High nitrogen removal in a constructed wetland receiving treated wastewater in a cold climate

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ABSTRACT

Constructed wetlands provide cost-efficient nutrient removal, with minimal input of human labor and energy, and their number is globally increasing. However, in northern latitudes, wetlands are rarely utilized, because their nutrient removal efficiency has been questioned due to the cold climate. Here, we studied nutrient retention and nitrogen removal in a boreal constructed wetland (4ha) receiving treated nitrogen-rich wastewater. On a yearly basis, most of the inorganic nutrients were retained by the wetland. The highest retention efficiency was found during the ice-free period, being 79% for ammonium-nitrogen (NH_4^+-N), 71% for nitrate-nitrogen (NO_3^--N) and 88% for phosphate-phosphorus (PO_4^{3-}-P). Wetland also acted as a buffer zone during disturbed nitrification process of the wastewater treatment plant. Denitrification varied between 106 and 252 mg N m^{-2} d^{-1} during ice-free period. During ice-cover period, total gaseous nitrogen removal was 147 mg N m^{-2} d^{-1}, from which 66% was removed as N_2, 28.5% as nitrous oxide (N_2O) through denitrification, and 5.5% as N_2 through anammox. Nearly 2600 kg N y^{-1} was estimated to be removed through microbial gaseous N-production which equaled 72% of NO_3^--N and 60% of TN yearly retention in the wetland. Wetland retained nutrients even in winter, when good oxygen conditions prevailed under ice. The results suggest that constructed wetlands are an efficient option for wastewater nitrogen removal and nutrient retention also in cold climates.

INTRODUCTION

Since the 1960s, growing human population has substantially increased excess nitrogen (N) in the environment causing health, environmental and economic problems through e.g. aerosols and ground-level ozone, loss of biodiversity, eutrophication and climate change.[1] Although a major part of anthropogenic nutrient loading comes from non-point sources, point sources (e.g. wastewater effluents) can cause significant environmental problems, especially in small water bodies.[2] In Finland, wastewater treatment plants (WWTPs) retain > 90% of the incoming phosphorus (P), while N removal efficiency is generally lower, varying between 8-92%.[3] Most modern WWTPs rely only on primary and secondary treatments[4], and treated wastewater still contains high amounts of nitrate-nitrogen (NO_3^--N) when entering the recipient water bodies.

Traditionally, P is considered as the key limiting nutrient for phytoplankton growth in freshwater systems[5-6], and therefore lake management has mainly focused on P reduction. However, freshwater systems can also be N-limited[7-11] and the limiting nutrient can vary seasonally and spatially.[12-14] Furthermore coastal seas, e.g. The Baltic Sea, are generally N-limited.[15] In Finland, natural N retention efficiency of the Baltic coast is low (0-20%)[16], so more effort should be made in inland nutrient removal. A huge potential for nutrient reduction exists in WWTPs in the Baltic Sea area[17], and recent cost-efficiency modeling results from Finland including both agricultural and WWTP sources suggest that N load abatement measures should be targeted at WWTPs that have low N reduction level.[18]

Constructed wetlands (CWs) can be an efficient and low-cost option for wastewater nutrient removal[19-21] and polishing treated wastewater[22], especially in small communities and in areas without centralized wastewater treatment. CWs require no chemical and energy inputs, so maintenance costs are lower as compared with conventional WWTPs.[20] Maintenance includes the removal of excess vegetation and sediment. However, it is not always clear whether the CWs can successfully be applied in a cold climate.[23,24]

In wetlands, the most important N removal process is microbial-driven denitrification, where NO_3^- is reduced to nitrous oxide (N_2O), and further to nitrogen gas (N_2) under anoxic conditions.[25] Traditionally, denitrification rates have been considered to decline with declining temperature[26], but previous studies conducted in boreal lakes have reported active denitrification also during cold seasons.[27-29] The relative production of the greenhouse gas N_2O is highly variable depending on environmental conditions (e.g. temperature, oxygen concentration, carbon (C)[30-32]) suggesting that the share of N_2O from denitrification could be substantial in seasonally variable boreal environments. Another pathway contributing to N removal is anaerobic ammonium oxidation (anammox), which is important in environments with low organic C[33-34] as well as in wastewaters.[35] Unlike heterotrophic denitrification, anammox produces no N_2O, requires no external C source[35], and may thus remove N year around.

In this study, we measured nutrient retention in a constructed wetland for two years at Lammi WWTP, southern Finland. Our study questions were: 1) how efficiently does the wetland retain nutrients from treated wastewater? 2) does the retention efficiency vary between seasons or study years? and 3) are anammox and incomplete denitrification (N_2O production) important in N removal during ice-cover period?

MATERIALS AND METHODS

Study site

The study was carried out in a constructed wetland (4 ha, with mean depth 1.0 m), residence time ca. 40 days (Figure 1) at Lammi WWTP of Hämeenlinnan Seudun Vesi Ltd, in southern Finland (61°08′83″N, 24°99′66″E). The annual mean temperature of air is approximately 4–5 °C. Originally, the wetland was constructed in 1963 to treat wastewaters from a dairy. Since the 1970s the wetland has been used for tertiary treatment of municipal wastewaters from October to April. From May to September, the treated wastewater has been discharged straight to the recipient Lake Ormajärvi, to avoid aesthetic impairment resulting from high algae biomass discharged from the CW. During this study, the treated wastewater was discharged into the study wetland all year round. The Lammi WWTP is a small unit treating domestic wastewater of 4000 inhabitants, ca. 350 000 m^3 y^{-1}. Average daily discharge (Q) to the WWTP is 871 m^3 d^{-1} (±335 m^3 d^{-1}) varying seasonally. Highest discharge is usually recorded during snow melt and in summer after heavy rains. The treatment process contains sand separation, P removal by iron (II)-sulfate ($FeSO_4$) and nitrification of ammonium-nitrogen (NH_4^+-N) to NO_3^--N. After the treatment, wastewater is first directed to two small (600 m^2 each) settling ponds with ca.12-hour residence time, then through a collection well gathering the waters before entering the wetland. During summer, ca. 10–15% of the surface area is covered with vegetation consisting mostly of *Typha latifolia* and *Phragmites australis.*

