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Faculty of Science and Technology, University of Tartu, Estonia

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CONTENTS

ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by Roman numerals. Published papers are reproduced in print with the permission of the publisher.

I. Kasak, K., **Kill, K.**, Pärn, J., Mander, Ü., 2018. Efficiency of a newly established on-stream constructed wetland treating diffuse agricultural pollution. *Ecological Engineering* 119, 1–7. https://doi.org/10.1016/j.ecoleng.2018.05.015

II. **Kill, K.**, Pärn, J., Lust, R., Mander, Ü., Kasak, K., 2018. Treatment efficiency of diffuse agricultural pollution with constructed wetland impacted by groundwater seepage. *Water* 10, 1601. https://doi.org/10.3390/w10111601

III. Kasak, K., Valach, A.C., Rey-Sanchez, C., **Kill, K.**, Shortt, R., Liu, J., Dronov, I., Mander, Ü., Szutu, D., Verfaillie, J., Baldocchi, D.D. 2020. Experimental harvesting of wetland plants to evaluate trade-offs between reducing methane emissions and removing nutrients accumulated to the biomass in constructed wetlands. *Science of the Total Environment* 715, 136960. https://doi.org/10.1016/j.scitotenv.2020.136960

IV. **Kill, K**., Grinberga, L., Koskiaho, J., Mander, Ü., Wahlroos, O., Lauva D., Pärn, J., Kasak, K. 2022. The phosphorus removal performance of in-stream constructed wetlands treating agricultural runoff in temperate climate. *Ecological Engineering*. Under review.

V. Kasak, K., **Kill**, K., Uuemaa, E., Maddison, M., Aunap, R., Riibak, K., Okiti, I., Teemusk, A., Mander, Ü. 2022. Low water level drives high nitrous oxide emissions from surface flow treatment wetland. *Journal of Environmental Management*. Under revision.

Author's contribution to the articles denotes: '*' a minor contribution, '**' a moderate contribution, '***' a major contribution.

Categories	Author's contribution				
	I	II	III	IV	V
Original idea	***	***	*	**	**
Study design	***	***	*	**	***
Data processing and analysis	**	***	*	***	*
Interpretation of the results	**	***	**	**	**
Writing the manuscript	**	***	*	***	**

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ABBREVIATIONS AND ACRONYMES

CH_4	methane
Cl^-	chlorine ion
CW	constructed wetland
ET	evapotranspiration
FWS	free water surface flow
GEP	gross ecosystem production
GHG	greenhouse gas
N_2O	nitrous oxide
NEE	net ecosystems exchange
NO_3-N	nitrate nitrogen
PCA	principal component analysis
PO_4-P	phosphate phosphorus
SO_4^{2-}	sulphate ion
TC	total carbon
TIC	total inorganic carbon
TN	total nitrogen
TOC	total organic carbon
TP	total phosphorus

ABSTRACT

The aim of this thesis was to investigate the efficiency of an in-stream free water surface flow, Vända constructed wetland (CW), in a temperate climate in order to reduce agricultural diffuse pollution and study the parameters affecting nutrient reduction and greenhouse gas (GHG) emissions. The Vända CW was established in 2015 and was monitored from March 2017 until September 2021. Water samples were collected biweekly from the inlet and outlet of Vända CW wetlands (Wetland1 and Wetland2). GHG samples were collected from six points in both wetlands. Flow velocity, turbidity, oxygen concentration, electrical conductivity, water temperature and pH were measured on site with portable devices.

The long-term results of this thesis show that in in-stream CWs, nutrient removal efficiency and GHG emissions are highly dependent on water parameters and wetland design. Vända CW effectively reduced phosphorus, the reduction of which had clear seasonal dynamics. Higher removal efficiency occurred during the warm period and lower values were seen during the cold period. Average annual total phosphorus (TP) removal was 32.1±3.59%, and for PO_4-P it was 22.5±4.4%. Higher removal efficiency values for phosphorus were seen when the flow rate was lower. With a lower flow rate, sedimentation and filtration processes are favoured.

The results for nitrogen removal were contrary to expectations because CW acted more as a source of nitrogen. The annual increase in nitrate nitrogen (NO_3-N) was 48.1±7.4%, being higher in summer. The addition of nitrogen was suspected to come from groundwater seepage. The concentration of total inorganic carbon (TIC), sulphate (SO_4^{2-}) and chlorine (Cl^-) ions, which are characteristic to groundwater, also increased in the wetland, especially during the low flow rate. This suggests a higher groundwater proportion in the wetland that was contaminated with NO_3-N during summer with a low flow rate.

Vegetation coverage was estimated each year and its spread increased exponentially up to 90% and 74% respectively in initially planted and not planted wetland within six years after the establishment thereof. Vegetation helps to remove nutrients in water by sedimentation, filtration and plant uptake, which was seen in Vända CW. Phosphorus removal had a strong positive correlation with vegetation coverage. Based on nutrient concentration in aboveground biomass and its translocation after the vegetation period, the optimal time for biomass harvesting is in autumn if the purpose is to remove nutrients from the wetland. Biomass harvesting timing is also crucial if low methane (CH_4) emissions are targeted, as its emissions increase after cutting and decrease after a couple of days. During the vegetation period, emissions are higher and vary from month to month, but emissions are lowest in autumn.

Overall, for CH_4 and nitrous oxide (N_2O), clear seasonal dynamics were seen, with higher emissions during the warm period and lower emissions during the cold period. Average annual CH_4 emissions were 97.3±11.6 kg of CH_4-C ha^{-1} y^{-1}, having an increasing trend. Average annual N_2O emissions were 7.6±0.5 kg of

N_2O-N ha^{-1} y^{-1}. N_2O emissions were strongly related to water depth, flow rate and water temperature. During summer, with high microbial activity, significantly higher N_2O emissions were seen. Sampling points where the water level was between 0–9 cm were considered N_2O hotspots. Annual mean emissions from this area accounted for 48.5% of the total mean annual N_2O emissions from the entire CW. Correlation between water level and N_2O emissions was confirmed by the flooding experiment in 2021. After the hotspot area was flooded, N_2O emissions decreased by 95.2% and increased back to the usual level after the experiment, when the water level was lowered to its usual level.

Long-term measurements give a good outcome for the future, when similar systems are designed and established to create CWs with better performance and low GHG emissions in a temperate climate.

1. INTRODUCTION

Water quality and its improvement has been one of the environmental issues in the Baltic Sea region and the main reason for this is eutrophication (HELCOM 2021). Food production in agriculture has increased due to the demand of a growing population (FAO 2017; Garcia et al., 2020). The proportion of agricultural land has increased in some regions (Jepsen et al., 2015; Pärn et al., 2018), therefore leading to more fertilizer use. If nitrogen and phosphorus are applied to arable land at the wrong time or in excessive amounts, plants cannot use it all and unused nutrients will reach water bodies by runoff or leaching from fields (Sharpley et al., 2015). Intensive or poorly timed fertilization affects surface waters and increases eutrophication in larger water bodies where smaller nutrient-rich streams flow (Bouraoui and Grizzetti, 2011; Huang et al., 2017). Diffuse agricultural pollution is difficult to locate and control due to its diffuse nature, climate conditions and strong anthropogenic influence (Withers et al., 2014). In order to maintain or improve surface water quality, it is important to find and use measures that can do this. Several legal regulations have been created, such as the Water Framework Directive (2000/60/EC), but nutrients still transfer to water bodies and only few EU water bodies have achieved good ecological and chemical status (European Commission, 2019). Therefore, more work must be done to achieve this goal. One of the best measures for improving water quality is using constructed wetlands (CWs) (Carstensen et al., 2020, Hoffmann et al., 2020).

Constructed wetlands help to improve water quality through natural treatment processes, among several other benefits. They create more habitats for birds, amphibians and mammals and therefore increase biodiversity (Kadlec and Wallace, 2009; Mitsch and Gosselink, 2015; Semeraro et al., 2015; Rannap et al., 2020). The main processes in CWs that help to reduce nutrients in the water are sedimentation, filtration, plant uptake (Gacia et al., 2019), nitrification, denitrification, ammonification and volatilization (Mitsch and Gosselink, 2015; Shi et al., 2018). The treatment processes are affected by several factors, for example changes in precipitation, flow rate, temperature, water depth, water residence time and vegetation (Tonderski et al., 2005; Tanner and Kadlec, 2013). CWs also help to buffer storm water and can be used as water reservoirs during periods of drought. There are several types of CWs: surface flow, subsurface flow and hybrid systems (Vymazal, 2007). For agricultural diffuse pollution, free water surface flow (FWS) CWs are mostly used (Kadlec and Wallace, 2009). FWS CWs are like natural areas; therefore, their appearance is more appealing in a rural environment, creating more green areas that attract birds and mammals (Ilyas and Masih, 2017).

There are two ways to establish FWS CWs – off-stream and in-stream. One option is to create a wetland next to a ditch or a stream and direct only part of its water into the wetland (Arheimer and Pers, 2017; Mander et al., 2017; Tournebize et al., 2017). An in-stream CW is established into a flow path by widening the ditch banks and creating a more meandering flow through wetland using small islands or baffles (Bendoricchio et al., 2000; Darwiche-Criado et al., 2017; Dal Ferro et al., 2018). Using in-stream FWS CWs is not as widespread as off-stream CWs (Borin and Tocchetto, 2007; Zheng et al., 2014, 2015; Hernandez-Crespo et al., 2017; Johannesson et al., 2017;) because they are more prone to flow rate fluctuations (Tanner and Kadlec, 2013; Darwiche-Criado et al., 2017) and in intensive agricultural areas, a limiting factor might be available land to achieve suitable wetland/catchment ratio. Therefore, in-stream CWs performance might be lower. On average, off-stream CWs have been reported to reduce total nitrogen (TN) and total phosphorus (TP) by 62.3–97% (Borin and Tocchetto, 2007; Zheng et al., 2014; Hernandez-Crespo et al., 2017) and 36–86% (Geranmayeh et al., 2018; Zheng et al., 2014; Johannesson et al., 2017; Hernandez-Crespo et al., 2017), respectively. While the effectiveness of in-stream CWs to reduce TN and TP is reported to be up to 67% (Koskiaho et al., 2003; Geranmayeh et al., 2018; Koskiaho and Puustinen, 2019) and 75% (Johannesson et al., 2011; Koskiaho and Puustinen, 2019), respectively. In-stream CWs are beneficial, as they help to reduce nutrient concentration from all water that flows in ditch, while off-stream CWs reduce nutrients only from part of the river or ditch water that is directed into the wetland (Arheimer and Pers, 2017). Thus, off-stream CWs might occasionally remain dry if there is little rainfall during summer and the flow rate and water depth lowers in the main stream bed (Darwiche-Criado et al., 2017). If the wetland dries out during a period, then the plants are under stress and therefore off-stream CWs have generally sparse vegetation coverage. Thus, the permanently flooded conditions of in-stream CWs increase vegetation coverage, which is beneficial for nutrient removal by vegetation (Darwiche-Criado et al., 2017).

Widely used aquatic plants in FWS CWs are the usual emergent macrophyte species, such as cattails (*Typha* sp.), common reed (*Phragmites australis*), reed canary grass (*Phalaris arundinacea*), rush (*Juncus* sp.), nut grass (*Cyperus* sp.) and mannagrass (*Glyceria* sp.) (Gorgoglione and Torretta, 2018). Cattail and common reed geographical distribution is wide, and they adapt well in various conditions. However, these plants prefer to grow in shallow water areas (Wiltermuth and Anteau, 2016). Vegetation in the CW is important as it helps to reduce nutrient concentration by plant uptake (Vymazal, 2013b), creates suitable conditions for sedimentation and filtration processes by calming the flow (Braskerud, 2001; Stottmeister et al., 2004) and prevents resuspension from sediments (Gargallo et al., 2017). Maintenance of vegetation by harvesting aboveground biomass is useful as nutrients can be removed from the system (Kadlec and Wallace, 2009; Vymazal, 2020), but only if it is followed by the removal of harvested biomass (Jabłońska et al., 2021). The harvested and removed biomass can be used for biogas production if a suitable C/N ratio for anaerobic conditions is met (Roj-Rojewski et al., 2019).

