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Carbon and nitrogen dynamics and greenhouse gas emissions in constructed wetlands treating wastewater: a review

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Abstract. The removal efficiency of carbon (C) and nitrogen (N) in constructed wetlands (CWs) is very inconsistent and frequently does not reveal whether the removal processes are due to physical attenuation or whether the different species have been transformed to other reactive forms. Previous research on nutrient removal in CWs did not consider the dynamics of *pollution swapping* (the increase of one pollutant as a result of a measure introduced to reduce a different pollutant) driven by transformational processes within and around the system. This paper aims to address this knowledge gap by reviewing the biogeochemical dynamics and fate of C and N in CWs and their potential impact on the environment, and by presenting novel ways in which these knowledge gaps may be eliminated. Nutrient removal in CWs varies with the type of CW, vegetation, climate, season, geographical region, and management practices. Horizontal flow CWs tend to have good nitrate (NO_3^-) removal, as they provide good conditions for denitrification, but cannot remove ammonium (NH_4^+) due to limited ability to nitrify NH_4^+. Vertical flow CWs have good NH_4^+ removal, but their denitrification ability is low. Surface flow CWs decrease nitrous oxide (N_2O) emissions but increase methane (CH_4) emissions; subsurface flow CWs increase N_2O and carbon dioxide (CO_2) emissions, but decrease CH_4 emissions. Mixed species of vegetation perform better than monocultures in increasing C and N removal and decreasing greenhouse gas (GHG) emissions, but empirical evidence is still scarce. Lower hydraulic loadings with higher hydraulic retention times enhance nutrient removal, but more empirical evidence is required to determine an optimum design. A conceptual model highlighting the current state of knowledge is presented and experimental work that should be undertaken to address knowledge gaps across CWs, vegetation and wastewater types, hydraulic loading rates and regimes, and retention times, is suggested. We recommend that further research on process-based C and N removal and on the balancing of end products into reactive and benign forms is critical to the assessment of the environmental performance of CWs.

1 Introduction

Increasing anthropogenic loading of reactive nitrogen (Nr; all forms of nitrogen (N) except di-nitrogen gas, N_2) along the N cascade in the environment raises many critical concerns for human health, drinking water quality (Gray, 2008), coastal and marine water degradation as well as algal blooms and hypoxia (Conley et al., 2009; Rabalais et al., 2010). Constructed wetlands (CWs) are artificial sinks for Nr (Galloway et al, 2003; Tanner et al., 2005), and have been successfully used to treat domestic sewage, urban runoff and storm water, industrial and agricultural wastewater, and leachate. While the biogeochemistry of wetlands in general has been discussed in the literature (Whalen, 2005; Reddy and Delaune, 2008), less is known about the delivery pathways of the transformation products of carbon (C) and N from CWs treating

wastewater. Although CWs have a proven potential for organic C and N removal, with few exceptions (Dzakpasu et al., 2014), studies have rarely quantified all relevant pathways. This has meant that reported removal efficiencies have been variable (Seitzinger et al., 2002). If the fate of C and N is accurately quantified, appropriate design and management strategies may be adopted.

Constructed wetlands are complex bioreactors that facilitate a number of physical, chemical, and biological processes, but are frequently evaluated as a *black box* in terms of process understanding (Langergraber, 2008). Many investigations target single contaminant remediation and disregard the reality of mixed contaminants entering and leaving CWs. They do not consider the dynamics of *pollution swapping* (the increase in one pollutant as a result of a measure introduced to reduce a different pollutant) driven by transformational processes within and around the system. This means that potential negative impacts that CWs may have on the environment, such as greenhouse gas (GHG) emissions (IPCC, 2013; Clair et al., 2002; Mander et al., 2008; Mitsch and Gosselink, 2000) or enhancement of pollution swapping (Reay, 2004), are not accounted for in analyses. There are many pathways by which the removed N can contribute to water and air pollution: accumulation and adsorption in soils, leaching of nitrate (NO_3^-) and ammonium (NH_4^+) to groundwater, emissions of nitrous oxide (N_2O) and ammonia (NH_3^+) to the atmosphere, and/or conversion to N_2 gas. Constructed wetlands significantly contribute to atmospheric N_2O emissions either directly to the atmosphere from the surface of the wetland (IPCC, 2013; Søvik et al., 2006; Ström et al., 2007; Elberling et al., 2011) or indirectly via dissolved N_2O in the effluent or groundwater upon discharge to surface waters. The IPCC (2013) has recognized the significance of indirect N_2O emissions from CW effluent that is discharged to aquatic environments, and estimate emission factors (EF) ranging from 0.0005 to 0.25. Production and reduction processes of N_2O in the environment are not yet fully understood.

Constructed wetlands receive organic C from the influent wastewater and from fixation by the photosynthetic hydrophytes, which are incorporated into soil as organic C. Soil organic C undergoes the biogeochemical processes that regulate C accretion in soil and microbial respiration, producing carbon dioxide (CO_2). Anaerobic mineralization of organic C by methanogenic archaea can produce methane (CH_4) (Laanbroek, 2010; Ström et al., 2007; Søvik et al., 2006; Pangala et al., 2010). Constructed wetlands can also contribute to the dissolved organic carbon (DOC) load transfer to ground- and surface waters, which may produce and exchange substantial amounts of CO_2 and CH_4 with the atmosphere (Clair et al., 2002; Elberling et al., 2011). Therefore, CWs can diminish the environmental benefits of wastewater treatment. The dynamics of dissolved N_2O, CO_2, and CH_4 in CWs is a key knowledge gap in global GHG budgets.

Surface emissions of GHG from CWs have been commonly measured by the closed chamber method (Johansson et al., 2003, 2004; Mander et al., 2005, 2008), but have rarely been measured by ebullition and diffusion methods (Søvik et al., 2006). The measured rates have shown high spatial, temporal, and diurnal variations due to the change in biogeochemistry of C and N and plant–microbe–soil interaction over time and space. Surface emissions cannot explain the kinetics of production and consumption rates of GHG, which we need to know in order to adopt better management practices to mitigate emissions. In addition, subsurface export of dissolved nutrients and GHG, an important pathway of nutrient loss (Riya et al., 2010), is frequently ignored. Mass balance analysis of the different components of the N cycle and kinetics of their transformation processes occurring within the treatment cells using the isotope-tracing ^{15}N technique can provide mechanistic information for N transformation products (Lee et al., 2009; O'Luanaigh et al., 2010) and may be used to start to answer such questions. Similarly, ^{14}C application and measurement of C species (e.g. CO_2, CH_4, and DOC) may elucidate the C mineralization and CO_2 and CH_4 production and consumption. Used in combination, these methods may provide a comparative analysis of the rates of C and N transformation processes and the role of these processes in delivering NO_3^-, NH_4^+, and DOC to ground/surface waters and N_2O, CO_2, and CH_4 to the atmosphere.

