, where inlet and outlet NO$_3$-N concentrations ($13.2 \pm 1.4$ and $11.1 \pm 2.3$ mg NO$_3$-N l$^{-1}$, respectively) otherwise indicated that nitrate removal should be a zero-order process. Hence, modelling nitrate removal as a first-order process using Eq. (1) yielded a stronger correlation between observed and predicted outlet concentrations ($r = 0.994$, $p < 0.0001$, $n = 24$) than if removal was modelled as a zero-order process using Eq. (2) ($r = 0.747$, $p < 0.0001$, $n = 25$). Furthermore, a plot of estimated yearly average NO$_3$-N removal rates along the wetland against average COD$_{PART}$ concentrations in the water column showed an asymptotic increase in nitrate removal rates as COD$_{PART}$ concentrations increased ($R^2$ of semi logarithmic regression = 0.984, $n = 5$, plot not shown), corroborating the finding that denitrification was carbon limited. A previous study by von Ahnen et al. (2020) similarly found denitrification in a slow-flowing MTF wetland to be limited by readily available carbon. Modulating the flows in 2 parallel wetland streams, the authors showed that TN removal rates were significantly higher in the sludge-fed wetland side stream ($8.4 \pm 1.4$ g N m$^{-2}$ d$^{-1}$) than in the parallel side stream ($1.8 \pm 1.0$ g N m$^{-2}$ d$^{-1}$) treating the same nitrate-rich effluent but receiving no carbonaceous sludge. As carbon availability and oxygen conditions vary within and between MTF wetlands, and as simple NO$_3$-N $k_{1,A}$ values do not account for this, simple NO$_3$-N $k_{1,A}$ values are strictly site specific (Reddy et al. 1982), which also applies to the present study. In addition to being carbon lim-
Table 3. Estimated and measured mass nutrient removal (mean ± SD, n = 26). Turnover + sedimentation derived as: (inlet concentrations – modelled outflow concentrations [using Eq. 1]) × inflow rates. Downward infiltration + transpiration derived using Eqs. (3) & (4). Measured removal is the difference between measured inlet and outflow masses (concentration × flow). See Table 2 for abbreviations.

| Nutrient | Turnover Removed (kg d⁻¹) | Estimated removal (kg d⁻¹) | Measured removal (kg d⁻¹) |
|----------|---------------------------|---------------------------|---------------------------|
|          | + Turf + Downward infiltration + Sedimentation + Transpiration | Total |                          |
|          |                           |                           |                           |
| TN_DISS  | 8.3 ± 4.4                 | 14.8 ± 11.6               | 23.1 ± 14.7               | 23.0 ± 14.6 |
| NO₃-N    | 7.1 ± 3.9                 | 11.5 ± 8.6                | 18.6 ± 10.4               | 18.3 ± 10.8 |
| NO₂-N    | 0.36 ± 0.34               | 0.43 ± 0.33               | 0.78 ± 0.55               | 0.67 ± 0.63 |
| TAN      | ND b                      | 3.1 ± 3.4                 | ND                        | 2.9 ± 5.7   |
| P_DISS   | 0.73 ± 0.50               | 0.18 ± 0.26               | 0.88 ± 0.62               | 0.86 ± 0.62 |
| COD_DISS | 29.9 ± 14.9               | 4.8 ± 4.4                 | 34.8 ± 17.3               | 34.6 ± 16.4 |
| COD_PART | 10.4 ± 5.9                | 16.8 ± 13.4               | 27.2 ± 14.0               | 27.8 ± 14.2 |
| BOD₅_DISS| 12.5 ± 6.9                | 2.6 ± 3.2                 | 15.2 ± 9.7                | 18.2 ± 11.4 |
| BOD₅_PART| 6.5 ± 4.9                 | 3.4 ± 2.5                 | 9.9 ± 5.0                 | 10.7 ± 5.4  |

aIncluding five instances of net negative removal counted as zero removal; bNot determined due to consistent periods of net negative removal not encompassed by Eq. (1)

4.3. P_DISS and TAN removal

A large portion of P_DISS and TAN in the wetland was apparently derived from mineralisation in the associated sludge basin (based on concentration measurements before and after the sludge basin inflow at Stn 1; data not shown). Phosphorus and nitrogen otherwise removed as solids in the production unit were thereby reintroduced as dissolved nutrients into the wetland. Here, they seemingly were incorporated in the annual growth cycle involving plant and microbial uptake and release, nitrification, and mineralisation (Simmons & Cheng 1985, Christensen et al. 1990, Kadlec & Reddy 2001). This resulted in small average net annual removal rates of TAN (0.398 ± 0.766 g m⁻² d⁻¹) and P_DISS (0.045 ± 0.129 g m⁻² d⁻¹) as also observed in other FWS wetlands (Kadlec & Wallace 2009). In fact, the wetland served as a net producer of TAN and P_DISS at times (Figs. 3f & 4d).