When establishing and maintaining FWS CWs, it is also important to consider GHG emissions from these systems, as wetlands are known to emit gaseous compounds into the atmosphere through microbial treatment processes (Mander et al., 2014). Among these gases are methane (CH_4) and nitrous oxide (N_2O). N_2O is produced in the nitrification process if oxygen is present and in the denitrification process in anoxic conditions (Vymazal, 2010). CH_4 is released from the organic matter mineralisation in anaerobic conditions (Bridgham et al., 2013). GHG emissions in CWs could be affected by several factors, such as oxygen concentration, nutrient availability, C/N ratio, vegetation, temperature and water level (Wu et al., 2009; Maucieri et al., 2016; Wu et al., 2017, Evens et al., 2021).

Therefore, the main objectives of the thesis are to: (i) estimate the performance of in-stream FWS CW in a temperate climate to reduce agricultural diffuse pollution; (ii) analyse the factors and parameters affecting performance (**Articles I, II and IV**); (iii) investigate CH_4 and N_2O emissions and their dynamics; and (iv) analyse the factors affecting this (**Articles III and V**) in order to provide guidance on the establishment of similar CWs based on the results from this thesis.

To achieve the objectives, multiple hypotheses were proposed:

– phosphorus removal is highly affected by flow rate and vegetation cover

– nutrient reduction in FWS CWs improves over time when the wetland is stabilized and vegetation has spread

– CH_4 and N_2O emissions have clear seasonal dynamics

– biomass harvesting increases CH_4 emissions in the short term

– N_2O emissions are higher from areas with low water level

2. MATERIALS AND METHODS (Publications I–V)

2.1 Fieldwork methodology

Most fieldwork was conducted in the Vända in-stream FWS CW (hereinafter referred to as the Vända CW) (Figure 1). Water purification and GHG dynamics were investigated in the Vända CW in order to understand their performance and analyse the various parameters affecting this. In addition, **Articles III and IV** included data collection and data analyses from wetlands located in Latvia, Finland and California, USA.

Figure 1. Vända in-stream FWS CW location in Estonia (top left); scheme of upper bed (Wetland1) and lower bed (Wetland2) (top right); and photos of vegetation development during different study periods (bottom from left to right: April 2017, May 2019 and May 2021).

2.1.1 Description of study sites

The treatment efficiency of the Vända CW was studied in **Articles I–II and IV**. The experimental site Vända CW (58°16'57"N 26°43'19"E) is situated in Southern Estonia, 12 km south of Tartu. The catchment is 2.2 km^2 of which arable land has the largest share (approximately 62%), followed by natural areas (forest and bog) (32%) and other land use types. It was established in a ditch in 2015 and the upper bed was planted with cattail (*Typha latifolia*) and common reed (*Phragmites australis*), while the lower bed was left to colonize naturally (Figure 1). **Articles I and II** investigate the nitrogen and phosphorus compound treatment efficiency a short period after the establishment, and in **Article II**, groundwater seepage influence was in focus. In **Article IV**, phosphorus removal efficiency was investigated. GHG fluxes from the Vända and United States wetlands were investigated in **Article III**, when biomass was harvested. In **Article V**, GHG dynamics were investigated during the study period and during an artificial flooding experiment in summer 2021, mimicking the impact of water level changes on GHG emissions.

The study in **Article III** was partly conducted in the Sacramento-San Joaquin Delta in California, USA (East Pond wetland) (38°06' N, 121°38'W). In 1997, the East Pond (Ameriflux US-Tw5) wetland with an area of 3 ha was constructed on a former agricultural field by excavating the surface soil and using the soil to build berms to contain the wetland. It was permanently flooded to a depth of 0.55 m and planted with stems and rhizomes of *Schoenoplectus acutus*, while *Typha angustifolia* and *Typha latifolia* colonized naturally from the surrounding fields and ditches (Miller and Fujii, 2010). The growing season typically extends from March to October. The mean air temperature is 15.9 °C and the mean annual precipitation is 336 mm, as recorded by a nearby climate station in Antioch (**Article III**).

The phosphorus treatment efficiency and the factors affecting this were also studied in Latvian and Finnish wetlands in addition to the Estonian (Vända CW) wetland in **Article IV**. Latvian wetland Mezaciruli (56°34'20"N 23°29'41"E) and three Finland wetlands – Hovi FWS CW (60°25'30"N 24°22'29"E), Rantamo-Seitteli FWS CW (60°26'16"N 25°01'51"E) and Nummela Gateway FWS CW (60°19'40"N 24°20'14"E) – are all in-stream CWs, similarly to the Vända CW, and are located in a temperate climate with four near-equal length seasons. The growing season typically extends from late April to September.

2.1.2 Water sampling and analysis

From March 2017, water samples from Vända CW were collected biweekly until September 2021, except during winter when there was ice cover. Each time, the samples were collected from the inlet and outlet of both wetlands and from the upper course of the Vända CW catchment, where it is natural with bog and forest and water is not yet affected by agriculture (reference point). A portable device (YSI ProDSS) (YSI Inc., Yellow Springs, OH, USA) was used to measure the parameters on site, such as pH, turbidity (from spring 2018), temperature, oxygen concentration, redox potential and electrical conductivity. For flow rate measurements, SonTek FlowTracker (YSI Inc., Yellow Springs, OH, USA) handheld acoustic Doppler velocimeter was used.

From the water samples, total nitrogen (TN), total organic carbon (TOC), total inorganic carbon (TIC) and total carbon (TC) were measured with a Vario TOC cube (Elementar GmbH, Langensenbold, Germany). Concentrations of TP and PO_4-P were determined by spectrophotometry. Concentrations of NO_3-N, SO_4^{2-} and Cl^- were analysed with ion chromatography. The analyses were performed following EVS-EN standards (www.evs.ee/en). Removal efficiency of nutrients was calculated based on the differences between concentrations in the inlet and outlet of the wetland (Equation 1).

$$RE = \frac{(Cin - Cout)}{Cin} * 100\% \qquad \text{Eq.1}$$

where: RE – removal efficiency; Cin – inlet concentration (mg L^{-1}); Cout – outlet concentration (mg L^{-1}).

2.1.3 GHG emissions measurements and sediment sampling

N_2O and CH_4 emissions from the water surface in the Vända CW were measured with white closed floating chambers with a volume of 65 L. Chambers were equipped with a floating device and anchored for stability. Samples were collected from 12 sampling points over the wetland, and boardwalks were created to avoid disturbing the underlying soil surface. At each sampling point, two PVC chambers were used. Samples were collected immediately after the closure of the chamber (0 mins) and after 20 mins, 40 mins and 60 mins using pre-evacuated (0.3 mbar) 50-mL gas bottles (see Kasak et al., 2016). Sampling was carried out biweekly from May 2018 through September 2021. Chamber measurements during some winters were not conducted due to ice coverage. N_2O and CH_4 concentrations from the samples were analysed using Shimadzu GC-2014 gas chromatography equipped with a flame ionization detector, an electron capture detector and Loftfield's auto sampler (Shimadzu Corporation, Kyoto, Japan) (Loftfield et al., 1997). Since the gas concentration in the chamber increased in a near-linear fashion, a linear regression was applied to calculate the gas fluxes. Flux measurements with a coefficient of determination (R^2) of 0.9 or greater were used in further analyses.

For this thesis, a total of 1759 chamber fluxes were used in further analyses for CH_4. For N_2O, 1896 chamber fluxes filled the criteria and were used in further analyses in **Article V**.

In **Article III**, continuous CH_4 fluxes in East Pond were measured between the ecosystem and atmosphere using the eddy covariance (EC) technique (Baldocchi et al., 1988). While in the Vända CW, fluxes were measured using manual closed chambers with a different volume for sampling spots (65 L) and control (260 L) in order to capture emissions before cutting plants. Fluxes in East Pond were measured by an open path infrared gas analyser (LI-7700, LiCOR Inc., Lincoln, NE, USA) and a sonic anemometer was used to measure air temperature and wind speed components (WindMaster Pro 1590, Gill Instruments Ltd., Lymington, Hampshire, England). Fluxes were calculated using the 30-min covariance of turbulence fluctuations in the vertical wind velocity and the scalar of interest after a series of standard and site-specific corrections were applied (Teh et al., 2011; Hatala et al., 2012; Knox et al., 2015). Data were used for the period during and after the cutting to see the clear impact of harvesting on CH_4 emissions.

Sediment samples were collected seasonally or annually from each sampling spot and analysed for pH and dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), dissolved nitrogen (DN), NH_4-N and NO_3-N. All samples were analysed following EVS-EN standards (www.evs.ee/en). Sediment sampling spots were associated with GHG sampling spots in the Vända CW.

2.1.4 Vegetation coverage and biomass analysis

Biomass harvesting was done in the Vända CW in February 2017 and then monthly from August through October 2018. Each month, a new location for harvesting was chosen. Harvesting was done manually using hedge shears and plants were cut approximately 5 cm above the water surface. After harvesting, GHG emissions were measured from five sampling spots using white closed floating chambers as described in the previous subchapter. For control, a taller chamber with a volume of 0.26 m^3 was used to capture the emissions before the plants were cut (**Article III**). Gas samples from the Vända CW were collected with manual chambers using the same method as described in chapter 2.1.3. In **Article III**, in order to understand the changes in CH_4 concentration, fluxes were measured before cutting, two sessions immediately after cutting, followed by once 24, 48 and 72 hours afterwards.

Harvested biomass from five sampling points (0.5 m^2 each) (**Article III**) was analysed for nutrient-uptake. Plant aboveground biomass was collected (a total of ten samples from wetland). Biomass samples were dried for 72 h at 70 °C to a constant weight in a Gallenkamp Sanyo OMT oven, and the dry weight was determined using the Kern GS 6200-1 analytical balance (reproducibility 0.1 g). The content of nitrogen (titrimetric determination), phosphorus (photometry) and total carbon (TC) (gravimetric) in the plant aboveground biomass was determined in the Estonian Environmental Research Center. Vegetation cover was analysed in both Vända CW wetlands using GPS mapping and the MapInfo professional 10.5 software (**Articles III–IV**).

2.2 Statistical analyses

Normality of variables was checked using Shapiro-Wilk tests (Shapiro and Wilk, 1965). As the distribution of data deviated from normality, the nonparametric Mann-Whitney U test (Mann and Whitney, 1947) and Spearman's rank correlation were applied to compare wetlands' performance during the study period. Spearman's rank correlation (Spearman, 1904) was used to determine relationships between water's physical and chemical parameters and N_2O and CH_4 emissions. The analyses were performed and the figures were constructed using Statistica 10.0 (StatSoft Inc., Tulsa, OK, USA).

Principal component analysis (PCA) (Pearson, 1901) was used to determine the relationship between water parameters and phosphorus removal efficiency in vegetated and non-vegetated wetlands. The analyses were performed using R software (version 3.4.4). The level of significance of $p<0.05$ was accepted in all cases.