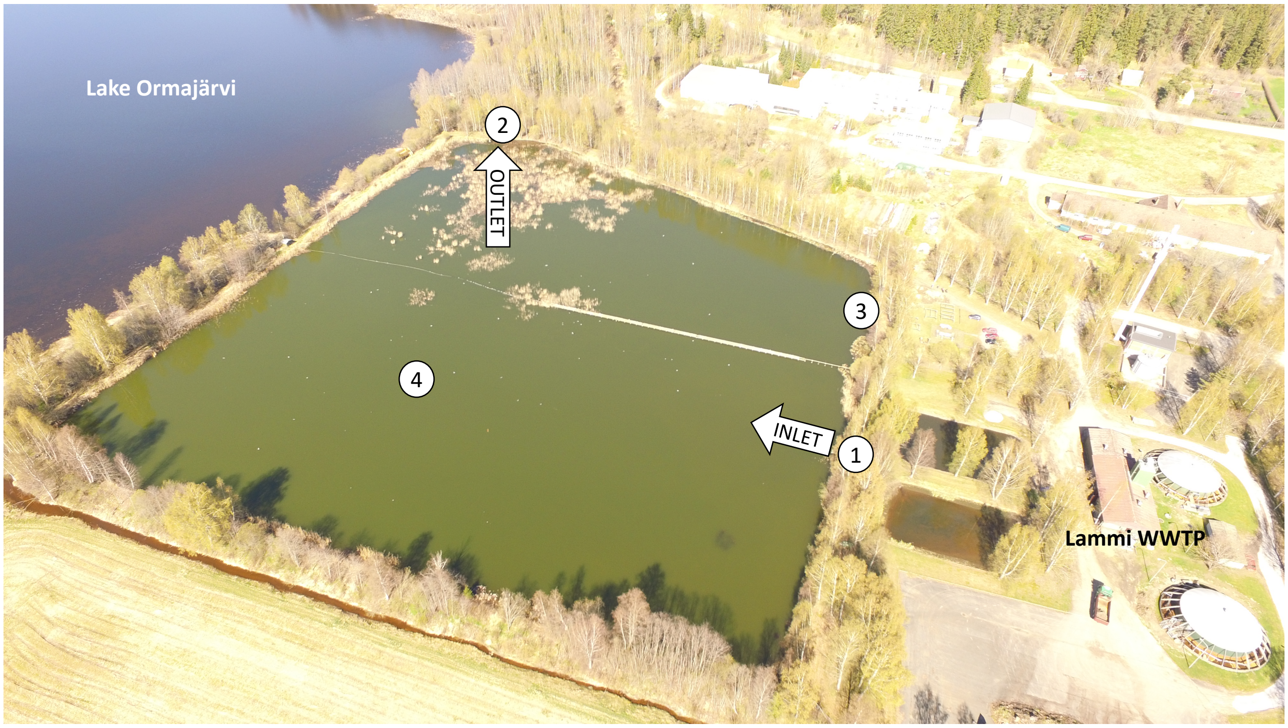

Figure 1. Constructed wetland of Lammi WWTP. Inlet is the place for treated wastewater discharge and outlet is the wetland discharge to Lake Ormajärvi. 1 = sampling of the WWTP discharge from a collection well, 2 = sampling of the wetland discharge, 3 = sediment sampling in the vegetated area in June and August 2015, 4 = sediment sampling in the non-vegetated area in June and August 2015 as well as February 2017.

Nutrient and chlorophyll-*a* sampling and analyses

Nutrient, dissolved organic carbon (DOC) and chlorophyll-*a* (Chl) samples were taken biweekly during two years (*n*=45), starting on 25 March 2015 continuing until 15 March 2017. Besides nutrients, we measured dissolved oxygen (O_2) and water temperature (T) with an oxygen meter (ProODO, YSI) 0.1 m from the bottom and 0.1 m below the surface close to the inlet (depth ca. 1.0 m) and 0.1 m below the surface close to the outlet (ca. 0.2 m) (Figure 1). WWTP discharge nutrient samples and DOC were taken from the collection well (Figure 1). Wetland discharge nutrients, DOC and Chl were sampled from the outlet (Figure 1). All water samples were kept dark and cold (+4 ºC), and analyzed in the laboratory within 1–3 hours. The samples were filtered through pre-rinsed (deionized water MQ; EMD Millipore, Billerica) 0.45 µm filters (Millex-HA, Merck Millipore). DOC was analyzed according to SFS-EN 1484 (SFS-EN; same standard for Finland and Europe), with carbon analyzer (Ordior TOC-V, Shimadzu). NO_2^--N + NO_3^--N (henceforth referred to as NO_x^--N) was analyzed following the SFS-EN ISO 13395 standard. NH_4^+-N analysis was performed according to SFS-EN ISO 11732 standard with a modification of using salicylate method. Total N (TN) was analyzed from a non-filtered sample according to SFS-EN ISO 11905-1. Total P (TP) and PO_4^{3-}-P were analyzed following ISO/DIS 15681-2 standard, except persulphate digestion was used in total P analysis. All nutrient fractions were measured with spectrophotometer (Gallery Plus, Thermo Scientific). Chl filters (Whatman GF/C glass-fiber, Whatman) were stored in a freezer -20 ˚C (SFS 5772) before spectrophotometrical analysis (UV-1800, Shimadzu). Particulate N (PN) and particulate P (PP) were obtained by subtracting the concentrations of dissolved fractions from totals (i.e. PN=TN - NO_x^--N - NH_4^+-N and PP=TP - PO_4^{3-}-P). Atmospheric N deposition to the wetland was estimated from data 2011–2016 provided by the Finnish Meteorological Institute (FMI) from a monitoring site situated (61°14.3926′N, 25°03.9161′E) ca. 6.5 km NE from the WWTP wetland. As the N deposition for the whole wetland (NO_x^--N + NH_4^+-N) was insignificant (29 g d^{-1}) compared with the WWTP TN discharge (23 kg d^{-1}), it was not taken into account in mass balance.

Sediment analyses

After determining the water content of the sediment, porosity of the surface sediment (0-3 cm) was calculated from a homogenized and dried (105 °C, 16–20h) subsample. The content of organic carbon was calculated from the loss on ignition (LOI%) from oven-dried (550 °C, 2h) surface sediment.

Denitrification and anammox measurements

Before stable isotope incubations, the underlying assumptions of the ^{15}N-isotope pairing technique (IPT)[35] were verified with a concentration series of ^{15}N-labeled potassium nitrate ($K^{15}NO_3$, 98 atom%, Sigma-Aldrich) 3, 9, 18 and 30 mg L^{-1} using the wetland sediment.

Sediment N_2-production rates were measured in June and August 2015 from a vegetated area as well as from a non-vegetated area. Intact sediment cores (height 16 cm, diameter 2.4 cm) were collected by hand from the vegetated area (*Typha latifolia*). In the non-vegetated area, larger sediment cores (height 34.1 cm, diameter 4.1 cm) were collected with a sediment sampler. Water-sediment ratio in the cores were approximately 70/30%. Samples were kept dark until delivered to the laboratory within an hour. Denitrification rates for N_2-production were obtained from cores incubated in the laboratory in dark at *in situ* temperature. After addition of the label, the water column was gently mixed with a glass rod to ensure homogenous distribution of the label, after which the cores were sealed with rubber stoppers and incubated for 3h with magnetic stirring (25 rpm). Assuming higher ambient NO_x^- concentration in the non-vegetated area (situated closer to the WWTP discharge) than in the vegetated area, we used different concentrations of $K^{15}NO_3$: 2.25, 5.25, 10.5 and 15 mg N L^{-1} in the vegetated area cores, and 6, 16.5, 27 and 37.5 mg N L^{-1} in the cores from the non-vegetated area. For each concentration, we had 2–3 replicate cores. Two unlabeled cores from each site were used for measuring background N_2 concentration. After incubation, the water and sediment of each core was stirred into slurry, and a 12 mL-subsample was taken from each core into a glass vial (Exetainer 12 mL 738W, Labco Limited) containing 100 µL of formaldehyde solution (37 wt%, Sigma-Aldrich) to terminate all microbial activity. Vials were stored upside down (+4 °C, dark). Before stable isotope analysis, a helium headspace (5mL) was added to each N_2 sample.[36]