Reflecting the annual growth cycle in the wetland, increased photosynthesis and CO₂ uptake during daytime may explain the higher pH and oxygen concentrations measured in spring (Fig. 2), while plant decomposition and ammonification may explain the lower pH and net TAN production measured in late summer, when temperatures were high and oxygen concentrations low (Reddy & Patrick 1984). Processes other than plant uptake and nitrification also remove TAN. As discussed in Section 4.2, anammox removes TAN directly (Kuenen 2008, Rambags et al. 2019) and minor removal of TAN by anammox possibly occurred downstream in the wetland where denitrification presumably was carbon limited. In addition, un-ionized ammonia is easily lost through volatilization (Reddy & Patrick 1984), but such losses were probably insignificant here given that pH was generally below 7.5.

4.4. OM_DISS removal

As for OM_PART, OM_DISS removal was largest in the first part of the wetland while it diminished in an exponential manner along the wetland. Unlike particu-
late matter, OM$_{\text{DISS}}$ $k_{1,A}$ values fluctuated in a seasonal manner, and using Eq. (1) it can be estimated that approximately 5 times more BOD$_{5,\text{DISS}}$ was removed during winter when $k_{1,A}$ values were high than in summer/autumn when they were low (applying similar inlet concentration). Other temperate FWS wetland studies have found similar seasonal patterns in OM$_{\text{DISS}}$ removal, coupling it to oxygen solubility at different temperatures (lower oxygen solubility at higher temperatures). In addition, leaching of less degradable OM from plant material may contribute to reducing net removal (Pinney et al. 2000, Kadlec & Reddy 2001). This probably also applied to the present study, where BOD$_{5,\text{DISS}}$ $k_{1,A}$ values were slightly negatively and positively correlated, respectively, with wetland water temperatures ($r = -0.634$) and oxygen concentrations ($r = 0.433$). Furthermore, longitudinal concentration profiles indicated that there was a net production of less degradable COD$_{\text{DISS}}$ at times (data not shown) presumably deriving from the decomposition of dead plant biomass and a release of less degradable humic substances (Kadlec & Wallace 2009). Humic substances are a natural source of acidity, and a production of humic substances would explain the reduction in pH observed in summer and autumn (Fig. 2d) when temperatures were high.

### 4.5. Nutrient mass removal by infiltration and evapotranspiration

It was an unusually hot summer in 2018, including the most extended drought ever registered in Denmark (Cappelen 2019), and up to 50% of wetland water was lost in April–July (Fig. 2c). Wetlands treating MTF effluents are non-lined, non-planted, stream-like systems and measuring water balances for 2 full years in 8 type III MTF wetlands, Svendsen et al. (2008a) deduced that influxes and downward infiltration dominated the balances, whereas impacts of evapotranspiration and rainfall were insignificant, contributing at maximum ±0.3 l s$^{-1}$ on a yearly basis. Consistent with this, we assume that water losses in the present study were primarily due to downward infiltration along with transpiration from macrophytes growing along the banks, as there was principally no emergent aquatic vegetation within the wetland channel (Fig. 1). Downward infiltration and transpiration do not affect dissolved nutrient concentrations in the water column per se, as water lost by infiltration and transpiration has the same nutrient concentration as the water column. In addition, dissolved nutrient turnover in an FWS wetland is predominantly associated with processes occurring in plants, biofilms, and at sediment interfaces rather than processes occurring in the water column. A change in water volume (i.e. water column depth) will therefore not proportionally affect the turnover of dissolved nutrients (Kadlec & Wallace 2009). Similarly, downward infiltration supposedly had a minimal effect on residual particulate nutrient concentrations given that most particulate nutrients, as discussed in Section 4.1, settled in the first part of the wetland with concentrations rapidly approaching a constant background level. We therefore assume that downward infiltration and transpiration had minimal effects on the particulate and dissolved $k_{1,A}$ values derived by fitting Eq. (1) to the measured nutrient concentrations. Mass removals calculated using these $k_{1,A}$ values will, however, be underestimated (i.e. a conservative measure), as dissolved nutrients removed by downward infiltration and transpiration are not accounted for. Combined downward infiltration and transpiration losses were therefore estimated separately using Eq. (3), and as seen in Table 3, there was high consistency between the estimated and measured total removal. This shows that nutrients removed by sedimentation and internal turnover processes were adequately accounted for by the simplistic first-order plug flow model (within observed concentration ranges), while predicted overall mass removal will be underestimated in the case of large downward infiltration and transpiration losses.