3. RESULTS AND DISCUSSION

Several studies have indicated that excessive or poorly timed usage of nitrogen and phosphorus fertilizers affects quality of waterbodies by increasing eutrophication (Bouraoui and Grizzetti, 2011; Sharpley et al., 2015; Huang et al., 2017). Therefore, it was necessary to investigate the water quality before it is affected by agriculture and reaches to the Vända CW. During the four-year study in the Vända CW, it was seen that agriculture had a significant impact on water quality parameters. It was observable that the concentration of most of the pollutants was higher at the inlet of the wetland than at the reference point, which was located 2 km upstream from the wetland. At the reference point, the ditch water was in a natural state and not affected by agriculture. The average annual TN and TP concentrations were 3.1±0.3 and 0.086±0.015 mg L^{-1}, respectively, and increased to 5.0±0.1 and 0.223±0.037 mg L^{-1} before entering the Vända CW (**Article IV**). A similar pattern was also observable with the average concentrations of SO_4^{2-} and Cl^- throughout the study period, which increased from 5.9±1.1 to 16.7±0.8 mg L^{-1} and 4.34±0.98 to 14.68±0.98 mg L^{-1}, respectively. The highest concentrations of SO_4^{2-} (up to 41.6 mg L^{-1}) and Cl^- (up to 33.9 mg L^{-1}) occurred in late summer, early autumn in 2018, which clearly show the impact of fertilizers on the surrounding water bodies (Molenat et al., 2002). The average TOC concentration was higher throughout the study period in reference point due to bog and forest in upper course of the catchment, being 87.1±3.4 mg L^{-1}, which decreased to 47.1±2.2 mg L^{-1} before entering the treatment system.

3.1 Treatment efficiency of diffuse agricultural pollution in free surface water flow constructed wetland (Articles I–II, IV)

3.1.1. Nitrogen removal efficiency

The Vända CW is in a valley surrounded by intensively managed agricultural fields, which were affecting nitrogen concentration in ditch and groundwater. Previous studies have reported good results for nitrogen removal, but only few wetlands are in-stream FWS CWs (Kieckbusch and Schrautzer, 2007; Koskiaho and Puustinen, 2019) treating agricultural diffuse pollution. Though, some studies in colder climates have also reported low removal efficiencies for TN (Braskerud, 2002; Grinberga and Lagzdins, 2017; Robotham et al., 2021) in insteam CWs.

Average nitrate nitrogen (NO_3-N) concentration increased through the entire wetland towards the outlet from 3.47±0.16 to 4.85±0.20 mg L^{-1}. The minimum NO_3-N concentration was 0.02 mg L^{-1} measured at the outlet of the lower bed and the maximum concentration was 15.0 mg L^{-1} measured at the inlet of the lower bed. The highest concentrations were usually observed in summer and early autumn in each study year, which indicates that the effluent concentration was 48% higher than the influent concentration (Table 1; Figure 2a). The same pattern was observable for TN, as average inflow concentration (4.99±0.14 mg L^{-1}) increased significantly after passing the wetland system to 6.44±0.19 mg L^{-1} (~38.3±5.7%) (Table 1; Figure 2a). For TN, the highest concentrations were also seen mostly in summer and the maximum concentration was 21 mg L^{-1} at the inlet of the lower bed indicating the addition of nitrogen between the two Vända wetlands. However, if the wetlands are observed separately (Wetland1 and Wetland2), one can see that the upper bed acts as a source of nitrogen, but the lower bed shows better results than upper bed despite the higher inflow concentrations (Figure 2a) and only acted as a source of nitrogen in the first study year. The yearly average TN removal efficiency has increased with time from –1.3±1.0% to 18.9±4.6% in Wetland2, while Wetland1 had the lowest average removal efficiency (–13.7±4.5%) in the second study year, and for the last study year, this had increased to 10.1±4.1%. For NO_3-N, a similar trend was seen. On average, for most study years, Wetland1 acted as a source of NO_3-N, but with the last study year, the average removal efficiency increased to 13.8±7.4%. Wetland2 also showed better results in the last study year compared to the entire study period, when the average removal efficiency was 16.0±6.6%.

Nitrogen removal efficiency is highly dependent on a variety of factors in FWS CWs. For example, temperature, water residence time and carbon and dissolved oxygen concentration are some of the important factors affecting the treatment processes for nitrogen removal (Mitsch and Gosselink, 2015). In aerobic conditions of CW, the nitrification process, where ammonium is oxidized to nitrate, takes place (Vymazal, 2013a) and in CW zones, where there are anoxic conditions and the oxygen concentration is significantly lower (Tanner et al., 2005; Tournebize et al., 2017), denitrification, i.e. reduction of NO_3-N to N_2O and N_2 (Mander et al., 2014; Tournebize et al., 2015), occurs. Most of the processes are more intense during warmer periods with the lower flow rate and the water residence time is longer, so even higher loads of nitrogen compounds could be decreased (O'Geen et al., 2010). Nitrification and denitrification depend on microbial activity, which is affected by temperature, dissolved oxygen concentration and pH level in the wetland (Mayo et al., 2018). Average annual water temperature in this study was 10.1±0.6 °C and during the summer 17.7±0.4 °C, which should be suitable for denitrification. In the Vända CW, water residence time was longer during the warmer period when there was less precipitation and the average flow rate decreased almost twofold. Therefore, during warmer period, there should have been enough time for treatment processes to take place. The average TN concentration increase was even slightly higher during the summer (–40.6±15.1%) than during the entire study period. However, if the CWs are observed separately, in Wetland1, the TN removal efficiency was up to 77.2%, and in Wetland2 even up to 85%. This shows that in the Vända CW, the nitrification and denitrification processes took place, but other factors limited the overall efficiency.

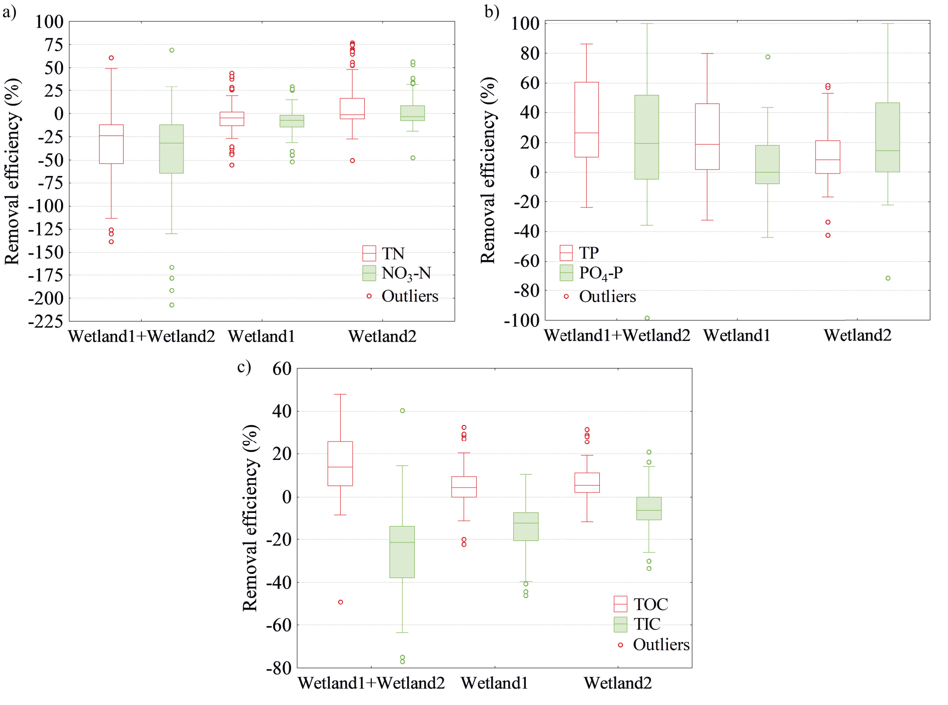

Figure 2. Nutrient and carbon removal efficiency in the Vända CW during the whole study period: a) total nitrogen (TN) and nitrate nitrogen (NO_3-N); b) total phosphorus (TP), phosphate phosphorus (PO_4-P); and c) total organic carbon (TOC) and total inorganic carbon (TIC). Median, 25 and 75% quartile and non-outlier min/max values are presented. Results for both wetland beds (Wetland1+Wetland2), upper bed (Wetland1) and lower bed (Wetland2) are shown. Negative removal efficiency indicates that the outflow has higher pollutant concentration than the inflow.

Table 1. Water parameters and GHG emissions from inlet (In) and outlet (Out) of the Vända CW in each study year with nutrient removal efficiency (RE%). Study year is from March to March. Nutrient concentrations are in mg L^{-1}, water flow rate (Q) is in L s^{-1}, electrical conductivity (EC) is in µS cm^{-1}, turbidity is in NTU and oxygen concentration (O_2) is in mg L^{-1}. GHG emissions are in kg of N_2O-N and CH_4-C ha^{-1} y^{-1}. Yearly average values and ± standard error are presented.

Site year	TN			NO_3-N			TP			PO_4-P			TOC			TIC			TC			Q (L s^{-1})	Temperature (°C)	EC (µS cm^{-1})	Turbidity (NTU)	O_2 (mg L^{-1})	GHG emissions	
	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE						N_2O	CH_4
	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%						kg ha^{-1} y^{-1}	kg ha^{-1} y^{-1}
2017–2018	6.19±0.18	7.42±0.23	−20.3±2.8	4.18±0.15	5.35±0.21	−28.8±3.9	0.25±0.10	0.24±0.12	11.7±4.8	0.06±0.01	0.05±0.01	12.6±5.7	49.3±3.0	44.1±3.1	11.1±1.8				70.2±3.0	69.0 ±2.9	1.5±0.7	4.7±0.9	9.0±1.4	318.6±29.5	10.4±1.9	11.03±0.51		
2018–2019	4.77±0.32	7.36±0.39	−67.7±13.7	3.33±0.42	5.64±0.57	−88.0±22.6	0.19±0.02	0.10±0.02	45.6±7.8	0.05±0.01	0.05±0.02	21.6±13.5	31.8±4.2	27.1±3.6	13.2±3.5	27.1±2.8	30.6±2.8	−11.1±1.9	60.1±2.8	58.7±2.7	1.7±2.4	2.0±0.8	9.8±1.6	343.8±36.3	10.0±1.0	10.10±0.38	9.8±1.4	16.5±12.3
2019–2020	4.47 ± 0.30	5.71± 0.39	−37.4 ±0.7	2.81± 0.41	4.49± 0.39	−82.4 ±1.0	0.16± 0.02	0.10± 0.02	32.4± 0.01	0.04± 0.01	0.03± 0.01	25.4± 0.01	52.7± 4.7	41.7± 5.0	16.5± 5.0	23.5± 3.5	32.3± 3.6	−24.4±	75.0± 1.8	72.9± 2.0	1.1± 1.7	3.0± 1.1	9.1± 1.2	303.0± 38.3	7.8± 1.2	10.02± 0.38	6.7±0.8	46.2±8.4

Site year	TN			NO_3-N			TP			PO_4-P			TOC			TIC			TC			Q (L s^{-1})	Temperature (°C)	EC (µS cm^{-1})	Turbidity (NTU)	O_2 (mg L^{-1})	GHG emissions	
	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE	In	Out	RE						N_2O	CH_4
	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%	mg L^{-1}		%						kg ha^{-1} y^{-1}	kg ha^{-1} y^{-1}
2020–2021	4.73 ± 0.19	5.81± 0.32	−31.6 ± 11.6	3.09± 0.29	3.87± 0.39	−20.3 ±8.8	0.25± 0.04	0.11± 0.02	46.0± 6.2	0.05± 0.01	0.03± 0.004	33.0±6.6	51.9± 4.0	43.8± 4.1	18.9± 2.3	29.1± 2.7	36.3± 2.9	−17.2± 12.2	79.9± 2.4	79.6± 2.1	−1.0± 2.5	2.6± 0.5	11.6± 1.0	327.4± 27.0	8.6± 1.6	8.67± 0.45	6.9±0.6	181.1±24.8
Whole study period	**4.99± 0.14**	**6.44± 0.19**	**−38.3 ±5.7**	**3.47± 0.16**	**4.85± 0.20**	**−48.1 ±7.4**	**0.22± 0.04**	**0.15± 0.04**	**32.1± 3.59**	**0.05± 0.004**	**0.04± 0.005**	**22.5±4.4**	**47.1± 2.2**	**39.8± 2.2**	**15.5± 1.3**	**27.0± 1.7**	**33.6± 1.8**	**−32.8± 8.5**	**72.5± 1.5**	**71.5± 1.4**	**0.5± 1.1**	**3.0± 0.4**	**10.1± 0.6**	**327.1± 16.1**	**8.8± 0.8**	**9.76± 0.24**	**7.6±0.5**	**97.3±11.6**