Past reviews on CWs, though very limited, summarize the performance of different types of CWs on C and N removal (Vymazal, 2007) and surface emissions of GHG (Mander et al., 2014), but have not discussed the mechanisms of nutrient removal and the fate of the nutrients delivered and removed to and from CWs. Therefore, the objectives of this review are to (i) understand the biogeochemical dynamics of C and N in CWs, (ii) better understand the fate of various C and N species in a holistic manner, in addition to the conventional influent/effluent balance for nutrient removal, (iii) identify the research gaps that need to be addressed to optimize nutrient removal and mitigate GHG emissions, and (iv) discuss emerging measurement techniques that may give insights into the production and reduction of GHG.

2 Removal efficiency, hydraulic loading, and retention time

In CWs, the efficiency of C and N removal is generally limited and highly variable over CW types, plant types, seasons, climatic regions, and management practices. On average, it appears that 50 and 56 % of the influent total nitrogen (TN) and total organic carbon (TOC), respectively, can be removed, but the removal rates are very inconsistent. Mean ($\pm$ standard error) TN removals, obtained from the literature cited in this paper, ranged from 31.3 ± 6.3 % in surface flow (SF) CWs to 40.4 ± 4.4 % in subsurface flow (SSF) CWs,

Table 1. TN input (mg N L^{-1}), TN output (TN, mg N L^{-1}), and TN removal (%) in various CWs treating wastewater; average standard error (± SE) is presented for TN removal; NA – data not available.

CW type	Treatment	N input (mg N L^{-1})			N output (mg N L^{-1})			N removal (%)			References
		TN	NH$_4^+$	NO$_3^-$	TN	NH$_4^+$	NO$_3^-$	TN	NH$_4^+$	NO$_3^-$	
SF_Finland	Municipal	1.4 ± 150	0.03 ± 5.8	0.3 ± 95	1.1 ± 48	0.01 ± 3.0	0.02 ± 6.7	21.4	66.7	93.3	Søvik et al. (2006)
SF_Finland	Agril. runoff	66.1 ± 1.9	63.5 ± 1.3	0.7 ± 0.13	64.7 ± 1.7	61.2 ± 1.7	0.3 ± 0.09	2.1	3.6	57.1	Søvik et al. (2006)
SF_Norway	Municipal	43.4 ± 3.6	41.5 ± 3.0	0.0 ± 0.0	36.7 ± 2.7	32.6 ± 1.9	0.9 ± 0.4	15.4	21.4	−800	Søvik et al. (2006)
SF	Municipal	NA	4.5	15.5	NA	NA	NA	61	NA	NA	Song et al. (2011)
SF	Domestic	NA	40	5	NA	NA	NA	97–98	NA	NA	Dzakpasu et al. (2011)
SF	Various	NA	39	4.4	NA	NA	NA	39–48	NA	NA	Vymazal (2007)
SF	Municipal	NA	36		NA	NA	NA	39	NA	NA	Vymazal (2010)
SF	Municipal	NA	196	<2	NA	NA	NA	35	NA	NA	Shamir et al. (2001)
SF	various	NA	80	<1	NA	NA	NA	>90	NA	NA	Harrington et al. (2007)
SF	Municipal	NA	0.95	1.54	NA	NA	NA	45	NA	NA	Toet et al. (2005)
SF	Dairy washout	227	NA	NA	NA	NA	NA	40	NA	NA	Van der Zaag et al. (2010)
All SF								31.3 ± 6.3			
HSSF_Estonia	Municipal	96.5 ± 3.0	83.9 ± 2.7	0.2 ± 0.02	46.2 ± 1.5	36.2 ± 1.4	5.9 ± 0.65	52.1	56.9	−2850	Søvik et al. (2006)
HSSF_Norway	Municipal	53.4 ± 4.3	38.4 ± 7.7	14.1 ± 7.5	45.0 ± 4.1	43.1 ± 4.7	1.0 ± 0.8	15.7	−12.2	92.9	Søvik et al. (2006)
HSSF	Dairy washout	306 ± 101*	NA	NA	177 ± 58*	NA	NA	42.2	NA	NA	Van der Zaag et al. (2010)
HSSF	Domestic	NA	74.9	3.9	NA	NA	NA	29	NA	NA	O'Luanaigh et al. (2010)
HSSF	Domestic	87						46–48			Mander et al. (2008)
HSSF	Dairy washout	227						28			Van der Zaag et al. (2010)
HSSF	Milk parlour	112	22	NA	24	11	NA	78	50	NA	Kato et al. (2006)
HSSF	Agriculture	67	40	0.85	27	11	1.1	47	39	−29	Vymazal and Kröpfelova (2010)
HSSF	Industry	124	65	8.5	103	31	7.4	20	20	8	Vymazal and Kröpfelova (2010)
HSSF	Landfill	157	149	1.5	147	98	1.3	30	33	31	Vymazal and Kröpfelova (2010)
HSSF	Municipal	43	24	2	24	14	1.2	40	30	33	Vymazal and Kröpfelova (2010)
All HSSF								40.4 ± 4.4			
VSSF_Estonia	Municipal	50.9 ± 9.2	35.7 ± 6.2	1.1 ± 0.32	43.1 ± 7.6	31.7 ± 5.5	1.7 ± 0.84	15.3	11.2	−54.5	Søvik et al. (2006)
VSSF_Norway	Municipal	52.6 ± 5.2	49.6 ± 4.0	0.0 ± 0.0	47.8 ± 6.9	21.4 ± 6.9	25.5 ± 1.3	9.1	56.9	−25 400	Søvik et al. (2006)
VSSF	Municipal	41.0 ± 0.5	NA	NA	20.7 ± 0.8	NA	NA	49.3 ± 1.8	NA	NA	Yan et al. (2012)
VSSF	Municipal	46 ± 13	NA	NA	NA	NA	NA	74 ± 3	NA	NA	Zhao et al. (2014)
All VSSF								37.0 ± 10.9			

SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow; * mg N m^{-2} h^{-1}.

Table 2. Total organic C (TOC) removal (%) in various CWs treating wastewater; average standard error (± SE) is presented for TOC removal; NA – data not available.

CWs type	Treatment	C input (TOC; mg C L^{-1})	C output (TOC; mg C L^{-1})	TOC Removal (%)	References
SF_Finland	Municipal	13.0 ± 0.3	14.0 ± 0.5	−7.7	Søvik et al. (2006)
SF_Finland	Agril runoff	25.0 ± 3.4	20.0 ± 3.4	20.0	Søvik et al. (2006)
SF_Norway	Municipal	26.7 ± 2.9	17.1 ± 1.8	36.0	Søvik et al. (2006)
SF	Dairy wash out	186[a]	136[a]	27	Van der Zaag et al. (2010)
All SF				18.8 ± 9.4	
HSSF	Domestic	150[b]	NA	NA	Garcia et al. (2007)
HSSF	Dairy wash out	186[a]	107.9[a]	42	Van der Zaag et al. (2010)
HSSF_Estonia	Municipal	62.8 ± 16.6[a]	41.0 ± 11.3[a]	34.7	Søvik et al. (2006)
HSSF_Norway	Municipal	40.5 ± 11.3	15.0 ± 2.4	63.0	Søvik et al. (2006)
All HSSF				46.6 ± 7.3	
VSSF_Estonia	Municipal	132.2 ± 32.2[a]	62.8 ± 16.6[a]	52.5	Søvik et al. (2006)
VSSF_Norway	Municipal	40.5 ± 11.3	15.0 ± 2.4	63.0	Søvik et al. (2006)
VSSF	Municipal	106 ± 35	74 ± 21	26 ± 4.6	Yan et al. (2012)
VSSF	Municipal	249 ± 49	NA	83 ± 1.0	Zhao et al. (2014)
All VSSF				56.2 ± 9.5	

SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow; [a] BOD; [b] mg m^{-2} h^{-1}.

whereas TOC removal ranged from 18.8 ± 9.4 % in SF CWs to 56.2 ± 9.5 % in vertical subsurface flow CWs (Tables 1 and 2). In European systems, for example, typical removals of ammoniacal N in long-term operation are around 35 %, but can be enhanced if some pre-treatment procedures are followed (Verhoeven and Meuleman, 1999; Luederitz et al.,

2001). Generally, TN removal is higher in SF CWs than SSF CWs (Table 1), but studies differ. For example, Van der Zaag et al. (2010) showed higher N removal in SF CWs than SSF CWs, but Søvik et al. (2006) and Gui et al. (2007) showed the opposite. In SSF CWs, limited removal can be caused by a reduced environment that enhances NH$_4^+$ accumulation and

limits NH_4^+ oxidation. In SF CWs, denitrification rates can be limited due to lack of NO_3^-. In vertical subsurface flow (VSSF) CWs, aeration can increase NH_4^+ oxidation to NO_3^-, which can be denitrified or converted to NH_4^+ by dissimilatory NO_3^- reduction to NH_4^+ (DNRA).

Plant species are important components of CWs, and affect C and N removals. Optimal species selection for best removal is difficult because some species are efficient in removing one pollutant but not the other (Bachand and Horne, 2000; Bojcevska and Tonderski, 2007; da Motta Marques et al., 2001). In some studies there are no inter-species differences at all (Calheiros et al., 2007). Mixed species perform better than monocultures to remove C and N pollutants because they increase microbial biomass and diversity. Payne et al. (2014a) discussed the role of plants in nutrient removal. Plants regulate CW hydrology (evaporation and transpiration) and temperature (insulating CWs from seasonal temperature change, trapping falling and drifting snow, and heat loss of wind). Some species can create a large surface area for microbial attachment and enhance microbial diversity, but experimental evidence is still scarce.

Soil physico-chemical properties, such as permeability (Dzakpasu et al., 2014) and cation exchange capacity (Drizo et al., 1999) are important factors controlling the purification capacity in CWs. Microbial activities and growth depend on substrate C quality and C : N ratios, which affect nutrient removal. Growth of heterotrophic microorganisms is a function of the wastewater C : N (Makino et al., 2003). High C : N ratios can enhance denitrification by providing electron donors for denitrifiers, but the opposite can increase nitrification. High C : N ratios can also encourage DNRA over denitrification. Yan et al. (2012) measured a high TN removal but low TOC removals at a C : N ratio 2.5 : 1, which indicates that removal of one parameter might lead to a problem with a different one. The uncertainty in the conditions for achievement of optimum removal suggests that the rates of C and N transformations and the fate of the removed nutrients within CWs should be investigated. However, to our knowledge, no study has provided a holistic evaluation of C and N attenuation and transformation.

The removal of pollutants in CWs depends on hydraulic loading rates (HLR) and hydraulic retention time (HRT) (Toet et al., 2005). The HLR and HRT are considered to be significant design parameters determining the nutrient removal efficiencies (Weerakoon et al., 2013). Longer HRTs of wastewater in CWs increase the removal of C and N (Wang et al., 2014) by increasing sedimentation and duration of contact between nutrients and the CWs. The effects of HLR and HRT can vary with the nature of the use of CWs, e.g. whether they are used for treating single or mixed pollutants. To reduce Nr delivery to the receiving waters or to the atmosphere, CWs need to be optimally designed with respect to HLR and HRT.

Fluctuating hydraulic loading influences all biotic and abiotic processes in CWs (Mander et al., 2011). For example, if the groundwater table is lowered through changes in hydraulic loading, soil aeration can increase or decrease. Ammonification and nitrification rates increase with increased soil aeration and this enhances C utilization by bacteria and, therefore, can stimulate the removal of C and N. Investigation into the effects of fluctuating hydraulic loadings (hydraulic pulsing) on C and N removals and their transformation products will provide information about the fate of the added nutrients in terms of their environmental benefits and/or pollution swapping potential. For example, if the dominant product is N_2, the system will be relatively benign in terms of its impact on the environment, but if it is NH_4^+, it can be fixed in the soils or transported to ground- and surface waters connected to CWs if the cation exchange sites become saturated. Several authors have used a wide range of HLRs and HRTs to measure nutrient removal efficiency, but experimental evidence linking HLR and HRT to removal efficiency is scarce (Toet et al., 2005). Luo et al. (2005) reported that low HLR results in incomplete denitrification, whereas Zhang et al. (2006) argued that low HLR increases NH_4^+ and chemical oxygen demand oxidation. The way in which the performance of a CW is assessed can lead to different conclusions regarding the removal of Nr. For future studies, evaluation of CWs in a holistic manner, which includes pollution swapping at different HLRs and HRTs, is important, particularly within the context of the changing hydrologic cycle in a changing climate. In addition, local legislative targets should be considered and weighting factors (e.g. the relative importance of, say, GHG over water quality targets) should be developed to evaluate the overall performance of CWs. In addition to the estimation of nutrient removal rates, investigation of the effect of HLR and HRT on the different forms of nutrients in the final effluent and their fate in the natural environment may help elucidate the pollution swapping potential of CWs.

3 Accumulation of C and N in CWs soils

The soil in CWs is a major sink for C and N (Mustafa and Scholz, 2011). However, although data on the influent and effluent N concentrations are available, data on N accumulation (dissolved organic nitrogen (DON), TN, NH_4^+, or NO_3^- – N) within the soil profile of various CWs are scarce. The wide range of N accumulation reported in the literature (e.g. 30–40 %, Shamir et al., 2001; 39 %, Harrington et al., 2007; 9 %, Mander et al., 2008; 2.5 %, Obarska-Pempkowiak and Gajewska, 2003) may be due to the variations in CW types and management strategies. The accumulated species of N are reactive unless they have been transformed to N_2 by biogeochemical processes. However, there is a dearth of information on the extent of Nr accumulation in soils and discharge to surface waters and air (Shamir et al., 2001). Accumulated organic N could be mineralized to NH_4^+ and NO_3^-,

depending on the physico-chemical properties of soil. The Nr could be assimilated by plants and microbes, which are recycled in a soil–plant–soil continuum. Nitrogen spiralling occurs from NH_4^+ to organic N and back to NH_4^+ within the CW (O'Luanaigh et al., 2010). Typically, N accumulation has been found to decrease with soil depth (Shamir et al., 2001). In terms of the conventional input–output balance, these are considered as removed N, but may, in fact, remain in such a biogeochemically active system. In addition to N, organic C accumulation occurs in CW soils (Nguyen, 2000).