In addition to summertime measurements, denitrification was measured in typically cold conditions (avg air temp. -7°C) in the non-vegetated area during winter in February 2017. Unlike in summer, we measured N_2 as well as N_2O. Sampling procedure was the same as in June and August. However, we used larger concentration series (n=12) of labeled $^{15}NO_3^-$-N from 25.5 to 57.4 mg N L^{-1}. Again, two unlabeled cores were used to determine the background N_2 and N_2O concentration. Due to cold conditions, incubation time was increased to 5.5h (in situ temperature +3 °C). For N_2O-production rate, a slurry sample of 30 mL was collected into a polypropylene syringe (60 mL) avoiding any air bubbles and 30 mL helium headspace was added in the syringes. After equilibration at 20˚C by vigorous shaking, 20 mL of the headspace was transferred to the pre-evacuated glass vial (12 mL, Labco Exetainer®) and stored over-pressurised in the dark until analysis.

Isotope analysis and calculation of denitrification and anammox

Sample isotope mass areas (m/z 28, 29, 30, 44, 45, and 46) as well as N_2 and N_2O concentrations were analyzed with isotope ratio mass spectrometer (CF-IRMS, Isoprime Ltd) connected to a TraceGas preconcentrator unit. For N_2, a modified N_2O method with no cryotrapping, and valves in CO_2 mode was used, while N_2O analysis was done following the standard Isoprime TraceGas N_2O procedure with cryotapping. For summer samples, we measured only N_2-production as previously reported.[36] For winter sampling, full and truncated denitrification (N_2+N_2O) and anammox rates were calculated with a different procedure.[37]

Wetland nutrient loading and retention

Wastewater discharge (Q, m^{-3} d^{-1}) entering the WWTP was used for wetland nutrient load calculations assuming, that equal amount of water enters the wetland. We calculated wetland N and P retention for the study years and for different periods (ice-free and ice-cover). From June to September, the decreasing effect of wetland surface evapotranspiration to the wetland discharge was taken into account.[38] Nutrient and DOC retention/release were calculated as the difference between load in and load out relative to the incoming load.

Data analysis

We investigated the differences in nutrient retention between the study years, but also between ice-free and ice-cover periods using non-parametric Wilcoxon signed rank tests. We used regression analysis to investigate the relationships between environmental factors (temperature, O_2, pH) and Chl concentration. In addition, the difference in nutrient concentration between wetland inlet and outlet relative to environmental factors was examined to clarify the prevailing processes in the wetland. Finally, we estimated yearly N removal in the wetland on the basis of the measured N removal process rates. Softwares used were SPSS 24 and Microsoft Excel for Mac 16.12.

RESULTS

Water temperature and oxygen conditions in the wetland

Water temperature and dissolved oxygen concentration near the wetland outlet were highest from June to August. Maximum temperature in the outflowing water was 20.3 °C during the first ice-free period (2015) and 20.6 °C during the second, both measured in August. During ice-cover, temperature and O_2 were similar in both study years, temperature being lowest in January. Lowest O_2 saturation in the wetland discharge was measured both years during ice-cover in February (2.8 and 8.0 %). During ice-free period, O_2 saturation was higher in the first year reaching up to 270%, while the maximum in the second year was 230%.

Nutrient loading and environmental conditions relative to open water and ice-cover periods

WWTP discharge to the wetland was highest in spring (Table 1). Mean inflowing TN concentration was 27.6 mg N L^{-1}. TN load from the WWTP to the wetland consisted mostly of NO_x^--N (67%), and the proportion of NH_4^+-N was 28%. During the study, NO_x^--N concentration of the incoming treated wastewater varied from 1.5 to 51.0 mg N L^{-1} being 19.6 mg N L^{-1} on average. The proportions of inorganic N fractions varied temporally (Figure 2), NO_x^--N loading from the WWTP being 0.9–57.8, and NH_4^+-N loading between 0.1–19.4 kg N d^{-1}(Table 1). NH_4^+-N loading was fourfold in the second year ice-free period as compared with the one measured in the first year. Most of the TP-load from the WWTP was in particulate form.

Table 1. WWTP discharge (Q, m^3 d^{-1}) and nutrient load (kg d^{-1}) to the wetland from 2015 to 2017 (periodical averages within a study year, as well as mean, minimum, maximum and standard deviation for the whole study). Ice-free period April–November, ice-cover period December–March. DOC = dissolved organic carbon, NH_4^+-N = ammonium-nitrogen, PO_4^{3-}-P = phosphate-phosphorus, NO_x^--N = nitrate+nitrite-nitrogen, TN = total nitrogen, TP = total phosphorus, PN = particulate nitrogen, PP = particulate phosphorus.

	Q (m^3 d^{-1})	**DOC** (kg d^{-1})	**NH_4^+-N** (kg d^{-1})	**PO_4^{3-}-P** (kg d^{-1})	**NO_x^--N** (kg d^{-1})	**TN** (kg d^{-1})	**TP** (kg d^{-1})	**PN** (kg d^{-1})	**PP** (kg d^{-1})
1st year									
Ice-free period	885	8.4	2.2	0.10	20.1	23.3	0.30	1.00	0.20
Ice-cover period	1122	9.5	7.4	0.068	14.8	23.9	0.32	1.67	0.25
2nd year									
Ice-free period	781	7.9	8.9	0.05	10.1	19.3	0.30	0.89	0.26
Ice-cover period	826	7.4	5.7	0.06	23.1	31.0	0.22	2.30	0.16
Mean	**871**	**8.2**	**5.8**	**0.07**	**16.0**	**22.7**	**0.29**	**1.19**	**0.22**
min	422	3.2	0.13	0.004	0.85	7.4	0.069	0.001	0.028
max	1790	18.8	19.4	0.30	57.8	70.6	0.95	5.86	0.69
st.dev	335	3.5	5.3	0.065	9.6	9.7	0.15	1.16	0.13