In **Article I**, the dissolved oxygen (DO) concentration and organic carbon supply for microorganisms were considered some of the limiting factors regarding the age of the wetland. During the first years after the construction, the vegetation was not fully developed and spread through the Vända CW. Therefore, there was less plant litter to decompose and thereby provide available carbon supply for microorganisms. Due to the lack of available carbon supply, denitrification in the bottom of the Vända CW might not have been promoted (Ding et al., 2018). In both Wetland1 and Wetland2, the C/N ratio was lowest during the first years (**Article I**), being on average 6.4±0.8 and 4.2±0.7, respectively. Thanks to vegetation development, the average C/N ratio had risen. In the last study year, it was on average 11.9±1.0 and 8.0±1.0, respectively. This confirms that during the first few years, the available carbon supply might have been one of the reasons denitrification was suppressed and nitrogen removal efficiency low. During the summer, average DO concentration fluctuated between 4.96–10.59 mg L^{-1} in the water column, while in the cool seasons it was above 10 mg L^{-1}, which could suppress the denitrifiers (Tiedje, 1988) in the sediment when the water level is low. Therefore, conversion of nitrate to gaseous nitrogen forms (N_2 and N_2O) could have been inhibited. The optimum pH value for the nitrification and denitrification processes is 7–7.5 (Saleh-Lakha et al., 2009 and 2017), which was in close range to results in this thesis. The average pH level during the study period was 7.8. However, some studies have shown that plants can acidify the surrounding area and lower pH, which can affect some denitrifying bacteria (Kasak et al., 2018). This shows that on some occasions, nitrogen removal processes might not be promoted; however, the high increase of TN and NO_3-N concentration between the wetlands and at the outlet indicates some other disturbances, such as contaminated groundwater seepage in the Vända CW.

3.1.1.1. The impact of groundwater

The year-round increase in nitrogen compounds, TIC concentration (Figure 3) and also the concentration of SO_4^{2-} and Cl^- ions (Figure 4) suggest that these compounds enter the Vända CW not only from ditch water, but also from other sources. This addition of these compounds affects the overall efficiency of the wetland, especially the removal of nitrogen. Due to the location in a valley, which is surrounded by intensively managed agricultural land (Figure 1), groundwater seepage has been the main reason for the increase in nitrogen compounds the Vända CW (**Article II**). The surrounding groundwater areas have been historically affected by the heavy use of fertilizers. During the Soviet period, fertilizer use was significantly higher (Mander and Järvet, 1998) and nitrate rich groundwater has been a problem ever since. Based on the measurement results, the increase in nutrient concentrations was highest between the two wetlands. Therefore, most seepage was assumed to come from there. To analyse nutrient concentration in groundwater, 10 piezometers were established on both sides of the ditch connecting the two wetlands. Sampling results from piezometers and from one local household well reveal that TN concentration was in the range of 0.24–27 mg L^{-1} (**Article II**). Thus, nitrogen rich groundwater seepage could explain most of the nitrogen increase in the Vända CW.

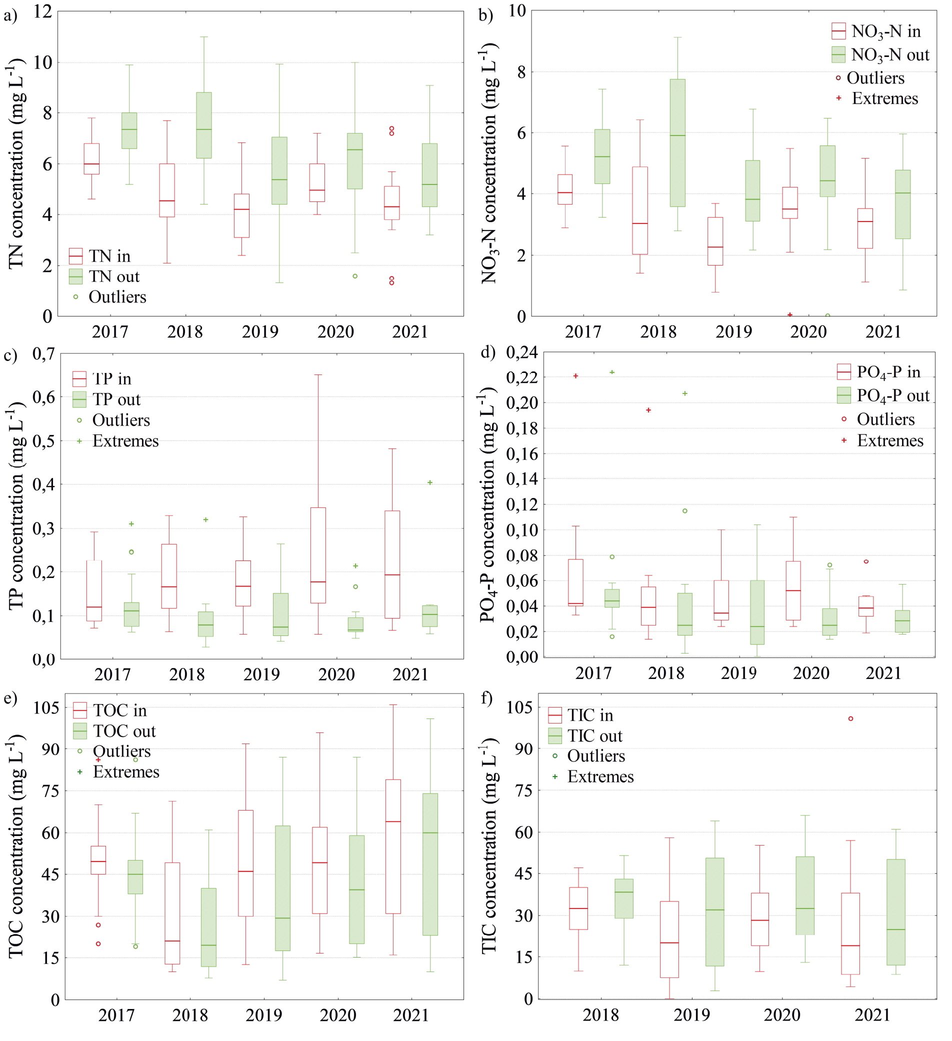

Figure 3. Yearly inflow and outflow concentrations in the Vända CW (Wertland1+ Wertland2) for a) total nitrogen (TN), b) nitrate nitrogen (NO_3-N), c) total phosphorus (TP), d) phosphate phosphorus (PO_4-P), e) total organic carbon (TOC), and f) total inorganic carbon (TIC). Median, 25 and 75% quartile and non-outlier min/max values are presented.

As seen in Figure 4a, the higher negative peaks of nitrogen removal efficiency occurred mostly in summer and spring. In spring, the higher nitrogen concentrations are expected due to higher nutrient runoff from fields due to snowmelt and rain events (Kadlec and Wallace, 2009). However, wetland acting as a source of nitrogen in the summer, when the flowrate was low, indicate disturbances in the system. The higher increase in outlet concentrations during summer for TN and NO_3-N was on average up to 40.6±15.1% and 53.8±13.5%, respectively. Within study years, the average TN removal efficiency in summer has had a negative trend. In 2017, the outlet concentration increased during summer by 32.4±4.9%, and in summer 2021, the increase was as much as 68.3±46.7%. However, in summer 2020, the average TN removal efficiency was 30.2±9.25%. For NO_3-N, the average removal efficiency in summer decreased during first three years. However, in summer 2020 and 2021, the outlet concentration increased by 13.6±6.1% and 13.4±39.6%, respectively. Looking at the two wetlands individually, it was seen that the lower bed had better results. In Wetland1, the average TN removal efficiency in summer changed between –11.3±2.7% and 35.8±8.4%, while in Wetland2 it changed between 3.4±2.3% and 52.7±8.0%.

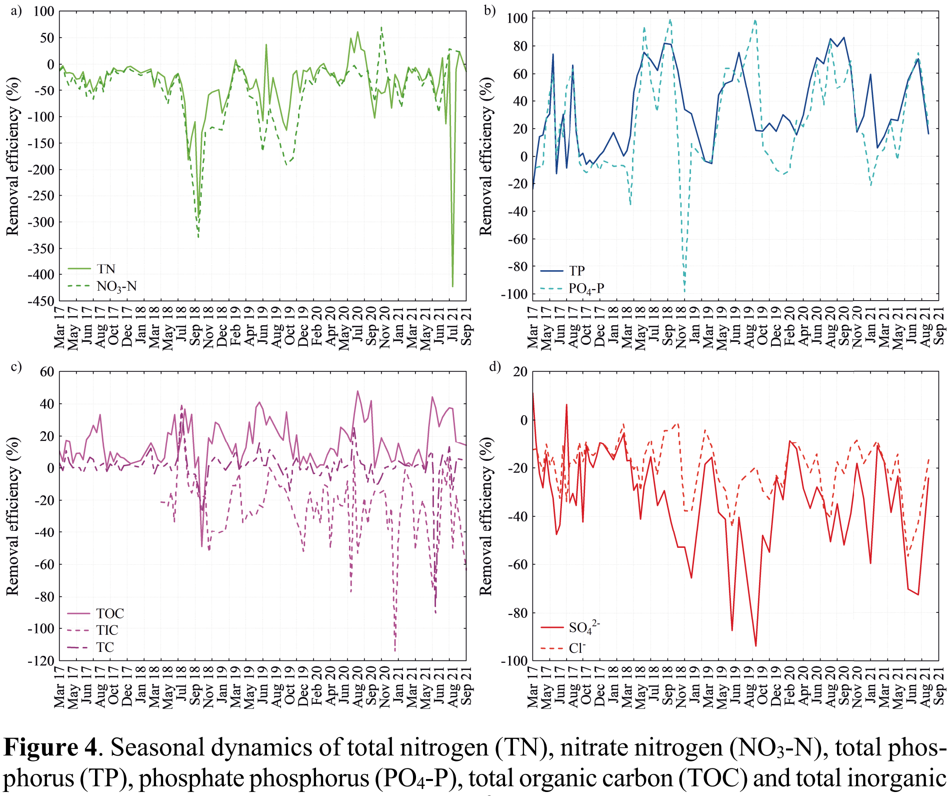

Figure 4. Seasonal dynamics of total nitrogen (TN), nitrate nitrogen (NO_3-N), total phosphorus (TP), phosphate phosphorus (PO_4-P), total organic carbon (TOC) and total inorganic carbon (TIC), total carbon (TC), sulphate (SO_4^{2-}) and chlorine (Cl^-) removal efficiency (%) during study period.