Soils of CWs represent organic C and Nr-rich systems, where the products of continuously occurring biogeochemical processes, such as accumulation in soil and transportation to fresh waters and to the atmosphere, need to be quantified. Such an approach will show the shortcomings of conventional removal efficiency estimation methods and will also demonstrate how the apparently removed C and N species can become a source of contamination. Estimation of the rates of nutrient accumulation in soils in various types of CWs under different management systems is important. The stability of the accumulated C and N under changing climatic scenarios also needs to be addressed to consider the long-term sustainability of CWs.

4   C and N dynamics and greenhouse gas emissions

Increased nutrient input to CWs increases the productivity of wetland ecosystems and the production of GHG. As CWs are designed to remove pollutants in an anaerobic/suboxic environment, they change the C and N biogeochemistry and contribute significantly to CH_4 and N_2O emissions (Johansson, 2002, Johansson et al., 2003; Mander et al., 2005, 2008; Stadmark and Leonardson, 2005; Liikanen et al., 2006). Søvic et al. (2006) measured N_2O, CH_4, and CO_2 emissions in various CWs in different European countries, and suggested that the potential atmospheric impacts of CWs should be examined as their development is increasing globally. Management of CWs must consider the negative climatic aspects of increased emissions of GHG in addition to their primary functions (Ström et al., 2007). Therefore, estimation of the contribution of CWs to global warming is required. In this regard, measurement of spatial and temporal variations (seasonal and diurnal) of GHG emissions is necessary to accurately estimate CW-derived GHG emissions. A holistic assessment of ecologically engineered systems has been outlined by Healy et al. (2011, 2014) and developed further by Fenton et al. (2014). Such assessments can be applied in evaluating nutrient dynamics in CWs. Moreover, plant mediated GHG emissions could be an important component of total emissions, but again research in this area is very limited. Effective modelling or up-scaling of GHG emissions from watershed to regional/national scales is important for the improvement of global GHG budgets. Such up-scaling needs an accurate estimation of C and N inputs and outputs, i.e. a

balance coupled with net GHG emissions, while considering all possible processes and pathways involved. A study of the dynamics of C and N in CWs is crucial, as the forms of removed C and N are particularly pertinent to their potential for pollution swapping, global warming, and water pollution.

Processes involved in N removal and N transformations in wetlands include sedimentation of particulates (Koskiaho, 2003), nitrification, denitrification, DNRA (Poach et al., 2003; Burgin et al., 2013), microbial assimilation and plant uptake–release (Findlay et al., 2003), and anammox (anaerobic ammonium oxidation) and deamox (DEnitrifying AMmonium OXidation). Constructed wetlands are complex systems that facilitate aerobic and anaerobic microsites (Wynn and Liehr, 2001). Nitrification, denitrification, and nitrifier denitrification are the processes responsible for the production of N_2O. Depending on the environmental conditions or management practices prevailing, a certain process will dominate; e.g., denitrification is the dominant process in SF CWs (Beaulieu et al., 2011), but nitrifier denitrification is dominant in VSSF CWs (Wunderlin et al., 2013). Generally, CWs are anaerobic but aquatic macrophytes can transport oxygen from the atmosphere to the rooting zone, where it can sustain nitrification. The existence of microsites that support high activity and promote denitrification has been shown in soils (Parkin, 1987). Such conditions are also likely to occur in CWs, which have patchy distributions of organic material (e.g. particulate organic carbon), due to rhizodepositions (Minett et al., 2013; Hamersley and Howes, 2002). Minett et al. (2013) found that simultaneous oxygenation of the rhizosphere, through radial oxygen loss, and enhanced oxygen consumption by the soil occurs in the area immediately surrounding the roots. Nitrate produced in the rooting zone can be taken up by plants or denitrified and/or converted back to NH_4^+ by DNRA.

Competition for NO_3^- may occur between denitrification and biotic assimilation. This is likely governed by the prevailing aerobic/anaerobic conditions and therefore dependent on the type of wetland. For instance, in storm water biofiltration systems, prolonged periods of inundation and dry periods may support bio-assimilation over denitrificaton (Payne et al., 2014a, b).

The conditions that favour the occurrence of either denitrification or DNRA are still in debate (Rütting et al., 2011). DNRA is thought to be favoured by a $C : NO_3^-$ ratio of > 12 (Rütting et al., 2011) and occurs at low levels of oxidation-reduction potential (Thayalakumaran et al., 2008). The differences between denitrification and DNRA may be due to the availability of organic matter, because DNRA is favoured at a high $C : NO_3^-$ ratio and denitrification is favoured when carbon supplies are limiting (Korom, 2002; Kelso et al., 1997). The fermentative bacteria that carry out DNRA are obligate anaerobes, and so cannot occupy all the niches that denitrifiers can (Buss et al., 2005). Takaya (2002) stated that a more reducing state favours DNRA over denitrification.

Pett-Ridge et al. (2006) showed that DNRA is less sensitive to dissolved oxygen (DO) than denitrification. Fazzolari et al. (1998) showed that the partitioning between DNRA and denitrification depends on the $C : NO_3^-$ ratio and C rather than DO.. Significant DNRA may occur only at a $C : NO_3^-$ ratio above 12 (Yin et al., 1998). Different numbers of electrons are required in the reduction of each NO_3^- molecule: five for denitrification and eight for DNRA. Therefore, more organic matter can be oxidized for each molecule of NO_3^- by DNRA than by denitrification. In addition, NO_3^- reduction is generally performed by fermentative bacteria that are not dependent on the presence of NO_3^- for growth under anaerobic conditions. Therefore, DNRA bacteria may be favoured in NO_3^--limited conditions (Laanbroek, 1990). Recent studies have suggested that DNRA may be an important process compared to denitrification in wetland sediments (Burgin and Hamilton, 2008). Van Oostrom and Russell (1994) found a 5 % contribution of DNRA to NO_3^- removal in CWs. Little is known about the eventual fate of the NO_3^- that is converted to NH_4^+ via DNRA pathways. In recent years, N-cycling studies have increasingly investigated DNRA in various ecosystems to explore its importance in N cycling (Rütting et al., 2011), but controls on DNRA are relatively unknown (Burgin et al., 2013), DNRA being probably the least studied process of N transformation in wetlands (Vymazal, 2007). However, DNRA can be a significant pathway of NO_3^- reduction that impacts on the CW ecosystem services and so should therefore be evaluated.