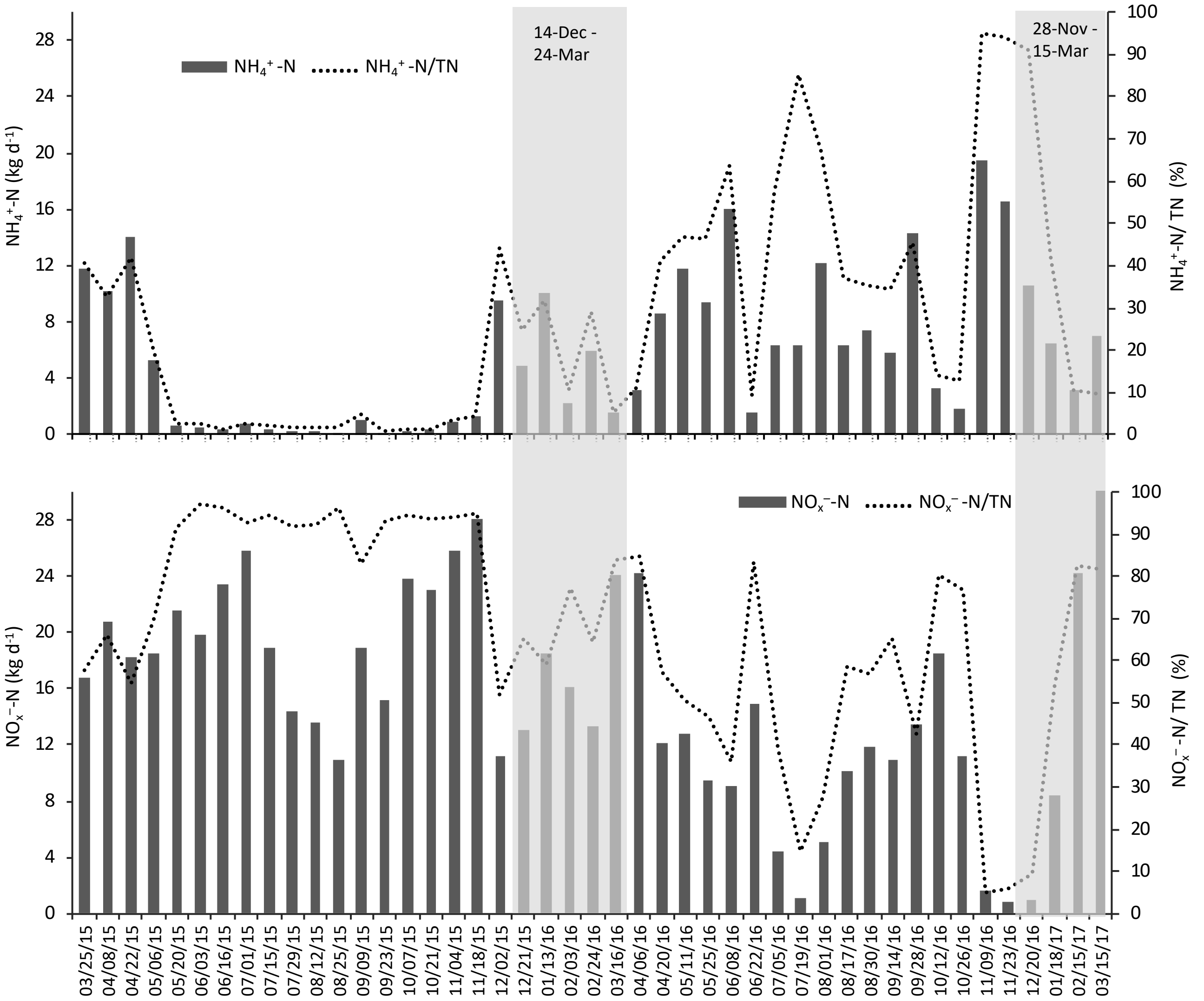

Figure 2. Daily ammonium-nitrogen (NH_4^+-N) and nitrite+nitrate-nitrogen (NO_x^--N) WWTP discharge (columns) to the wetland and the proportion (%) of the inorganic N fractions from the total nitrogen (TN) discharge (dotted line) between 2015 and 2017. Ice-cover periods are indicated with gray areas.

Nutrient retention in the wetland

During the two study years, the retention efficiency was positive for inorganic as well as for total nutrient fractions (Table 2). More than half of the incoming TN, NH_4^+-N, NO_x^--N and PO_4^{3-}-P was retained, whereas the retention of TP was lower. Q and N retention had rather similar annual patterns, whereas the retention of DOC, PO_4^{3-}-P and PP differed (Table 2). N retention varied also seasonally: highest proportions of inorganic N fractions were retained during ice-free period, while the amount of PN discharged from the wetland increased. Similarly, PP was produced during ice-free periods, but retained during the ice-cover periods. The amount of particulate N and P increased with Chl (R^2=0.83 and 0.84, respectively). Maximum Chl concentration (1044 µg L^{-1}) was measured during the second year, whereas during the first year the highest concentration was 525 µg L^{-1}. Annual N retention in the wetland was nearly 4300 kg for TN, 3600 kg for NO_x^--N, nearly 1300 kg for NH_4^+-N, meaning 108, 90 and 33 g N m^{-2}, respectively. PN release from the wetland to Lake Ormajärvi was > 500 kg N y^{-1} (Table 2). The release of NH_4^+-N and PO_4^{3-}-P from the wetland to the lake during the second ice-cover period can partly be explained with low O_2 saturation ($R^2 = 0.33$, p-value < 0.005).

Table 2. Mean nutrient retention (%) and removed or released (-) average nutrient load (kg y^{-1}) in the wetland. Ice-free period April–November, ice-cover period December–March. DOC = dissolved organic carbon, NH_4^+-N = ammonium-nitrogen, PO_4^{3-}-P = phosphate-phosphorus, NO_x^--N = nitrite+nitrate-nitrogen, TN = total nitrogen, TP = total phosphorus, PN = particulate nitrogen, PP = particulate phosphorus. Results of the non-parametric Wilcoxon signed rank test with Z- and P-values.

		DOC	**NH_4^+-N**	**PO_4^{3-}-P**	**NO_x^--N**	**TN**	**TP**	**PN**	**PP**
1st year (n=23)									
Open water period (%)		-10.7	75.2	89.9	73.7	63.2	13.9	-188.2	-19.9
Ice-cover period (%)		-2.8	48.5	16.4	19.4	24.2	45.0	-40.9	52.9
2nd year (n=22)									
Open water period (%)		10.1	83.4	86.3	68.3	60.6	8.2	-225.3	-6.7
Ice-cover period (%)		3.0	-54.4	-32.0	51.8	32.0	16.3	36.8	34.0
Avg change (kg y^{-1})		-114.2	1286.0	17.2	3580.5	4280.7	16.7	-528.1	-0.6
Difference between open water (n=34) and ice-cover (n=11) periods	Z	-0.09	-2.22	-2.93	-2.85	-2.85	-0.09	-2.58	-1.60
	P	0.93	*0.03*	*0.003*	*0.004*	*0.004*	0.93	*0.010*	0.72
Difference between study years	Z	-1.96	-0.60	-2.26	-0.99	-0.31	-0.31	-0.96	-2.03
	P	*0.050*	0.55	*0.024*	0.32	0.76	0.76	0.34	*0.042*

Statistically significant test values (P < 0.05) are written in italics.