The addition of nitrogen to the wetland was assumed to come from groundwater seepage. During summer, when flow rate was low and occasionally no runoff came by ditch, only leaching or groundwater seepage could occur. This hypothesis, also considered in **Article II**, was supported by the increase of other pollutants, such as SO_4^{2-}, Cl^- and TIC, which refer to groundwater seepage, as TIC shows the concentration of carbonates in the water characteristic to groundwater (Jarvie et al., 2017). Thus, the increase in the concentration suggests that the Vända CW received additional inorganic carbon from another source, probably from groundwater. In Figure 2c, negative removal efficiency can be seen for total inorganic carbon, and its concentration in wetland increases. Similarly, in Figure 3f, it can be seen that each study year there is higher TIC concentrations at the outlet of the wetland. During summer when flow rate was low, high TIC concentrations were observed (Figure 5b). Thus, the addition of TIC did not come with flow from the ditch, but instead originated from groundwater seepage, which did not affect flow rate in wetland. Higher SO_4^{2-} and Cl^- concentrations in the outlet were also observable in summer during the low flow rate. Figure 5a illustrates the strong positive linear correlation of NO_3-N concentration with SO_4^{2-}, Cl^- and TIC. This indicates that the nitrate addition to the wetland originates from groundwater seepage, which lowers the overall nitrogen treatment efficiency, and wetland becomes a source of nitrogen. However, at some point, the Vända CW showed good nitrogen removal efficiency, but the addition of nutrients had a greater effect.

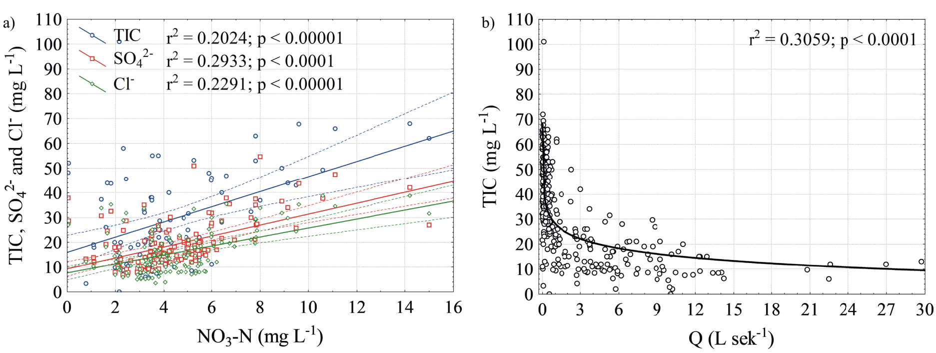

Figure 5. Correlations between water parameters: a) linear correlations between sulphate (SO_4^{2-}), chlorine (Cl^-), total inorganic carbon (TIC) and nitrate (NO_3-N) concentrations measured from the inlets of the wetlands. Dashed lines represent 95% confidence interval; b) TIC concentration and flow rate (Q) correlation.

3.1.2. Phosphorus removal efficiency

Throughout the study period, average TP concentration decreased from 0.223± 0.04 to 0.147± 0.04 mg L^{-1}, resulting in an average removal efficiency of 32.1±3.6% (Table 1). The first study year had the lowest average removal efficiency (11.7±4.8%), but within years, it increased. In the last study year, average TP removal efficiency was 46.0±6.2%. Average PO_4-P concentration decreased from 0.054±0.004 to 0.042±0.005 mg L^{-1}, achieving an average removal efficiency of 22.5±4.4% (Table 1). PO_4-P had a similar trend to TP for removal efficiency over the study years. The average removal efficiency was at first 12.6±5.7%, the next study year 21.6±13.5%, and in last study year it had increased to 33.0±6.6%. These results are in close range with some other in-stream CW studies (Johannesson et al., 2011 and 2017; Koskiaho and Puustinen, 2019). In Figure 3c, one can see that with each study year, median TP inflow concentration slightly increases, being 0.121±0.108 mg L^{-1} in 2017, 0.166±0.022 mg L^{-1} in 2018, 0.167±0.024 mg L^{-1} in 2019, 0.177±0.05 mg L^{-1} in 2020, and in the last year (2021) 0.193±0.055 mg L^{-1}. However, outflow concentration remains lower; therefore, good results for phosphorus removal are seen. With each study year, the average removal efficiency for phosphorus increased (**Article IV**). Figure 4b shows that over the years, the negative peaks are not that extreme, suggesting that similar wetlands take few years to stabilize and reach a certain desired level for phosphorus removal. The best results for TP and PO_4-P removal occurred during summer, when the flow rate was lowest and water residence time in wetland highest. Wetland showed clear seasonal dynamics, having better results for phosphorus removal during the warmer period and poorer results during the colder months (Figure 4b). During the summer months, removal efficiency on average was for TP and PO_4-P 50.9% and 51.7%, respectively. However, annual TP removal was twice as low due to the fluctuating flow rate, which can significantly reduce residence time. From Figure2b, one can see that for TP, the upper bed had better results than the lower bed, which suggests that most of TP from the water was already removed in the upper bed by sedimentation and filtration process and uptake by vegetation. Vegetation spread in the upper bed was higher, and as the lower bed did not receive such high TP concentration and had lower vegetation cover, its removal was moderate (Figure 6a). On the contrary, phosphate (PO_4-P) removal was higher in the lower bed, as this removal is also associated with vegetation uptake and microbial processes (Vymazal, 2002), which need more time to finish.

Higher phosphorus removal efficiency during the warmer months with a lower flow rate was confirmed by significant logarithmic correlations between log TP or log PO_4-P removal efficiency and flow rate (R^2=0.29 and 0.33, respectively) (**Articles I–II, IV**), showing that phosphorus removal efficiency was negatively dependent on flow rate. Taking this into account, phosphorus removal processes like sedimentation, filtration and uptake by vegetation were favoured when the flow rate did not exceed 5 L s^{-1}. Higher flow rate mostly occurred during colder periods with rainfall and snowmelt events that decreased water residence time in wetland; additionally, more runoff from fields was expected during this time. With decreased temperature and increased flow rate, phosphorus removal through microbial processes and vegetation uptake was also inhibited. Turbid and fluctuating flow could also result in sediment resuspension from the bottom sediments, especially in anaerobic conditions (Nguyen and Maeda, 2016; Johannesson et al., 2017). However, since the sedimentation and filtration processes seem to be the main processes for phosphorus removal, flow rate should be relatively low to achieve high efficiency throughout the year.

3.2 Impact of vegetation on nutrient balance in constructed wetlands (Articles III–IV)

In treatment wetlands, it is important to have vegetation, as this helps to slow the flow (Braskerud, 2001) and decrease the effect of wind fetches towards the outlet (Kadlec, 2016). Therefore, vegetation helps to increase water retention time, which is beneficial for sedimentation processes, and resuspension from sediments is prevented (Gargallo et al., 2017). Some previous studies have indicated that vegetation could influence the performance of wetland treating nutrient rich water (Gacia et al., 2019). Vegetation helps to reduce nutrient concentration in water, using these for its growth and life. However, if the plant dies and its litter decays in the bottom of wetland, some of the nutrients that were not stored in the sediment are released back into the water (Kadlec and Wallace, 2009). Therefore, it is necessary to consider when and how maintenance of the wetland should be done. Biomass harvesting is done to take out the nutrients from the wetland. The harvested biomass could be used for composting, potting media, and bioenergy (Wichtmann et al., 2016).

To estimate vegetation development and its effect on nutrient reduction in the Vända CW, vegetation cover estimates were carried out each year after establishment. Results about vegetation spread could be useful for estimating vegetation importance in wetlands and estimating time for planting and maintaining it. Annual vegetation spread estimations in the Vända CW showed that the macrophyte coverage increased rapidly from 30% to 90% in Wetland1 (initially planted) and from 1% to 74% in Wetland2 (initially not planted) within six years after establishment. For both wetlands, an exponential increase was observable (Figure 6a), although the initial planting made the spread more uniform in Wetland1 than in Wetland2. In Wetland2, vegetation coverage increased rapidly after 2018, which means that it takes more than three years for vegetation to spread over the wetland and fully develop by natural colonization (Mitsch et al., 2012). Some wetlands studied in **Article IV** had also developed relatively high vegetation coverage over the years (80% and 70%, respectively for Hovi and Nummela-Gateway CW), while the other wetlands – Mezaciruli and Rantamo-Seitteli CWs – had less than 20% vegetation coverage, as these wetlands were on average deeper than the others. In the studied wetlands, cattail (*Typha latifolia*), common reed (*Phragmites australis*) and willow (*Salix sp.*) are common, especially cattail in the Vända CW. These common wetland plants prefer to grow in shallow water areas that are constantly wet (Wiltermuth and Anteau, 2016). Therefore, the spread of macrophytes in the Mezaciruli and Rantamo-Seitteli CWs were limited by the depth of the wetland. Vegetation spreads more easily in shallow and small wetlands.

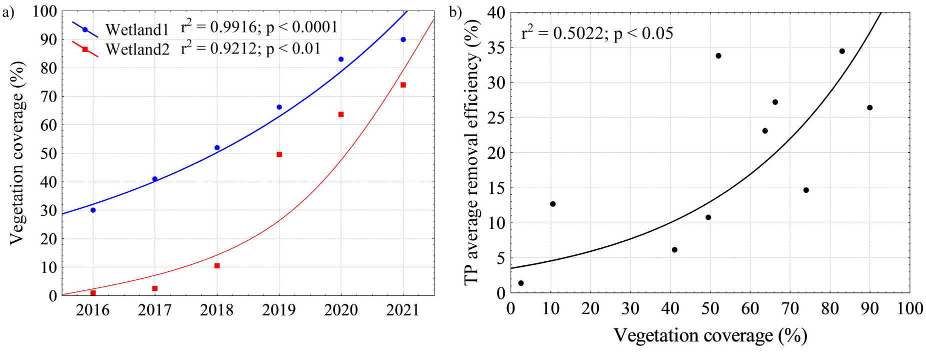

Figure 6. Vegetation development in the Vända CW during the study period (a) and its impact on TP removal efficiency (b). Wetland1 – the upper bed (initially planted), Wetland2 – the lower bed (initially not planted).

In **Article IV**, the effect of vegetation cover on phosphorus removal was assessed, which showed that non-vegetated wetlands (Mezaciruli and Rantamo-Seitteli) are more susceptible to changing weather conditions. Changes in flow rate and temperature affect removal efficiency more in non-vegetated wetlands. Non-vegetated wetlands had more fluctuating removal efficiencies (Figure 7) due to rain events and snowmelt, and resuspension may even occur (Johannesson et al., 2017). From the Vända CW results, it was clearly seen that within vegetation development and spread, the efficiency of wetland in reducing phosphorus increased exponentially (Figure 6b). The upper bed of the Vända CW had a higher average TP removal efficiency more quickly than the lower bed, as the vegetation in the lower bed took more time to develop and spread. If the wetland is not vegetated after its establishment, its acclimatization period is longer and, during this time, it is more receptive to changes in weather and its performance in reducing nutrients may be lower (Newman et al., 2015; Robotham et al., 2021).

In addition, plants use nutrients and therefore help to reduce the concentration of nutrients in the water (Maucieri et al., 2020). In the Vända CW, the dominant macrophyte species was *Typha latifolia* with some patches of *Phragmites australis*. Since the proportion of *Phragmites* was less than 10% of the total vegetated area during the experiment, only the *Typha* biomass was collected and analysed for TC, TN and TP concentrations (**Article III**). Nutrient concentrations in the aboveground biomass have been reported to follow seasonal dynamics with the highest concentrations in late summer and early autumn (Di Nasso et al., 2013; Lee et al., 2013), which was consistent with our biomass analyses. TN and TP concentrations varied between months and had a clear seasonal dynamic (Table 2). TN and TP concentrations in August were 20.3±0.7 g N kg^{-1} and 2.8±0.3 g P kg^{-1}, which decreased significantly to 15.8±0.8 g N kg^{-1} ($p<0.05$) and 1.7±0.1 g P kg^{-1} ($p<0.01$) in February, constituting a reduction of 25% and 42%, respectively. In late summer, the plant growth rate slowed down (Kadlec and Wallace, 2009) and nutrient translocation from leaves to rhizomes occurred.