### 4.6. Comparison with other MTF wetlands

Table 2 summarizes the nutrient loads (g m$^{-2}$ d$^{-1}$) and constant removal rates (g m$^{-2}$ d$^{-1}$) obtained in different MTF wetland studies. Loading rates in the present study were largely comparable to those in a faster-flowing type I MTF wetland (Dalsgaard et al. 2018), where lower inlet concentrations typically accompany a higher inflow. The faster-flowing wetland was, however, evidently less efficient, especially in removing nitrate. As in the present study, nitrate entered the faster-flowing wetland in seemingly non-rate limiting concentrations (>3 mg NO$_{3}$-N l$^{-1}$) and carbon loading was similar, if not higher, than in the present study, suggesting favourable conditions for denitrification. Hydraulic conditions in the faster-flowing wetland, however, resulted in higher oxygen levels (inlet oxygen concentrations >8 mg l$^{-1}$ throughout the year), presumably promoting nitrification rather than denitrification and leading to a net pro-
duction of nitrate at times. Nutrient loadings and removal rates were also largely comparable to those obtained by Svendsen et al. (2008a) in 8 similar slow-flowing MTF wetlands. Therefore, we assume that the $k_{1,A}$ values obtained in the present study generally apply to other type III MTF wetlands, except for nitrate, where $k_{1,A}$ values depend on site-specific carbon availability and oxygen conditions.

5. CONCLUSIONS

Danish MTF effluent regulation is based on wetland discharges of TN, TP, and total BOD$_5$ and it is assumed that nutrients are removed at constant rates independent of wetland size and season (Danish Ministry of the Environment 2016). As shown in the present study, nutrient removal is, however, a combination of 2 processes happening at different rates. Particulate nutrient removal largely takes place by sedimentation and typically happens at faster rates than that of dissolved nutrients. Dissolved nutrient removal largely relies on biologically mediated turnover, and $k_{1,A}$ values tend to fluctuate in a seasonal manner, coupling to biotic and abiotic processes. Improving wetland treatment performance must therefore take account of these differences.

Approximately half of the P and OM entering a type III MTF wetland is typically in particulate form, and as concentrations rapidly approach a residual background level, enlarging a wetland beyond this point will not improve particulate nutrient removal any further. Rather, a larger wetland/longer HRT may augment the growth of phytoplankton and elevate discharge concentrations of OM$_{\text{PART}}$ and P$_{\text{PART}}$. Improving wetland treatment performance therefore principally comes down to enhancing dissolved nutrient removal. Nitrate is typically the ‘first limiting nutrient’ in Danish MTF wetlands with respect to production expansions. Denitrification in MTF wetlands is presumably restrained by readily available carbon, and while a C:N ratio of ~2.5 may enable complete denitrification using methanol as carbon source (Halling-Sørensen & Jørgensen 1993), a C:N ratio of at least 5 is recommended under field conditions to account for carbon lost in other processes (Baker 1998, Hang et al. 2016). In comparison, the COD$_{\text{DISS}}$:NO$_3$-N ratio in the present study (measured in the water column) averaged 1.4 ± 0.4 across all sampling stations and sampling days, indicating that denitrification was limited by readily available carbon. Previous studies have demonstrated that, with little effort, fish sludge can be used as an endogenous carbon source to improve denitrification in MTFs (Suhr et al. 2013, Letelier-Gordo et al. 2015, von Ahnen et al. 2020). To optimize the utilization of sludge for denitrification in wetlands, freshly obtained sludge ideally hydrolysed for about 3 d to maximize the formation of volatile fatty acids is preferable compared to non-optimized sludge basin overflows. Installation of woodchip bioreactors in an MTF wetland is an additional way to supplement carbon and achieve year-round denitrification (e.g. Christianson & Schipper 2016, Hang et al. 2016, Lepine et al. 2018, von Ahnen et al. 2018). Woodchip bioreactors should preferably receive effluents with a low C:N ratio to reduce risks of clogging, making woodchip bioreactors especially suited in situations where there is a shortage of hydrolysed sludge.

The present study revealed that there was hardly any removal of P$_{\text{DISS}}$ or TAN in the wetland averaged over a year. In such a case, and if an MTF cannot meet discharge limits, the amounts of P$_{\text{DISS}}$ and TAN entering the wetland should be reduced. In some cases, that might be achieved relatively simply by emptying the overflowing sludge basin more frequently. With respect to phosphorus specifically, inflow concentrations might be reduced by adding some kind of chemical precipitant or binding/adhesion agent, or establishing a biological phosphate removal unit where applicable, taking system-specific flows and concentrations into account. Furthermore, phosphorus entering an MTF wetland originates from fish feed in the first place, and previous studies have shown that dietary phosphorus levels can be optimized (reduced) to minimize the excretion of phosphorus without harming the fish (Dalsgaard et al. 2009, Dalsgaard & Pedersen 2011), essentially solving subsequent removal issues. Finally, as all type III MTFs operate with nitrifying biofilters, TAN discharges can be reduced by optimizing biofilter size and performance.

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