Denitrification and anammox rates

Results from the $^{15}NO_3^-$ concentration series proved that denitrification (D14) based on the natural $^{14}NO_3^-$ was not affected by the added label $^{15}NO_3^-$ (S1). In summer, the highest denitrification rate (252 mg N m^{-2} d^{-1} as N_2) was found in June in the vegetated area (Figure 4a), when the bottom water temperature was 15.5 °C. In the non-vegetated area, denitrification was fueled by both water column NO_x^- and coupled nitrification-denitrification, whereas in the vegetated area, denitrification was completely based on the water column NO_x^-. NO_x^--N concentrations were 6.4 and 6.1 mg N L^{-1} in vegetated and non-vegetated area, respectively. In August (Figure 4b), the bottom water temperature had increased to 18 °C, but the N_2-production rate was lower than in June, following NO_x^--N concentration (non-vegetated area 3.4, vegetated area 2.4 mg N L^{-1}).

In winter (water temp. +3°C), NO_x^--N concentration in the WWTP discharge reached the maximum and the concentration above the sediment was nearly 10-fold (24.9 mg N L^{-1}) as compared with August. N_2-production rates were 94.7 for denitrification and 8.3 mg N m^{-2} d^{-1} for anammox, respectively. In addition, 44.0 mg N m^{-2} d^{-1} was released as N_2O from incomplete denitrification, so the total N removal was 147.0 mg N m^{-2} d^{-1} (Figure 4c). In average, complete denitrification contributed 66% (45.5–75.1%), incomplete denitrification (N_2O) 28.5% (20.4–46.2%), and anammox 5.5% (0.21–12.8%) to the total NO_x^--N removal.

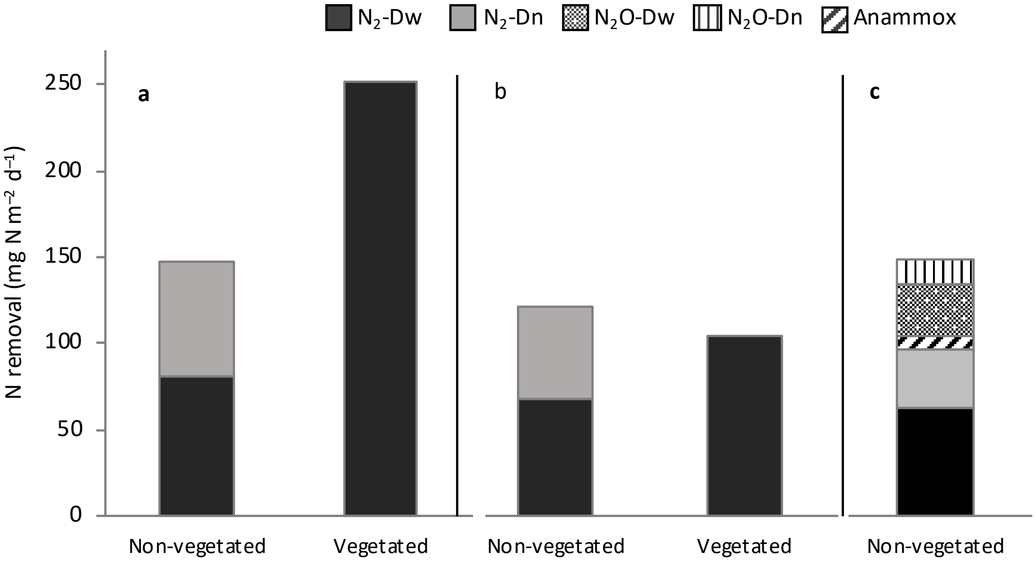

Figure 3. N removal rates by denitrification based on the original IPT in June (a), and in August (b). In February (c), N removal rates by denitrification, incomplete denitrification (N_2O) and anammox only in the non-vegetated area. D_w = denitrification based on water column nitrate, D_n = denitrification based on coupled nitrification-denitrification.

Based on the measured N_2 production rates, and assuming: 1) the proportional N_2O and anammox rates relative to N_2 being stable year round, 2) vegetation cover being 15% during ice-free period, the calculated mean N removal was 8.5 kg N d^{-1} for the whole wetland, totaling in 1869 kg N during ice-free period (8 months). During ice-cover period (4 months), the water temperature above the sediment was ca. 12-15 °C lower when compared with the summer measurements. Calculated N removal rate was 5.9 kg N d^{-1}, totaling 717 kg N. To conclude, the 4-ha wetland removed 2586 kg N y^{-1} through the microbial gaseous N-production (Figure4). This equals 72% of the NO_x^--N retention and 60% of the TN retention per year calculated from mass balance.

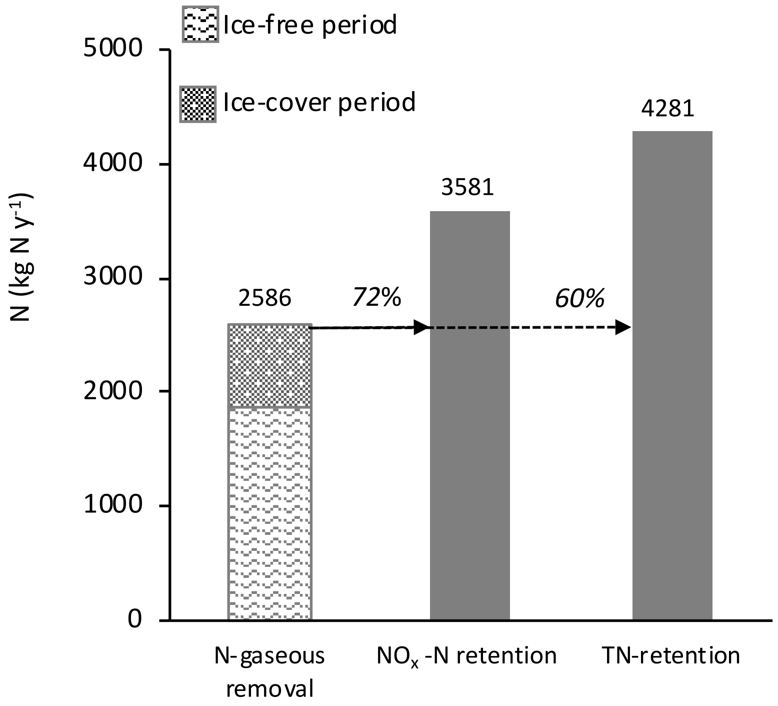

Figure 4. The removal of N (kg y^{-1}) based on gaseous N_2- and N_2O-production. Ice-free period April–November, ice-cover period December–March. Nitrite+nitrate-nitrogen (NO_x^--N) and total N (TN) retention (N kg y^{-1}) based on mass balance in the 4-ha wetland.