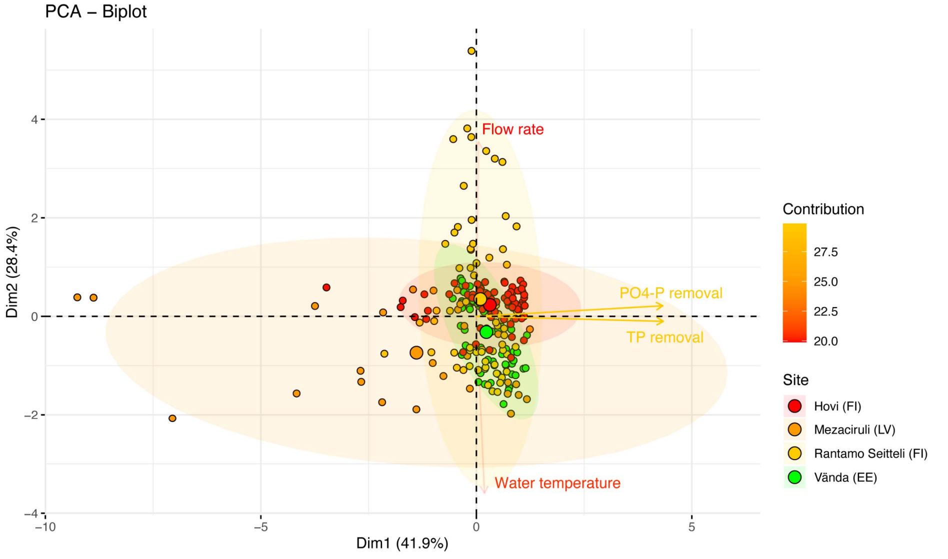

Figure 7. Principal component analysis (PCA) of environmental variables (flow rate and water temperature) and TP and PO_4-P removal efficiencies in four in-stream FWS CWs. Hovi and Vända CWs are vegetated. Mezaciruli and Rantamo-Seitteli where vegetation cover is <20% are considered non-vegetated CWs.

Thus, the highest nutrient concentration in aboveground biomass is found in summer (Di Nasso et al., 2013; Vymazal, 2007). As phosphorus is more mobile (Di Nasso et al., 2013), its relocation started earlier and the concentration decreased by over 40% from August to September. Nitrogen concentration did not decrease that quickly, and even in October the concentration was relatively high (**Article III**). TC concentration did not show similar dynamics with TN and TP; however, the average concentration was slightly higher in February. Due to the C/N changes in biomass during the year, harvest timing is also important, when the harvested biomass will be used for biogas production, in order to have the optimal C/N ratio (Fricke et al., 2007). The C/N ratio in biomass increased from August to February from 23.6±1.1 to 30.9±1.5, respectively, which stay in the range of the optimal C/N ratio needed if harvested biomass is used for biogas production (Fricke et al., 2007). Too low or high a nitrogen concentration might be limiting. Therefore, it is necessary to keep the optimal C/N ratio in order to ensure the degradation of carbon and sufficient nitrogen supply (Fricke et al., 2007). Based on the results, the total biomass harvesting potential in the Vända CW was around 4.4 t ha^{-1} (dry weight) (**Article III**). The results of this thesis and **Article III** showed that the optimal time to harvest aboveground biomass in a temperate climate is September-October. This was the time when nutrient re-location from aboveground biomass to belowground biomass was not completed. However, this process is dependent on the length of the growing season (Kadlec and Wallace, 2009; Vymazal, 2007) and is therefore climate- and region-specific.

Table 2. Plant biomass total nitrogen (TN), total phosphorus (TP), total carbon (TC) and carbon to nitrogen ratios (C/N) at the Vända CW from Jun to Feb (n=10 per month). Average values with ± standard error is presented.

Site	Sampling time	TN (g kg^{-1} dry weight)	TP (g kg^{-1} dry weight)	TC (g kg^{-1} dry weight)	C/N ratio
Vända CW	Aug	20,3±0,7	2,82±0,3	472±5,5	23,6±1,1
	Sept	19.0±1,1	2,11±0,2	470±2,6	25,6±1,8
	Oct	18,1±0,8	1,73±0,2	472±2,9	26,6±1,5
	Feb	15,8±0,8	1,71±0,1	478±8,3	30,9±1,5

3.3 Nitrous oxide (N_2O) and methane (CH_4) emissions (Articles III and V)

Greenhouse gas (GHG) samples were collected biweekly from the Vända CW for 3.5 years in order to investigate long term emissions from treatment wetland and to identify the influencing factors (**Article V**). Samples were collected from 12 points throughout the wetland and, on average, the results showed clear seasonal dynamics for N_2O and CH_4 emissions, having higher values during the warm period and lower emissions during the cold period (Figure 8 and Figure 9, respectively). The average annual N_2O emissions from the Vända CW were 7.6±0.5 kg N_2O-N ha^{-1} y^{-1}, which has a decreasing trend over the study years. During the first study year, average N_2O emissions were 9.8±1.4 kg N_2O-N ha^{-1} y^{-1}, but in last study year, this decreased to 6.9±0.6 kg N_2O-N ha^{-1} y^{-1}. Despite the decreasing trend, the mean annual emissions remained significantly higher than in previous studies (Batson et al., 2012; Hernandez and Mitsch, 2006; Mander et al., 2021; McNicol et al., 2017). Average higher emissions were caused by some sampling points where the emissions of N_2O were over four standard deviations higher than the monthly mean emissions. These areas were considered hotspots for emissions. The main hotspot was the outlet of the upper bed, where the average annual N_2O emissions were 44.7±4.5 kg N_2O-N ha^{-1} y^{-1}. Maximum fluxes in this area were up to 246.1 kg N_2O-N ha^{-1} y^{-1}. Thus, the hotspot average emissions were almost six times higher than the mean annual emissions from the entire wetland. Therefore, the hotspot area accounted for around 48.5% of the mean annual emissions from the entire wetland. The water level measured each time during sampling in this area stayed mostly below 10 cm.

Water's physical parameters were measured each time along with N_2O sampling. The strongest correlation with N_2O emissions had water depth (r_s= –0.63, p<0.01) followed by flow rate (r_s = –0.85, p<0.01), water temperature (r_s = 0.38, p<0.01) and other parameters. Over the study years, the highest emissions occurred during the warm period and from areas where the water level was between 0–9 cm. Some previous studies have also indicated that N_2O emissions are affected by changes in soil moisture and water level (Goldberg et al., 2010;

Leppelt et al., 2014; Pärn et al., 2018; Anthony and Silver, 2021). Thus, higher N_2O emissions occurred with a lower water level (Couwenberg et al., 2011). Higher N_2O emissions are seen during summer when the temperature is increasing, as the activity of denitrifiers increases with temperature (Braker et al., 2010).

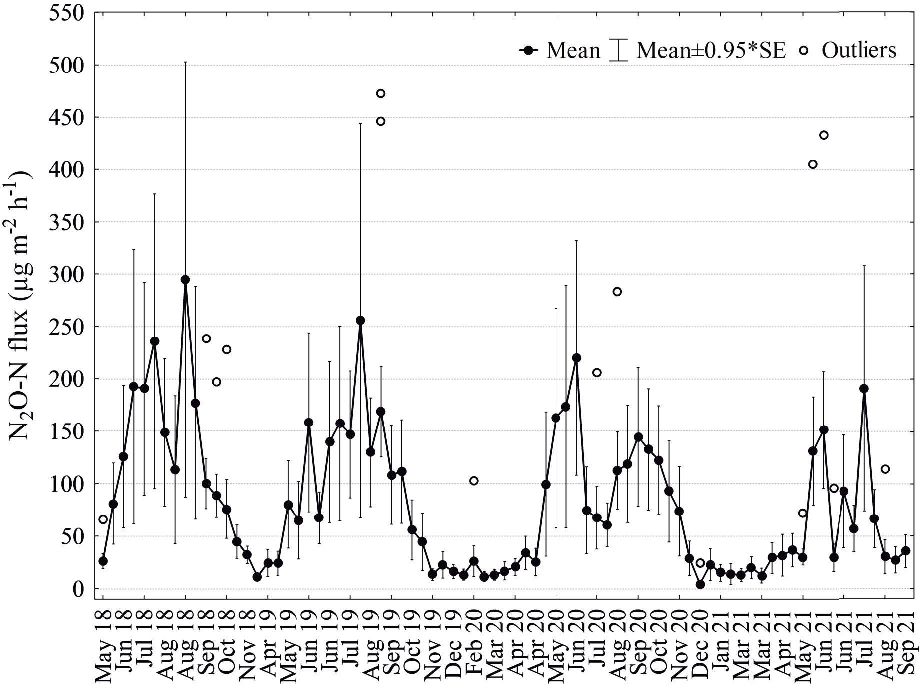

Figure 8. Seasonal dynamics of N_2O emissions in the wetland (n=1896).

To confirm that the main factor increasing N_2O emissions in the Vända CW was water level, the flooding experiment presented in **Article V** was carried out. The results confirmed the hypothesis that with a higher water level, N_2O emissions are lower. N_2O emissions decreased by 95.2% from 33.4±11.1 kg N_2O-N ha^{-1} yr^{-1} to 1.6±0.4 kg N_2O-N ha^{-1} yr^{-1} on average due to the rise in the water level by approximately 13 cm. After the experiment, N_2O emissions increased back to the usual level, once the water level was dropped.

Similarly, to N_2O, CH_4 emissions also had higher emissions during the warm period and lower emissions during the colder period (Figure 9). The average annual CH_4 emissions from the Vända CW were 97.3±11.6 kg CH_4-C ha^{-1} y^{-1}, which had an increasing trend over the study years. During the first study year, the average CH_4 emissions were 16.5±12.3 kg CH_4-C ha^{-1} y^{-1}, but in the last study year this had increased to 181.1±24.8 kg CH_4-C ha^{-1} y^{-1}. The increase in CH_4 emissions might be caused by increased plant litter decomposition, which provides carbon content in wetland bottom layers (Ye et al., 2016). Chamberlain et al.,

(2018) reported that CH_4 emissions were strongly related to plant physical processes, such as net ecosystem exchange, gross ecosystem production and evapotranspiration, especially when the wetland and its soil developed during that time. In the Vända CW, the increase in CH_4 was several times higher during the last study year, probably due to the increased amount of plant litter. Vegetation had spread rapidly, and plant litter accumulated over the years could decompose more intensively due to the higher water level, as it created more suitable conditions for microorganisms (Ye et al., 2016; Chamberlain et al., 2018). During the last study year, individual fluxes were even up to 12082.9 kg CH_4-C ha^{-1} yr^{-1}, but during the other study years, individual fluxes did not exceed 3855 kg CH_4-C ha^{-1} yr^{-1}. The water's physical parameters measured each time along with GHG sampling showed the strongest correlation with CH_4 emissions were water temperature (r_s= –0.53, p<0.01) followed by oxygen concentration (r_s = –0.49, p<0.01). The microorganisms in wetland are more active in warmer periods and decomposition in anaerobic conditions favours CH_4 emissions (Bridgham et al., 2013).