Denitrification has been estimated to be a significant N removal process, but actual quantification data are scarce. Few studies have estimated N losses by denitrification, e.g. 19 % (Mander et al., 2008) and 86 % (Obarska-Pempkowiak and Gajewska, 2003) of the total N input based on the mass balance study. To our knowledge, no data are available on denitrification measurements in soil/subsoils of surface flow CWs. While many of these pathways transfer Nr (mainly NH_4^+ and N_2O) to the environment, other pathways can convert Nr to N_2 (e.g. denitrification, anammox, and deamox). Anammox can remove NO_2^- and NH_4^+ as N_2 when the existing environment is hypoxic. Deamox can remove NO_3^- and NH_4^+ as N_2, where NO_3^- is converted to NO_2^- by autotrophic denitrification with sulfide (Kalyuzhnyi et al., 2006). In CWs, anammox and deamox are not well understood, so it is crucial to identify which of the processes are occurring in a specific type of CW and the rate at which they occur. Once a process that provides N_2 as the end product is determined, then the management of the CW can be directed towards enhancement of that process. Hence, quantifying the rates of these processes for various types of CW is required for improved N management towards lowering Nr in the environment.

The various components of the C cycle include: fixation of C by photosysnthesis, respiration, fermentation, methanogenesis ,and CH_4 oxidation with reduction of sulfur, iron, and NO_3^-. Anaerobic methane oxidation coupled with denitrification, a recently proposed pathway of the C cycle (á Norði and Thamdrup, 2014; Haroon et al., 2013; Islas-Lima et al., 2004), can reduce CH_4 emissions in CWs. The C removal processes are sedimentation, microbial assimilation, gaseous emissions, dissolved C losses through water to ground- and surface water bodies, and chemical fixation (bonding with chemical ions). Net primary productivity of wetland hydrophytes varies across CW type, season, climatic region, and local environmental conditions. For example, results can vary remarkably for CWs containing the same plant species in different geographical regions (Brix et al., 2001). The rate of carbon mineralization in CW sediments depends on the redox chemistry of soil, the bio-availability of organic C and temperature. In particular, areas of sediment subjected to prolonged low redox conditions (e.g, -150 mV) are conducive to methanogens and rates of CH_4 emissions exceeding 132 mg m^{-2} d^{-1} (Brix et al., 2001), but this is highly variable depending on $C : N$ ratio of the influent water and wetland seasonality. In summer, oxygen diffusion to the topsoil can reduce methanogenesis and stimulate CH_4 oxidation (Grünfeld and Brix, 1999). However, an increase in temperature can decrease DO in deeper subsoil layers, which can enhance CH_4 production. As in all biochemical reactions, temperature increases C and N turnover in CWs, causing high variations in GHG emissions in different types of CWs in different regions (temperate/tropical/arctic). This warrants the acquisition of more measurement data across CW types and regions for the better extrapolation of GHG emissions. The $C : N$ ratios of wastewater affect microbial growth and development that, in turn, affect their response to C and N cycles and GHG emissions. Previous research on the effects of $C : N$ ratios on nutrient removal and GHG emissions is limited. A few examples include Yan et al. (2012) and Zhao et al. (2014), who measured lower CO_2 and CH_4 emissions at $C : N$ ratios of between 2.5 : 1 and 5 : 1, but this lower range of $C : N$ ratios decreased TOC removal. Hence, investigation of the influence of $C : N$ ratio on nutrient removal efficiencies and GHG emissions across CW and management types is crucial.

Emissions of GHG in CWs can vary across CW typologies, e.g. surface flow or subsurface flow (Tables 3 and 4). Generally, CH_4 emissions are higher in SF CWs than in SSF CWs (Table 3), but may vary with season, which requires investigation. Nitrous oxide and CO_2 emissions are higher in VSSF CWs than horizontal subsurface flow (HSSF) and SF CWs. The N_2O EF (N_2O / TN input $\times$ 100) ranged from 0.61 ± 0.21 % in SF CWs to 1.01 ± 0.48 % in VSSF CWs. The EF for CH_4 emissions ranged from 1.27 ± 0.31 % in VSSF CWs to 16.8 ± 3.8 % in SF CWs. The GHG from CWs can vary between vegetated and non-vegetated systems (Table 5).

Aquatic plants play an important role in GHG production and transport to the atmosphere by releasing GHG through their interconnected internal gas lacunas (Laanbroek, 2010). Emergent plants can transport atmospheric oxygen to the

Table 3. Nitrous oxide (N_2O) emissions ($mg\,N\,m^{-2}\,d^{-1}$); N_2 emissions ($mg\,N\,m^{-2}\,d^{-1}$) and N_2O emission factor (N_2O / TN input $\times$ 100) in various type of CWs; mean standard error ($\pm$ SE) was presented for N_2O emission factor; NA – data not available.

CW type	Treatment	Denitrification		N_2O-N / TN (%)	N_2-N / TN (%)	References
		N_2O emissions ($mg\,N\,m^{-2}\,d^{-1}$)	N_2 emissions ($mg\,N\,m^{-2}\,d^{-1}$)			
HSF	Agril. tile drainage	0.01–0.12	NA	0.19–1.4	NA	Xue et al. (1999)
HSF	Treated municipal	2.0 ± 3.3	NA	0.02–0.27	NA	Johansson et al. (2003)
HSF	Agril. drainage	-0.2–1.9	NA	-0.14–0.52	NA	Wild et al. (2002)
HSF	Dairy wash out	16.8 ± 7.0	NA	0.33 ± 0.12	NA	Van der Zaag et al. (2010)
HSF_Finland	Municipal	0.01 ± 0.01	NA	1.6 ± 1.3	NA	Søvik et al. (2006)
HSF_Finland	Agril. runoff	0.40 ± 0.25	NA	0.37 ± 0.18	NA	Søvik et al. (2006)
HSF_Norway	Municipal	4.0 ± 1.6	NA	1.5 ± 4.4	NA	Søvik et al. (2006)
All SF		2.78 ± 1.72		0.61 ± 0.21		
HSSF	Domestic	0.2–17.0	NA	0.06–3.8	NA	Mander et al. (2005)
HSSF_Estonia	Municipal	7.1 ± 1.2	NA	0.05 ± 0.31	NA	Søvik et al. (2006)
HSSF_Norway	Municipal	6.9 ± 4.3	NA	0.24 ± 0.53	NA	Søvik et al. (2006)
HSSF	Domestic	1.3–1.4	160–170	0.37–0.60	15.2–22.7	Mander et al. (2008)
HSSF	Domestic	0.003–0.001	0.01–5.42	NA	NA	Teiter and Mander (2005)
HSSF	Domestic	0.13	NA	0.008	NA	Fey et al. (1999)
HSSF	Dairy wash out	9.5 ± 1.5	NA	0.18 ± 0.12	NA	Van der Zaag et al. (2010)
HSSF	Domestic	0.17	NA	0.23	NA	Liu et al. (2009)
VSSF	Domestic	0.17	NA	0.01		Mander et al. (2011)
All HSSF		4.23 ± 1.87		0.62 ± 0.38		
VSSF	Domestic	0.001–0.002	0.01–5.0	NA	NA	Teiter and Mander (2005)
VSSF	Domestic	4.6	150	0.45–0.50	NA	Mander et al. (2008)
VSSF	Domestic	11.0	NA	0.29	NA	Mander et al. (2005)
VSSF	Domestic	1.44	NA	0.03		Mander et al. (2011)
VSSF	Domestic	0.005	NA	0.09	NA	Gui et al. (2007)
VSSF	Domestic	0.003	NA	0.04	NA	Liu et al. (2009)
VSSF_Estonia	Municipal	15 ± 3.9	NA	04.3 ± 0.95	NA	Søvik et al. (2006)
VSSF_Norway	Municipal	960 ± 40	NA	1.4 ± 0.72	NA	Søvik et al. (2006)
All VSSF		123.8 ± 106		1.01 ± 0.48		

SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow.

rooting zone and contribute to increased N_2O and CO_2 production and CH_4 consumption (Brix, 1997). Vascular plants can exchange GHG between the rooting zone and atmosphere (Yavitt and Knapp, 1998). Vegetation and its composition affect the nutrient dynamics and the production, consumption, and transport of GHG and hence their exchange between wetlands and atmosphere (Ström et al., 2003, 2005; Søvic et al., 2006; Johansson et al., 2003). They can also affect the biogeochemistry of CWs due to the differences in their growth and development, longevity, root systems, root density, root depth, and microbial ecology in the rhizosphere. As some plant litter decomposes, organic matter with lignocellulose and humic compounds may be released that are more or less labile or stable in nature than others. Release of low molecular weight organic matter that is labile in nature is more likely to produce GHGs than stable forms. For example, *Z. latifolia* showed higher nutrient removal and CH_4 fluxes than *P. australis* (Inamori et al., 2007). The *Z. lotifolia* root system is shallow and the activity of methanotrophs is primarily confined to the top soil. The root systems of *P. australis* are deeper, which is more favourable for the oxidization of CH_4.

A fluctuating water table in CWs has significant impacts on GHG dynamics. Pulsing hydrologic regimes decreases CH_4 but increases N_2O emissions (Mander et al., 2011). In aerobic and anaerobic conditions caused by pulsing hydrology, incomplete nitrification and denitrification increase N_2O emissions (Healy et al. 2007). However, the effects of pulsing hydrologic regimes on GHG emissions are contradictory. For example, intermittent hydrologic regimes decrease both N_2O (Sha et al., 2011) and CH_4 emissions (Song et al., 2010). Highly contrasting results on gas emissions with fluctuating water levels have been reported and the controlling mechanisms are unclear (Elberling et al., 2011).

Therefore, the assessment of GHG emissions in various types of CWs (surface flow, subsurface flow, vertical and horizontal), vegetation cover (vegetated, non-vegetated) and species type, and management system employed (HLR, HRT, soil used, and water table), is necessary in light of the national and global GHG budgets. In addition, such measurements will help scientists, environmental managers, and policy makers adopt environmentally friendly construction and management of CWs. The enhanced reduction of N_2O to N_2 needs further elucidation.

Table 4. Carbon dioxide (CO_2; $mg\,C\,m^{-2}\,d^{-1}$), CH_4 ($mg\,C\,m^{-2}\,d^{-1}$), and CH_4 emission factor (CH_4–C / TOC input $\times$ 100) in various types of CWs; mean standard error ($\pm$ SE) was presented for CH_4 emission factor; NA – data not available.

CWs type	Treatment	CO_2 emissions ($mg\,C\,m^{-2}\,d^{-1}$)	CH_4 emissions ($mg\,C\,m^{-2}\,d^{-1}$)	CH_4 / TC (%)	References
SF	Municipal	NA	5.4	NA	Tai et al. (2002)
SF	Domestic	0.19	NA	26	Gui et al. (2007)
SF	Domestic	1.13	NA	16	Liu et al. (2009)
SF	Agril. drainage	NA	0.88	31	Wild et al. (2002)
SF	Dairy wash out	4250 ± 550	223 ± 35	9.45	Van der Zaag et al. (2010)
SF_Finland	Municipal	1200 ± 420	29 ± 6.4	19 ± 4.3	Søvik et al. (2006)
SF_Finland	Agril runoff	3200 ± 560	350 ± 180	11 ± 5.5	Søvik et al. (2006)
SF_Norway	Municipal	1400 ± 250	72 ± 28	4.8 ± 2.2	Søvik et al. (2006)
All SF		1675 ± 703	113 ± 58	16.8 ± 3.8	
HSSF	Domestic	NA	1.7–528	NA	Mander et al. (2005)
HSSF	Domestic	2.54–5.83	0.03–0.40	NA	Teiter and Mander (2005)
HSSF	Domestic	5.33	0.001	0.03	Garcia et al. (2007)
HSSF	Domestic	NA	0.03	4.3	Gui et al. (2007)
HSSF	Domestic	NA	0.29	4.0	Liu et al. (2009)
HSSF	Dairy wash out	3475 ± 375	118 ± 9.0	4.4	Van der Zaag et al. (2010)
HSSF	Domestic	0.6–1.7	1.4–4.1	0.12–0.23	Søvik et al. (2006)
HSSF	Domestic	600	0.48	0.02	Mander et al. (2011)
HSSF_Estonia	Municipal	3800 ± 210	340 ± 240	NA	Søvik et al. (2006)
HSSF_Norway	Municipal	790 ± 170	130 ± 43	9.5 ± 3.3	Søvik et al. (2006)
All HSSF		1010 ± 672	112 ± 74	3.23 ± 1.4	
VSSF	Domestic	5.83–12.13	0.60–5.70		Teiter and Mander (2005)
VSSF	Domestic	NA	16.4	NA	Mander et al. (2005)
VSSF	Domestic	NA	0.013	1.68	Gui et al. (2007)
VSSF	Domestic	NA	0.13	1.73	Liu et al. (2009)
VSSF	Municipal	2662 ± 175	33.5 ± 3.2	NA	Mander et al. (2008)
VSSF	Domestic	1080	3.36	0.05	Mander et al. (2011)
VSSF_Estonia	Municipal	8400 ± 2100	110 ± 35	NA	Søvik et al. (2006)
VSSF_Norway	Municipal	22000 ± 5000	140 ± 160	0.39 ± 0.27	Søvik et al. (2006)
All VSSF		6616 ± 3779	42.9 ± 23.7	1.27 ± 0.31	

SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow.