DISCUSSION

Here we demonstrated, that CWs can improve N removal also in northern WWTPs. Lammi WWTP wetland had a TN retention of 52% with an average in-load of 207 g m^{-2} (27.6 mg N L^{-1}). More than half of the inorganic nutrients were retained, and the highest retention efficiency was measured for N: 61–86% during ice-free period and 19–52% during ice-cover period. Although the retention efficiencies of inorganic nutrient fractions were lower during ice-cover periods, they were still mainly positive. Only under poor oxygen conditions, ammonium-nitrogen and phosphate-phosphorus release from the wetland was found. During the first ice-cover period, phosphate-phosphorus release was found between January and March, when oxygen saturation in the wetland discharge was 2.8–30.1%. The second ice-cover period was similar and phosphate-phosphorus was released between late November and February under oxygen saturation in the wetland discharge between 8 and 35%. At present, Lammi WWTP has N reduction ca. 50% without the CW, so it can easily achieve the 70% N reduction target set by EU directive (91/271/ETY)[39] and national regulation (888/2006)[40], although the present environmental permission allows a lower N reduction due to local conditions in the recipient lake. Our findings are in line with previous studies. Four treatment wetlands were studied in South Sweden and TN removal varied between 700–1500 kg ha^{-1} y^{-1}.[41] TN retention was studied in 85 wetlands situated in variable climatic conditions with an average yearly in-load of 466 g m^{-2} (14.3 mg N L^{-1}), and mean retention was 41% .[42]

Microbial processes, which are applied in wastewater technology, are sensitive to seasonal changes. We found an increase in the ammonium-nitrogen load to the wetland from the WWTP, likely resulting from a cold shock in bacterial community[43], leading to an insufficient nitrification process at the start of the ice-cover periods. Furthermore, we found high ammonium-nitrogen load from the WWTP released to the wetland during the second ice-free period (shown in Fig. 2). This was caused by a sudden significant increase in WWTP inflow coming from the wastewaters of a large Scout camp with 17 000 participants, which most likely disturbed the nitrification process in the WWTP. The WWTP nitrification efficiency remained low until October, but ammonium was successfully nitrified in the wetland. This indicates that northern treatment wetlands may not only improve the overall N retention efficiency under undisturbed situations, but can also buffer situations like disturbed WWTP nitrification process. Our results also show, that phosphate-phosphorus was mostly taken up by algae and bacteria during the ice-free periods. Wetland N removal was driven by denitrification. The results suggest that denitrifying bacteria were active also under ice in winter, agreeing with the few studies conducted in cold climate[44,45]. It seems denitrification rates are not controlled only by temperature, but also by nitrate availability[27,44,46]. In winter, ice-cover and snow can act as an insulator in shallow wetlands supporting microbiological processes in the sediment.[47] The presence of anammox in the studied sediment was not shown as an increased denitrification rate along with the added label amount in the $^{15}NO_3$ concentration series prior to the experiments. However, modest contribution of anammox may still be present even if not shown[48]. Slow growing anammox bacteria may be suppressed by fluctuations in environmental factors like oxygen, high nitrate and ammonium concentrations[49-50], or by low temperature (< 30 °C)[51]. In addition, when organic C is not limiting, heterotrophic denitrification bacteria generally out-compete anammox bacteria.[49]

Nitrous oxide production in aquatic systems can be promoted by different conditions, such as high N[30,52-53] and low availability of organic C[55]. However, the knowledge of nitrous oxide generation is mostly limited to open water seasons. In our winter measurements, the contribution of nitrous oxide in the total N removal was notable, which could be explained with organic C limiting the last denitrification step[54-55]. Furthermore, low temperate has been found to affect more to nitrous oxide reductase than enzymes producing nitrous oxide.[30] Therefore, the portion of N_2O from total denitrification being stable all year round, may be an overestimation in the calculated N budget. Denitrification in the vegetated area was entirely based on the nitrate diffusing from the water (D_w), even though earlier studies have reported plant roots to increase nitrification by oxygenation of the sediment[56]. *Typha latifolia* might have been so efficient in its nutrient uptake from the sediment in June and August that no ammonium was left available for nitrifiers. *T. latifolia* has been found to be well adapted to grow in wetland soils where ammonium is the prevailing nitrogen compound.[57] In general, macrophytes have been reported to be beneficial in many ways to microbial N removal in CWs.[24,42,58]

Nitrous oxide rate from denitrification measured in February stands within the range from studies performed in Norway, Sweden, Finland and Estonia reporting emissions in CWs treating wastewater: -5.3 to 110 mg N m^{-2} d^{-1}.[59] In comparison with the winter N_2 denitrification rates measured by similar technique in eutrophied Lake Ormajärvi (ca. 2.7 mg N m^{-2} d^{-1})[60], and in boreal agricultural stream water (1.7 mg N m^{-2} d^{-1})[45], the measured N_2 rate in the studied WWTP wetland was approximately 20-fold most likely due to a higher nitrate availability. In addition to the measured high denitrification rate, one of the key factors explaining efficient nutrient retention in the wetland is the relatively long residence time among CWs[61-62], although as compared with the average residence time in Finnish lakes (ca. 2 years)[63], it is very short. This highlights the efficiency of nutrient cycling in shallow wetlands.

In summary, constructed wetlands in northern latitudes have been largely ignored as a way to treat wastewaters, mainly because their efficiency in cold climate is questioned. Lammi WWTP wetland significantly reduces nutrient loading to Lake Ormajärvi. This study shows that microbial activity can have an impressive impact on nitrogen removal despite low temperatures and cold winter if the water residence time is sufficient. However, more information is needed about the relative nitrous oxide production throughout a year, so that generation of this strong greenhouse gas can be mitigated.

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SUPPORTING INFORMATION

Figure S1. Results from the $^{15}NO_3^-$ concentration series experiment.

Figure S2. Temperature and oxygen saturation in the wetland discharge from 25 March 2015 to 15 March 2017.

REFERENCES

(1) Sutton M.A.; Bleeker A.; Howard C.M.; Bekunda M.; Grizzetti B.; de Vries W.; van Grinsven H.J.M.; Abrol Y.P.; Adhya T.K.; Billen G.; Davidson E.A.; Datta A.; Diaz R.; Erisman J.W.; Liu X.J.; Oenema O.; Palm C.; Raghuram N.; Reis S.; Scholz R.W.; Sims T.; Westhoek H.; Zhang F.S. *Our Nutrient World: The challenge to produce more food and energy with less pollution. Global Overview of Nutrient Management.* Centre for Ecology and Hydrology, Edinburgh on behalf of the Global Partnership on Nutrient Management and the International Nitrogen Initiative. 2013.

(2) Carey R.O.; Migliaccio K.W. Contribution of Wastewater Treatment Plant Effluents to Nutrient Dynamics in Aquatic Systems: A Review. *Environ. Manage.* **2009***, 44*, 205–217.

(3) Tattari S.; Puustinen M.; Koskiaho J.; Röman E.; Riihimäki J. Vesistöjen ravinne- kuormituksen lähteet ja vähentämismahdollisuudet. *Suomen Ympäristökeskuksen raportteja* **2015***, 35, 1–73.* (In Finnish).