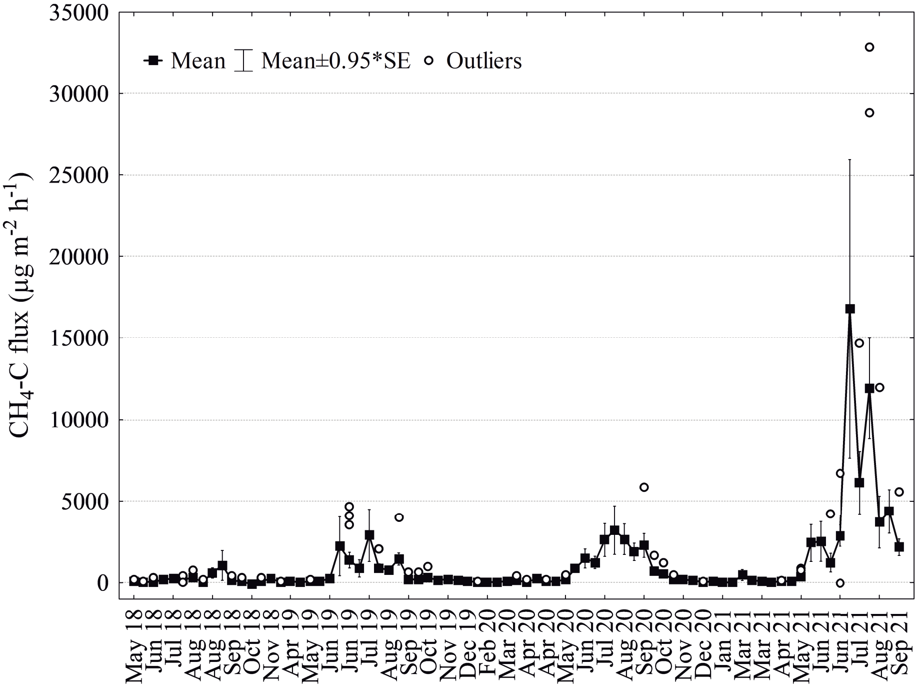

Figure 9. Seasonal dynamics of CH_4 emissions in the wetland (n=1758).

The CH_4 emissions were studied in **Article III**, as some studies have shown that biomass harvesting could increase CH_4 emissions (Barbera et al., 2015; Keyport et al., 2019; Maucieri et al., 2016; Xu et al., 2019), especially during the peak vegetation growing season. An excessive amount of CH_4 could be released during plant harvesting in the growing season. Accumulated CH_4 can be released from the plant aerenchym due to the loss of stomatal resistance to the gas flow (Yavitt and Knapp, 1998). Results from the Vända CW clearly showed that harvesting *Typha* during summer resulted in significantly higher CH_4 emissions than in autumn. This was also confirmed by ecosystem-scale measurements in the Sacramento-San Joaquin Delta (**Article III**). As in previous studies, the CH_4 emissions increased right after cutting the plants (Zhu et al., 2007) and decreased after a couple of days (Keyport et al., 2019). However, some studies have reported that after harvesting, CH_4 emissions remained higher for longer periods (Maucieri et al., 2016; Xu et al., 2019). Such a trend was not seen in the Vända CW. Higher CH_4 emissions after harvesting might be related to the slow vegetation recovery and changes in soil properties and the taxonomic composition of microbial communities (Angeloni et al., 2006). It would be important to maintain and harvest biomass in autumn when the vegetation period is ending in order to keep CH_4 emissions lower after harvesting. However, the concentration of nutrients accumulated in the aboveground biomass is also important when choosing the time for harvesting (Di Nasso et al., 2013). Based on the results in **Article III**, if the harvest is done in autumn, the nutrient translocations are not yet completed and the GHG emissions stay lower due to the colder weather.

4. CONCLUSIONS

The long-term results of this study from 2017 until 2021 show that nutrient removal efficiency and greenhouse gas emissions in the Vända in-stream CW are highly dependent on water parameters and wetland design.

The results demonstrate that the Vända in-stream CW can effectively reduce nutrient concentrations in water, especially the phosphorus content. However, regarding nitrogen compounds, due to nitrate-contaminated groundwater seepage, the wetland acted more as a source of nitrogen. The average annual inlet concentration of TN and NO_3-N increased by 38.3±5.7% and 48.1±7.4% respectively, whereas an even higher increase of the outlet concentration was observed during summer, when the temperature was higher and flow rate lower. The increase in nitrogen concentration was considered due to groundwater seepage. The impact of groundwater was more significant in summer with a low flow rate because its proportion increased in the CW as minimal or no inflow came from the ditch. Total inorganic carbon concentration also increased towards the outlet and had a strong correlation with flow rate, showing higher concentrations with a lower flow rate. Thus, additional nitrogen and inorganic carbon probably originated from the same source. In addition, the concentration of SO_4^{2-} and Cl^- ions characteristic to groundwater also increased throughout the CW. TIC, SO_4^{2-} and Cl^- also had a strong correlation with NO_3-N, which suggests that the addition of nitrogen to the CW came from the groundwater. Although the overall efficiency of CW opposed expectations, the lower bed (Wetland2) of Vända CW showed considerably good results for nitrogen removal. After the second study year, the TN removal efficiency rose to over 15.0% and that of NO_3-N to over 11.7% in the lower bed. This indicates that maturing wetlands can effectively reduce nitrogen compounds from both surface water and groundwater seepage if the conditions for removal processes are suitable.

Based on our results, the Vända CW effectively reduced phosphorus concentration in the water, and within each year, efficiency increased and stabilized. The annual removal efficiency in the first study year was 11.7±4.8% for TP and 12.6±5.7% for PO_4-P, which increased to 46.0±6.2% and 33.0±6.6%, respectively, for the fourth study year. Phosphorus removal efficiency showed clear seasonal dynamics, being higher in the summer when the flow rate was lower and water residence time longer. A strong logarithmic correlation was seen between log TP or log PO_4-P removal efficiency and flow rate, showing that phosphorus removal efficiency was negatively dependent on flow rate. Since the sedimentation and filtration processes seem to be the main processes for phosphorus removal, flow rate should be relatively low in order to achieve high efficiency throughout the year. Based on the Vända CW, the flow rate should not exceed two litres per second in order to give enough time for purification processes take place and retain good removal efficiency.

In this study, several parameters were analysed to investigate their impact on the performance of in-stream CWs. One of these parameters was vegetation coverage and the dynamics thereof. Nutrient concentration in collected aboveground biomass showed the highest nutrient concentration in late summer, whereas lower concentrations were observed in autumn and winter after the end of the vegetation period and when aboveground biomass had died. Nutrients are translocated from the leaves to the roots and rhizomes in autumn, which explains the lower concentrations in this period. If nutrients have to be removed from wetland by biomass harvesting, this should be done before the nutrient translocation has been completed. Based on TN and TP concentration in aboveground biomass, the optimal time for biomass harvesting in a temperate climate seems to be September-October.

Vegetation coverage estimations were carried out each year after construction to find out how quickly the lower bed will be colonized naturally by vegetation and how its spread affects wetland performance. Vegetation coverage spread up to 90% and 74% in initially vegetated and non-vegetated wetland, respectively. For both, the increase in vegetation coverage was exponential during the study period. However, the initially planted wetland had a more uniform spread. Based on the results from the Vända CW, it takes more than three years for vegetation to spread naturally and after five years it might have reached its maximum spread. In the Vända CW, a strong correlation between TP removal efficiency and vegetation coverage was seen. The upper bed received higher removal efficiency more quickly than the lower bed due to higher vegetation coverage. Vegetation helps to remove nutrients from the water by its uptake and helps to slow the flow, which in turn is beneficial for sedimentation and filtration processes.

Wetlands are known to be GHG emitters; therefore, it is important to understand what promotes GHG emissions from CWs and how. Based on the results from the Vända CW, N_2O and CH_4 emissions had clear seasonal dynamics, showing higher emissions during summer and lower emissions during the cold period. The annual N_2O emissions had a slightly decreasing trend, while CH_4 annual emissions had an increasing trend, especially in the last study year. CH_4 emissions increased due to more vegetation litter accumulating and decomposing in the bottom of the wetland. CH_4 emissions are also important to consider when harvesting biomass. Our results confirmed that CH_4 emissions increased immediately after cutting the plants and decreased during couple of days after cutting. The increase in CH_4 emissions after harvesting varied from month to month and was lowest in autumn. This correlates well with the biomass nutrient concentration. Therefore, autumn is the optimal time to harvest biomass while still removing a great amount of nutrients from the wetland with lower CH_4 emissions.

According to results from the Vända CW, N_2O emissions were strongly related to water depth, flow rate and water temperature. During summer, when microbial activity was high, significantly higher N_2O emissions were seen from sampling points where water level was lower. The highest emissions were seen when the water temperature was over 10 °C and water depth between 0–9 cm. These conditions were seen mostly at the outlet of the upper bed, which was the N_2O hotspot. The hotspot's annual mean N_2O emissions were around 48.5% of the total mean annual emissions from the entire CW. Correlation between water level and N_2O emissions was also confirmed by the flooding experiment in 2021. After the hotspot was flooded, N_2O emissions decreased by 95.2% due to the rise in the water level. After the experiment, when water level decreased back to its usual level, N_2O emissions also increased close to the emission values before the experiment.

Based on the results of this PhD thesis, the following recommendations for the better design and establishment of in-stream CWs can be provided:

- CW treating agricultural diffuse pollution should have a wetland/catchment area ratio of at least 0.5 in order to provide enough space and time for water treatment processes.
- CW should be vegetated after establishment in order to enhance vegetation spread and nutrient uptake by vegetation and reduce flow rate with denser vegetation to promote sedimentation and filtration processes.
- CW water depth should vary throughout wetland, but in shallow areas, it should exceed 10 cm for most of the year. Thus, GHG emissions are lower with a higher water depth.
- Flow rate should remain relatively low so as to avoid fluctuations in CW performance. Lower flow rate increases water residence time in wetland and therefore promotes nutrient removal processes, especially phosphorus removal by sedimentation.

Further research is needed to study the microbial processes and communities in in-stream treatment CWs in order to get a better understanding of the regulation of nutrient reduction and GHG emissions in these systems.

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SUMMARY IN ESTONIAN

Toitainete voogude reguleerimine põllumajanduslikku hajureostust puhastavas tehismärgalas

Ühiskonna suurenenud toidunõudluse tõttu on põllumajandus intensiivistunud, mistõttu kasutatakse rohkelt väetisi. Nende liigne või valel ajal kasutamine soodustab veekogude eutrofeerumist, kui toitained jõuavad põldudelt veekogudesse. Põllumajandusliku hajukoormuse leevendamiseks ja vee kvaliteedi parandamiseks on tõhusaks meetmeks kasutada tehismärgalasid, mis aitavad parandada vee kvaliteeti looduslike puhastusprotsesside abil ning pakuvad muidki ökosüsteemi hüvesid.

Töö uurib vahetult voolusängi ehitatud (*in-stream*) Vända tehismärgala efektiivsust, vähendamaks põllumajanduslikku hajukoormust ning toitainete vähenemist ja kasvuhoonegaaside (KHG) emissioone mõjutavaid parameetreid. Vända tehismärgala rajati kahe järjestikuse basseinisüsteemina (sängiga) samanimelisse kuivenduskraavi 2015. aastal ja seirati 2017. aasta märtsist 2021. aasta septembrini. Tegemist on tüüpilise täisläbivoolulise tehismärgalaga, millest voolab läbi kogu kraavivesi. Veeproove koguti Vända märgala sisse- ja väljavooludest iga kahe nädala möödudes. KHG proovid koguti mõlemal märgalal kuuest punktist. Kohapeal mõõdeti kaasaskantavate seadmetega vee voolukiirust, hägusust, lahustunud hapniku sisaldust, elektrijuhtivust, temperatuuri ja pH-d.