5   Surface emissions vs. subsurface export of C and N

Dissolved GHG produced in soils and subsoils can be emitted to the atmosphere by transpiration of vascular plants (from within the rooting zone), ebullition, and diffusion from soils. Elberling et al. (2011) reported that in wetlands, the transport of gases through subsoil occurs both via diffusive transport in the pores and through the vascular plants. Surface emissions of GHG from CWs have recently been recognized and have been commonly measured by chamber methods (Mander et al., 2008, 2011). As is the case with other dissolved pollutants (Dzakpasu et al., 2014), the GHG produced in CWs can also be transported to the groundwater with the percolating water and emitted to the atmosphere upon discharge to surface waters (Riya et al., 2010). It can also flow towards surface waters by advective transport and/or by dispersion of groundwater. Dissolved nutrients can be preferentially leached down into deeper soil layers and groundwater via different pathways (e.g. root channels). The Nr delivered

to groundwater can be transformed in situ to other reactive or benign forms. Hence, quantification of such Nr loadings to groundwater and their in situ consumption (e.g. N_2O to N_2 or CH_4 to CO_2) is necessary to understand their environmental consequences. In addition, DON, NO_3^-, NH_4^+, and DOC delivered to surface waters can undergo biochemical reactions and produce N_2O, CO_2, and CH_4 in streams and estuaries. Ström et al. (2007) measured a considerable quantity of CH_4 in porewater and found a correlation between the surface emissions and porewater CH_4 concentrations in vegetated wetlands. Measuring only the surface emissions of GHG can omit substantial quantities of GHG released from CWs. For example, Riya et al. (2010) measured emissions of CH_4 and N_2O, accounting for 2.9 and 87 % of the total emissions. Measuring porewater GHG and linking these to the surface emissions and subsurface export to groundwater below CWs will help to estimate a better GHG balance from both a national and global context. Elberling et al. (2011)

Table 5. Nitrous oxide (N$_2$O; mg N m^{-2} d^{-1}), CO$_2$, and CH$_4$ emissions (mg C m^{-2} d^{-1}) in various type of CWs under different plant types; NA – data not available.

CW type	Wastewater type	Plant type	N$_2$O (mg N m^{-2} d^{-1})	CH$_4$ (mg C m^{-2} d^{-1})	CO$_2$ (mg m^{-2} d^{-1})	Reference
HSF	Secondary treated	No plant	3.79 ± 2.64	163 ± 209		Johansson et al. (2003); Johansson et al. (2004)
	municipal	*Typha lotifolia*	2.64 ± 4.09	109 ± 185	NA	
		Phalaris arundinacea	3.79 ± 3.44	212 ± 151	NA	
		Glyceria maxima	0.76 ± 1.01	112 ± 178	NA	
		Lemna minor	1.45 ± 1.18	450 ± 182	NA	
		Spirogyra sp.	0.98 ± 1.25	107 ± 135	NA	
HSF	Sewage treatment water	No plant	-0.26 ± 2.53	-4.76 ± 61.8	4.32 ± 0.73	Ström et al. (2007)
		Typha atifolia	4.94 ± 2.00	225 ± 47.7	25.3 ± 4.08	
		Phragmites australis	7.80 ± 2.53	333 ± 76.6	25.1 ± 4.74	
		Juncus effusus	3.87 ± 1.86	489 ± 46.3	26.1 ± 3.00	
HSSF	Domestic	No plant	0.04 ± 0.02	87 ± 6.3	80 ± 6.3	Maltais-Landry et al. (2009)
		Phragmites	0.06 ± 0.03	50 ± 7.5	200 ± 35	
		Typha	0.03 ± 0.01	28 ± 3.0	235 ± 32	
		Phalaris	0.01 ± 0.01	45 ± 6.0	195 ± 31	
VSSF	Municipal	*Phragmites australis*	15 ± 3.9	110 ± 35	8400 ± 2100	Søvik et al. (2006)
VSSF	Municipal	*Phragmites australis*	264	384		Mander et al. (2005)

SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow.

linked subsurface gas concentrations in wetlands to the surface fluxes using a diffusion model. This demonstrates the need for future studies on subsurface GHG production, consumption and net GHG emissions in CWs within a climate change context.

It is important to characterize soils and subsoils' physical (e.g. texture, bulk density) and hydraulic (development of a soil water characteristic curve) properties and to assess their potential to percolate dissolved nutrients and gases in the solute phase to the underlying groundwater. To our knowledge, the indirect pathway of GHG emissions from CWs has never been reported, despite the fact that this would appear to have a high biogeochemical potential to produce and exchange GHG. The balance between N and C input and output flows between CWs and aquatic and atmospheric environments, together with the direct and indirect emissions of C and N species, could be an important input to global C and N budgets.

6 Hydrogeochemistry below CWs

Constructed wetlands can be designed with or without a clay liner or a compacted soil bed at the base, which can lead to large differences in permeability of the underlying layers. The variation in permeability of a CW soil bed will affect solute, nutrient, and GHG flows, and their interactions with the underlying groundwater (Dzakpasu et al., 2012, 2014). Groundwater hydrogeochemistry below CWs can therefore provide a unique insight into such interactions. An example of such interactions would be between nutrient-rich water discharging from CW cells mixing with laterally moving regional groundwater. It should be noted that groundwater can also discharge into CWs depending on the hydraulic gradi-

ents. This means that fully screened, multi-level piezometers or boreholes should be installed at such sites to elucidate groundwater flow direction, hydraulic gradients, and conductivities. Such monitoring networks allow water samples to be collected and the sources of nutrients in groundwater bodies below CWs to be identified. The local site hydrology (precipitation, groundwater table fluctuations, and evapotranspiration) has a large impact on the pollutant removal. Hydrogeochemical studies at an accurate spatial and temporal resolution should explain the effects of precipitation on nutrient removal by dilution as well in situ nutrient turnover. Effective CW management requires an understanding of the effects of wetland hydrology on the physical and biochemical attenuation of nutrients in order to assess their impacts on the surface emissions and subsurface export of nutrients and GHG. Data on the species of N in groundwater below the CWs are required to provide an in-depth understanding of wetland ecosystem services, particularly if CWs have the potential to leak pollutants down into the groundwater (Dzakpasu et al., 2014). Higher NH$_4^+$ concentrations in groundwater below the CW than the effluent are often reported (Harrington et al., 2007; Dzakpasu et al., 2012). Therefore, questions arise with respect to NH$_4^+$ concentrations in groundwater below the CWs if they have been transported from CWs. Linking geochemistry of groundwater below CWs to site hydrology, water table fluctuations, and soil/subsoil physico-chemical properties is required to elucidate the major environmental drivers of C and N removal, and/or pollution swapping. The quality of groundwater underlying CWs with regards to the Nr species is largely unknown.

7   Methodological developments

To improve the ecosystem services and to minimize the pollution swapping of CWs, quantification of N cycling is crucial. Measurement of GHG using the closed chamber method is widely used, but has large uncertainty in estimating the diurnal variability due to internal changes in temperature and physical access to the chambers over a 24 h time period. Gas ebullition and diffusion measurements are quite challenging in CWs covered by vegetation, because of the difficulties in estimation of gas transfer velocity. Application of the eddy-covariance method is not appropriate for most CWs, as it requires a large surface area ($>$ several ha) to avoid contribution of surrounding area and complication of GHG foot printing. A combination of chamber, ebullition, and diffusion methods in a single system could minimize the uncertainly in GHG estimation. The methane ebullition measurement was found to be similar to surface emissions by the chamber method, but N_2O and CO_2 ebullition measurements were lower than the surface emissions (Søvik et al., 2006).