(4) Morris L.; Colombo V.; Hassell K.; Kellar C.; Leahy P.; Long S.M.; Myers J.H.; Pettigrove V. Municipal wastewater effluent licensing: A global perspective and recommendations for best practice. Review. *Sci. Tot. Environ.* **2017**, *580*, 1327–1339.

(5) Schindler D.W. Evolution of Phosphorus Limitation in Lakes. *Science, New Series* **1977**, *195*, (4275), 260–262.

(6) Schindler D.W.; Hecky R.E.; Findlay D.L.; Stainton M.P.; Parker B.R.; Paterson M.J.; Beaty K.G.; Lyng M.; Kasian S.E.M. Eutrophication of lakes cannot be controlled by reducing nitrogen input: results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 11254–11258.

(7) Elser J.J.; Bracken M.E.S.; Cleland E.E.; Gruner D.S.; Harpole W.S.; Hillebrand H.; Ngai J.T.; Seabloom E.W.; Shurin J.B.; Smith J.E. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* **2007**, *10*, 1135–1142.

(8) Lewis W.M.; Wurtsbaugh W.A.; Paerl H.W. Rationale for control of anthropogenic nitrogen and phosphorus to reduce eutrophication of inland waters. *Environ. Sci. Technol.* **2011**, *45*, 10300–10305.

(9) Pietiläinen O.-P.; Räike A. Typpi ja fosfori Suomen sisävesien minimiravinteena. *Suomen ympäristö*, **1999**, *313*, 1–64. (In Finnish).

(10) Paerl H.W. Controlling Eutrophication along the Freshwater–Marine Continuum: Dual Nutrient (N and P) Reductions are Essential. *Estuar. Coasts.* **2009**, *32*, 593–601.

(11) Paerl H.W. It Takes Two to Tango: When and Where Dual Nutrient (N & P) Reductions Are Needed to Protect Lakes and Downstream Ecosystems. *Environ. Sci. Technol.* **2016**, *50*, 10805–10813.

(12) Bergström A.-K.; Blomkvist P.; Jansson M. Effects of atmospheric nitrogen deposition on nutrient limitation and phytoplankton biomass in unproductive Swedish lakes. *Limnol. Oceanogr.* **2005**, *50(3),* 987–984.

(13) Heini A.; Puustinen I.; Tikka M.; Jokiniemi A.; Leppäranta M.; Arvola L. Strong dependence between phytoplankton and water chemistry in a large temperate lake: spatial and temporal perspective. *Hydrobiologia* **2014**, *731*, 139–150.

(14) Kolzau S.; Wiedner C.; Rücker J.; Köhler J.; Köhler A.; Dolman A.M. Seasonal Patterns of Nitrogen and Phosphorus Limitation in Four German Lakes and the Predictability of Limitation Status from Ambient Nutrient Concentrations. *PLoS ONE* **2014**, *9(4)*, 1–14.

(15) Funkey C.P.; Conley D.J.; Reuss N.S.; Humborg C.; Jilbert T.; Slomp C.P. Hypoxia sustains cyanobacteria blooms in the Baltic Sea. *Environ. Sci. Technol.* **2014**, *48*, 2598–2602.

(16) Lepistö A.; Granlund K.; Kortelainen P.; Räike A. Nitrogen in river basins: Sources, retention in the surface waters and peatlands, and fluxes to estuaries in Finland. *Sci. Tot. Environ.* **2006**, *365*, 238–259.

(17) Hautakangas, S.; Ollikainen, M.; Aarnos, K.; Rantanen, P. Nutrient abatement potential and abatement costs of waste water treatment plants in the Baltic Sea region. *Ambio* **2014**, *43(3)*, 352–360.

(18) Rankinen, K.; Huttunen, M.; Kinnunen, P. Efficiency for nitrogen abatement through modelling. p. 4–5, In: Bergström, I.; Huotari, J., (eds.), EU Life+ N-SINK, Reduction of waste water nitrogen load: demonstrations and modelling. **2017**. Layman's report of project LIFE12 ENV/FI/597, 1–12.

(19) Lee C.; Fletcher T.D.; Sun G. Review. Nitrogen removal in constructed wetland systems. *Eng. Life Sci.* **2009**, *9 (1)*, 11–22.

(20) Vymazal J. Constructed Wetlands for Wastewater Treatment: A Review. *Water* **2010**, *2*, 530–549.

(21) Wu S.; Kuschk P.; Brix H.; Vymazal J.; Dong R. Development of constructed wetlands in performance intensifications for wastewater treatment: A nitrogen and organic matter targeted review. *Water Res.* **2014**, *57*, 40–55.

(22) Ghermandi A.; Bixio D; Thoeye C. The role of free water surface constructed wetlands as polishing step in municipal wastewater reclamation and reuse. *Sci. Tot. Environ.* **2007**, *380*, 247–258.

(23) Arheimer B.; Pers B.C. Lessons learned? Effects of nutrient reductions from constructing wetlands in 1996–2006 across Sweden. *Ecol. Eng.* **2017**, *103*, 404–414.

(24) Wang M.; Zhang D.; Dong J.; Tan S.K. Application of constructed wetlands for treating agricultural runoff and agro-industrial wastewater: a review. *Hydrobiologia* **2018**, *805*, 1–31.

(25) Mitsch W.J.; Gosselink J.G. *Wetlands.* 5th ed. John Wiley & Sons, Inc, USA, 2015.

(26) Dawson R.W.; Murphy K.L. The temperature dependency of biological denitrification. *Water Res.* **1972***, 6*, 71–83.

(27) Aalto S.L.; Saarenheimo J.; Ropponen J.; Juntunen J.; Rissanen A.J.; Tiirola M. Sediment diffusion method improves wastewater nitrogen removal in the receiving lake sediments. *Water Res.* **2018***, 138*, 312–322.

(28) Holmroos H.; Hietanen S.; Niemistö J.; Horppila J. Sediment resuspension and denitrification affect the nitrogen to phosphorus ratio of shallow lake waters. *Fundam. Appl. Limnol.* **2012**, *180(3)*, 193–205.

(29) Rissanen A.J.; Tiirola M.; Ojala A. Spatial and temporal variation in denitrification and in the denitrifier community in a boreal lake. *Aquat. Microb. Ecol*. **2011**, *64*, 27–40.

(30) Holtan-Hartwig L.; Dörsch P.; Bakken L.R. Low temperature control of soil denitrifying communities: kinetics of N_2O production and reduction. *Soil Biol. Biochem*. **2002**, 34, 1797–1806.

(31) Weymann D.; Geistlinger H.; Well R.; von der Heide C.; Flessa H. Kinetics of N_2O production and reduction in a nitrate-contaminated aquifer inferred from laboratory incubation experiments. *Biogeosciences* **2010**, *7*, 1953–1972.

(32) Wrage N.; Velthof G.L.; van Beusichem M.L.; Oenema O. Role of nitrifier denitrification in the production of nitrous oxide. *Soil Biol. Chem.* **2001**, *33*, 1723–1732.