Töö näitab, et taolistes ökosüsteemides sõltuvad toitainete eemaldamise efektiivsus ja ka kasvuhoonegaaside heitkogused vee parameetritest ja märgalade kujundusest. Vända tehismärgala vähendas tõhusalt fosforisisaldust vees, millel oli selge hooajaline dünaamika ning iga aastaga efektiivsus tõusis kuni stabiliseerumiseni. Keskmine puhastusefektiivsus esimesel uurimisaastal oli üldfosfori ($P_{üld}$) puhul 11,7±4,8% ja fosfaatfosfori (PO_4-P) puhul 12.6±5.7%, mis tõusis neljandal uurimisaastal vastavalt 46,0±6,2%-ni ja 33,0±6,6%-ni. Fosfori eemaldamise efektiivsusel oli selge sesoonne dünaamika, olles suurem suvel, kui vooluhulk oli väiksem ja vee viibeaeg pikem. Logaritmitud $P_{üld}$ ja PO_4-P puhastusefektiivsuse ja voolukiiruse vahel oli tugev logaritmiline seos, mis näitas, et fosfori puhastusefektiivsus sõltus negatiivselt voolukiirusest. Kuna settimis- ja filtreerimisprotsessid on peamised fosfori eemaldamise protsessid, peaks voolukiirus olema suhteliselt väike, tagamaks suure efektiivsuse kogu aasta vältel. Vända märgalal saadud tulemustele põhinedes ei tohiks voolukiirus ületada kahte liitrit sekundis, et puhastusprotsesside toimumiseks oleks piisavalt aega ja säiliks suur puhastusefektiivsus.

Lämmastiku eemaldamise tulemused olid ootustele vastupidised, kuna märgala oli suure nitraadisisaldusega põhjavee sissetungi tõttu pigem lämmastiku allikaks. Üldlämmastiku ($N_{üld}$) ja nitraatlämmastiku (NO_3-N) keskmine aastane kontsentratsioon tõusis vastavalt 38,3±5,7% ja 48,1±7,4%. Veelgi suuremat kontsentratsiooni tõusu väljavoolu suunas täheldati suvel, mil temperatuur oli kõrgem ja vooluhulk väiksem. Põhjavee mõju oli suurem suvel, sest selle osakaal suurenes märgalas, kuna vee sissevool kraavist oli minimaalne või puudus üldse. Anorgaanilise süsiniku üldkontsentratsioon suurenes samuti väljavoolu suunas ja see seostus voolukiirusega ehk kõrgemaid kontsentratsioone võis näha koos madalama voolukiirusega. Seega lisandus lämmastikku ja anorgaanilist süsinikku tõenäoliselt samast allikast. Peale selle suurenes märgalas ka põhjaveele iseloomulike ioonide – sulfaatiooni (SO_4^{2-}) ja klooriooni (Cl^-) kontsentratsioon. Anorgaaniline süsinik, SO_4^{2-} ja Cl^- olid samuti tugevas seoses NO_3-N-iga, mis viitab, et märgalasse lisandus lämmastikku põhjaveest. Kuigi Vända märgala üldine efektiivsus lämmastiku vähendamisel ei vastanud ootustele, näitas Vända märgala alumine säng lämmastiku eemaldamisel märkimisväärselt häid tulemusi. Pärast teist uuringuaastat tõusis alumises sängis $N_{üld}$ eemaldamise efektiivsus üle 15,0% ja NO_3-N puhul üle 11,7%. See näitab, et märgalad suudavad tõhusalt vähendada lämmastikuühendeid nii pinna- kui ka põhjaveest, kui eemaldamise protsesside jaoks on tingimused sobivad.

Taimkatte katvust hinnati igal aastal ja selle levik kasvas eksponentsiaalselt kuue aasta jooksul pärast rajamist. Vända märgala ülemine säng taimestati peale rajamist, kuid alumine säng jäeti loomulikul teel taimestuma. Taimedega kaetud ala oli viimaseks uuringuaastaks neis kasvanud vastavalt 90%-ni ja 74%-ni. Ülemises sängis oli taimestiku levik kiirem ja ühtlasem. Vända tehismärgalas oli tugev positiivne seos $N_{üld}$ puhastusefektiivsuse ja taimkatte leviku vahel. Taimed aitavad märgalas toitaineid vähendada neid omastades, rahustades voolu ja tekitades takistusi; need protsessid soodustavad settimis- ja filtreerimisprotsesse. Kui toitaineid soovitakse märgalast eemaldada, võib biomassi koristada sügisel, kui toitainete siire maapealsest biomassist risoomidesse pole veel lõppenud. Biomassi koristamise ajastus on oluline ka juhul kui metaani (CH_4) heitkogused on üldiselt madalad, sest vahetult pärast taimede lõikamist CH_4 emissioon tõuseb, vähenedes märkimisväärselt alles paari järgneva päeva jooksul. Vegetatsiooniperioodil on CH_4 heitkogused peale lõikamist suuremad ja varieeruvad kuude lõikes, kuid sügisel on heitkogused vähimad.

Teatud tingimustes on märgalad kasvuhoonegaaside allikad, mistõttu on oluline uurida, mis tingimused ja millisel määral soodustavad märgaladel KHG-de heitkoguseid. Vända tehismärgala tulemuste põhjal oli CH_4 ja dilämmastikoksiidi (N_2O) puhul näha hooajalist dünaamikat: soojal perioodil olid heitkogused suuremad ja külmal perioodil väiksemad. Keskmine aastane CH_4 heitkogus oli 97,3±11,6 kg ha^{-1} a^{-1}, millel oli tõusutrend. Aasta keskmine N_2O heitkogus oli 7,6±0,5 kg ha^{-1} a^{-1}. N_2O heitkogused olid tugevalt seotud vee sügavuse, voolukiiruse ja temperatuuriga. Suvel, kui mikroobne aktiivsus oli suurem, oli N_2O emissioon tunduvalt suurem. Proovivõtukohtades, kus veetase oli vahemikus 0–9 cm, mõõdeti suurimad N_2O heitkogused ajal, mil veetemperatuur oli üle 10 °C. Taolised tingimused ja suuremad N_2O emissioonid esinesid peamiselt ülemise sängi väljavoolualas. Selle piirkonna aastane keskmine heitkogus moodustas 48,5% kogu märgala keskmisest aastasest N_2O heitkogusest. Veetaseme ja N_2O emissioonide vahelist seost kinnitas 2021. aastal läbi viidud üleujutuskatse: pärast ala üleujutamist vähenes N_2O emissioon 95,2% ja pärast katse lõppu, kui veetase alandati uuesti tavapärasele tasemele, tõusis ka N_2O emissioon katse-eelse perioodi emissioonide tasemele.

Pikaajaliste mõõtmiste tulemused on kasulikud sarnaste süsteemide kavandamisel ja rajamisel, tagamaks tehismärgala suurema puhastusefektiivsuse ja väiksema kasvuhoonegaaside emissiooni. Lisauuringuid on vaja voolusisestel tehismärgaladel mikroobsete protsesside ja mikroobikoosluste dünaamika selgitamiseks. Ehkki nendest sõltuvad oluliselt nii toitainete vähendamise efektiivsus kui ka kasvuhoonegaaside emissioon, on teadmisi sellest veel vähe.

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2019 Dora Plus short study visits support for participation in 8th International Symposium on Wetlands Pollutant Dynamics and Control, WETPOL 2019

Conference presentations

30.09–04.10.2018 16th International Conference of the IWA Specialist Group on Wetland Systems for Water Pollution Control. Oral presentation "Potential of an in-stream free water surface constructed wetland to reduce agricultural diffuse pollution", Valencia, Spain.

28.–31.05.2019 The Society of Wetland Scientists' 2019 Annual Meeting "The Role of Wetlands in Meeting Global Environmental Challenges: Linking Wetland Science, Policy, and Society". Oral presentation "Nitrous oxide and methane emissions from surface flow constructed wetland treating diffuse agricultural pollution", Baltimore, Maryland, USA.

17.–21.06.2019 8th International Symposium on Wetlands Pollutant Dynamics and Control, WETPOL 2019. Oral presentation "Greenhouse gas emissions and nutrient reduction in a surface flow constructed wetland treating agricultural diffuse pollution", Aarhus, Denmark.

13.–17.09.2021 9^{th} International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2021 virtual). Oral presentation “The impact of vegetation and flow rate on phosphorus retention in constructed wetlands treating agricultural runoff”, online.

Publications

Kasak, K., Kill, K., Uuemaa, E., Aunap, R., Maddison, M., Riibak, K., Okiti, I., Teemusk, A., Mander, Ü., 202X. Low water level drives high nitrous oxide emissions from surface flow treatment wetland. *Journal of Environmental Management*. (Under revision).

Kill, K., Grinberga, L., Koskiaho, J., Mander, Ü., Wahlroos, O., Lauva D., Pärn, J., Kasak, K., 202X. The phosphorus removal performance of in-stream constructed wetlands treating agricultural runoff in temperate climate. Ecological Engineering. (Under revision).

Kasak, K., Espenberg, M., Anthony, T.L., Tringe, S.G., Valach, A.C., Hemes, K.S., Silver, W.L., Mander, Ülo., Kill, K. McNicol, G., Szutu, D., Verfaillie, J., Baldocchi, D.D., 2021. Restoring wetlands on intensive agricultural lands modifies nitrogen cycling microbial communities and reduces N_2O production potential. *Journal of Environmental Management*, 299, ARTN 113562. https://doi.org/10.1016/j.jenvman.2021.113562.

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Rannap, R., Kaart, M.M., Kaart, T., Kill, K., Uuemaa, E., Mander, Ü., Kasak, K., 2020. Constructed wetlands as potential breeding sites for amphibians in agricultural landscapes: A case study. *Ecological Engineering*, 158, 106077. https://doi.org/10.1016/j.ecoleng.2020.106077.

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2019	Kristjan Jaak välislähetuste stipendium osalemaks konverentsil "The Society of Wetland Scientists' 2019 Annual Meeting" Baltimoreis, Marylandis, Ameerika Ühendriikides
2019	Dora Plus lühiajalise õpirände stipendium osalemaks konverentsil "8th International Symposium on Wetlands Pollutant Dynamics and Control, WETPOL 2019" Aarhusis, Taanis

Konverentsie ettekanded

30.09–04.10.2018	16th International Conference of the IWA Specialist Group on Wetland Systems for Water Pollution Control. Suuline ettekanne "Potential of an in-stream free water surface constructed wetland to reduce agricultural diffuse pollution", Valencia, Hispaania.
28.–31.05.2019	The Society of Wetland Scientists' 2019 Annual Meeting "The Role of Wetlands in Meeting Global Environmental Challenges: Linking Wetland Science, Policy, and Society". Suuline ettekanne "Nitrous oxide and methane emissions from surface flow constructed wetland treating diffuse agricultural pollution", Baltimore, Maryland, Ameerika Ühendriigid.
17.–21.06.2019	8th International Symposium on Wetlands Pollutant Dynamics and Control, WETPOL 2019. Suuline ettekanne "Greenhouse gas emissions and nutrient reduction in a surface flow constructed wetland treating agricultural diffuse pollution", Aarhus, Denmark.

13.–17.09.2021 9th International Symposium on Wetland Pollutant Dynamics and Control (WETPOL 2021 virtuaalne). Suuline ettekanne "The impact of vegetation and flow rate on phosphorus retention in constructed wetlands treating agricultural runoff", virtuaalne.

Publikatsioonid

Kasak, K., Kill, K., Uuemaa, E., Aunap, R., Maddison, M., Riibak, K., Okiti, I., Teemusk, A., Mander, Ü., 202X. Low water level drives high nitrous oxide emissions from surface flow treatment wetland. *Journal of Environmental Management*. (ülevaatamisel).

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