(33) Thamdrup B.; Dalsgaard T. Production of N_2 through Anaerobic Ammonium Oxidation Coupled to Nitrate Reduction in Marine Sediments. *Appl. Environ. Microbiol*. **2002**, *68(3)*, 1312–1318.

(34) Jetten M.S.M.; van Niftrik L.; Strous M.; Kartal B.; Keltjens J.T.; Op den Camp H.J.M. Biochemistry and molecular biology of anammox bacteria. *Crit. Rev. Biochem Molec. Biol.* **2009**, *44(2-3)*, 65–84.

(35) Hu Z.; Lotti T.; van Loosdrecht M.; Kartal B. Nitrogen removal with the anaerobic ammonium oxidation process. *Biotechnol. Lett*. **2013**, *35*, 1145–1154.

(36) Nielsen L.P. Denitrification in sediment determined from nitrogen isotope pairing. *FEMS Microb. Ecol.* **1992**, *86*, 357–362.

(37) Hsu T.-C.; Kao S.-J. Technical Note: Simultaneous measurement of sedimentary N_2 and N_2O production and a modified ^{15}N isotope pairing technique. *Biogeosciences* **2013**, *10*, 7847–7862.

(38) Järvinen, J.; Kuusisto, E. Astiahaihdunta Suomessa 1961-1990. *Vesi- ja ympäristöhallinnon julkaisuja –sarja A,* **1995**, 1–220. (In Finnish).

(39) EU directive 91/271/EEC. http://data.europa.eu/eli/dir/1991/271/oj

(40) Finnish National Legislation (888/2006). https://www.finlex.fi/en/laki/kaannokset/2006/en20060888.pdf

(41) Andersson J.L.; Kallner Bastviken S.; Tonderski K.S. Free water surface wetlands for wastewater treatment in Sweden – nitrogen and phosphorus removal. *Water Sci. Tech.* **2005**, *51(9)*, 39–46.

(42) Vymazal J. Removal of nutrients in various types of constructed wetlands. *Sci. Tot. Environ.* **2007**, *380*, 48–65.

(43) Barria C.; Malecki M.; Arraiano C.M. Bacterial adaptation to cold. Review. *Microbiology* **2013**, *159*, 2437–2443.

(44) Cavaliere E.; Baulch H.M. Denitrification under lake ice. *Biogeochemistry* **2018**, *137*, 285–295.

(45) Uusheimo S.; Tulonen T.; Aalto S.L.; Arvola L. Mitigating agricultural nitrogen load with constructed ponds in northern latitudes: A field study on sedimental denitrification rates. *Agric. Ecosyst. Environ*. **2018**, *261*, 71-79.

(46) McCrackin M.L.; Elser J.J. Atmospheric nitrogen deposition influences denitrification and nitrous oxide production in lakes. *Ecology* **2010**, *91(2)*, 528–539.

(47) Wittgren H.B.; Mæhlum T. Wastewater treatment wetlands in cold climate. *Water Sci. Tech.* **1997**, *35(5)*, 45–53.

(48) Trimmer M.; Risgaard-Petersen N.; Nicholls J.C.; Engströn P. Direct measurement of anaerobic ammonium oxidation (anammox) and denitrification in intact sediment cores**.** *Mar. Ecol. Prog. Ser.* **2006,** *326,* **37–47.**

(49) Jin R.-C.; Yang G.-F.; Yu J.-J.; Zheng P. The inhibition of the Anammox process: A review. *Chem. Eng. J.* **2012**, *197*, 67–79.

(50) Terada A.; Zhou C.; Hosomi M. Presence and detection of anaerobic ammonium-oxidizing (anammox) bacteria and appraisal of anammox process for high-strength nitrogenous wastewater treatment: a review. *Clean Tech. Environ. Policy*. **2011**, *13*, 759–781.

(51) Tomaszewski M.; Cema G.; Ziembińska-Buczyńska A. Influence of temperature and pH on the anammox process: A review and meta-analysis. *Chemosphere* **2017**, *182*, 203–214.

(52) Saarenheimo J.; Rissanen A.J.; Arvola L.; Nykänen H.; Lehmann M.F.; Tiirola M. Genetic and Environmental Controls on Nitrous Oxide Accumulation in Lakes. PLoS ONE **2015**, *10(3), e0121201,* 1-14.

(53) Hefting M.M.; Bobbink R.; de Caluwe H. Nitrous Oxide Emission and Denitrification in Chronically Nitrate-Loaded Riparian Buffer Zones. *J. Environ. Qual.* **2003**, *32*, 1194–1203.

(54) Tiedje J.M. Ecology of denitrification and dissimilatory of nitrate reduction to ammonium. In: Zender A.J.B. (ed.), Environmental microbiology of anaerobes. John Wiley & Sons Inc. N.Y., 1988.

(55) Songliu L.U.; Hongying H.; Yingxue S.; Jia Y. Effect of carbon source on the denitrification in constructed wetlands. *J. Environ. Sci.* **2009**, *21,* 1036-1043.

(56) Reddy K.R.; Patrick W.H.; Lindau C.W. Nitrification-denitrification at the plant root-sediment interface in wetlands. *Limnol. Oceanogr.* **1989**, *34(6)*, 1004–1013.

(57) Brix H., Dyhr-Jensen K., Lorenzen B. Root-zone acidity and nitrogen source affects Typha latifolia L. growth and uptake kinetics of ammonium and nitrate. *J. Experiment. Botany*, **2002**, *53(379),* 2441–2450.

(58) Kallner Bastviken S.; Weisner S.E.B.; Thiere G.; Svensson J.M.; Ehde P.M.; Tonderski K.S. Effects of vegetation and hydraulic load on seasonal nitrate removal in treatment wetlands. *Ecol. Eng*. **2009**, *35,* 946–952.

(59) Søvik A.K.; Kløve B. Emission of N_2O and CH_4 from a constructed wetland in southeastern Norway. *Sci. Tot. Environ*. **2007**, *380*, 28–37.

(60) Rissanen A.J.; Tiirola M.; Hietanen S; Ojala A. Interlake variation and environmental controls of denitrification across different geographical scales. *Aquat. Microb. Ecol.* **2013,** *69*, 1–16.

(61) Arheimer, B.; Wittgren, H.B. Modelling N retention in potential wetlands at the catchment scale. *Ecol. Eng*. **2002**, *19 (1)*, 63–80.

(62) Toet S.; Van Logtestijn R.S.P.; Kampf R.; Schreijer M.; Verhoeven J.T.A. The effect of hydraulic retention time on the removal of pollutants from sewage treatment plant effluent in a surface-flow wetland system. *Wetlands* **2005**, 25(2), 375–391.

(63) Niemi J.; Lepistö L.; Mannio J.; Mitikka S.; Pietiläinen O.-P. Quality and trends of inland waters. In: *Inland and Coastal Waters of Finland;* Eloranta P. University of Helsinki, Saarijärvi, 2004.