The use of in situ microcosm studies and soil core incubation methods may give a better estimation of N_2O, CO_2, and CH_4 production and consumption than existing methods. With the recent advancement of isotope pairing and dilution techniques, single or simultaneously occurring C and N transformation processes can be quantified in laboratory or in situ conditions (Huygens et al., 2013; Müller et al., 2014). The isotope technique relies on the introduction of a known amount of ^{14}C and or ^{15}N into the CW and then quantification of C and N concentrations and isotopic compositions through different C and N pools after incubation for a specific period. Laboratory methods involve collection of intact soil/sediment cores, with subsequent incubation in the laboratory. In situ field techniques involve the release of a $^{14}C / ^{15}N$ solution in the CW soils. Incubation of intact soil cores with differentially labelled $^{15}NH_4^{14}NO_3$ and $^{14}NH_4^{15}NO_3$ can be used to quantify the rates of different N transformation processes (Rütting and Müller, 2008). The quantification of simultaneously occurring N transformation rates rely on the analysis with appropriate ^{15}N-tracing models. In recent years, ^{15}N-tracing techniques have evolved, and are now able to identify process-specific NO_2^- pools (Rütting and Müller 2008), pathway-specific N_2O production, and emission, as well as $N_2O : N_2$ ratios (Müller et al., 2014). Traditional techniques for investigation of gross N dynamics in sediments (Blackburn, 1979) may be combined with the latest ^{15}N-tracing techniques, where all N transformation rates are included (Huygens et al., 2013). Thus, current models should consider processes such as anammox and/or deamox, and then be tested in CWs under various operational conditions.   Denitrification in porewater samples can be measured by analysing samples for dissolved N_2 in a membrane inlet mass spectrometer (MIMS; Kana et al., 1994) and N_2O in a gas chromatograph (GC; Jahangir et al., 2012). The studies of natural abundance of ^{15}N and ^{18}O ($\delta^{15}N$ and $\delta^{18}O$) in NO_3^- is an insightful tool for the investigation of the sources, fate, and transformational processes of N in a system (e.g. in shallow groundwater; Baily et al., 2011). The in situ NO_3^- push–pull method has been used to determine denitrification in shallow groundwater (< 3 m) in riparian wetlands (Addy et al., 2002; Kellogg et al., 2005) and in deep groundwater in arable/grassland (Jahangir et al., 2013).

Isotope-based techniques can also be extended to other elements; e.g., a ^{33}P-tracing model has been developed recently to study phosphorus (P) cycle in soil (Müller and Bünemann, 2014). These techniques can be applied in the study of C, N, and P biogeochemistry in aquatic environments. In addition, measurements of DOC and gases (CO_2 and CH_4) will provide insights into the C consumption and transformation associated with the N transformations. Carbon and N dynamics are influenced by the interacting effects of soil conditions with microbial community structure and functioning. Microbial functioning involves transcription of genes, translation of messenger RNA, and activity of enzymes (Firestone et al., 2012). As such, activities of microbial communities under various environmental conditions and how these contribute to C and N dynamics is a very important area of future research (Müller and Clough, 2014). Molecular approaches can be important tools for identifying and quantifying the genes that code for enzyme-mediating C and N cycles (Peterson et al., 2012). These tools help assess the relationships among genes, environmental controllers, and the rates of C and N processes. The scientific tools and multi-disciplinary techniques are now available to better understand C and N transformation rates, processes, and factors controlling the unwanted emission of N and C products to the environment.

8   Conclusions and recommendations

The transformational processes on a mixture of contaminants within and below CWs can cause pollution swapping. A holistic assessment of C and N dynamics in CWs is needed to fully understand their removal, transport, and impact on water quality and emissions to atmosphere. Mixed contaminants entering CWs and those formed within and underneath CWs during transformational processes must be considered in future studies. The overall balance of these constituents will determine whether a CW is a pollution source or a sink. This will necessitate a higher degree of multi-level spatial and temporal monitoring and the use of multi-disciplinary in and ex situ techniques to fully characterize all pathways of C and N loss. At this time we cannot suggest any design optima in terms of nutrient removal and GHG mitigation because empirical information is not yet abundant. To do this, transformation kinetics of C and N and net GHG emissions through all possible pathways are required to provide a holistic assessment. However, a combination of various types of CW and plant types could provide higher removals and lower
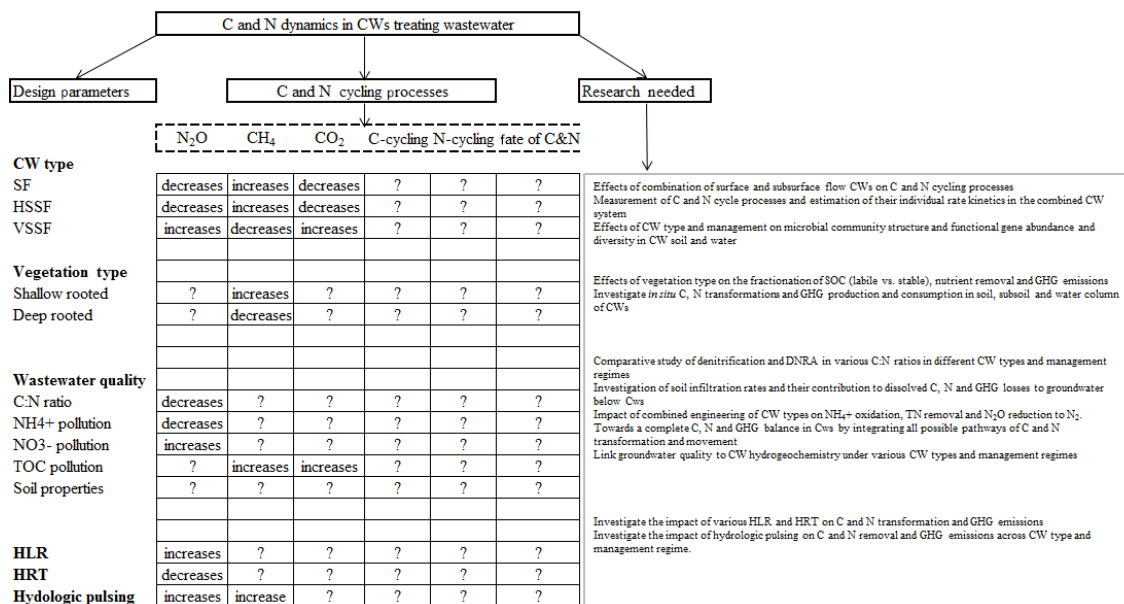
Conceptual model flowchart: C and N dynamics in CWs treating wastewater → Design parameters | C and N cycling processes | Research needed. C and N cycling processes subdivided into: N_2O, CH_4, CO_2, C-cycling, N-cycling, fate of C&N.

	N_2O	CH_4	CO_2	C-cycling	N-cycling	fate of C&N
CW type						
SF	decreases	increases	decreases	?	?	?
HSSF	decreases	increases	decreases	?	?	?
VSSF	increases	decreases	increases	?	?	?
Vegetation type						
Shallow rooted	?	increases	?	?	?	?
Deep rooted	?	decreases	?	?	?	?
Wastewater quality						
C:N ratio	decreases	?	?	?	?	?
NH_4+ pollution	decreases	?	?	?	?	?
NO_3- pollution	increases	?	?	?	?	?
TOC pollution	?	increases	increases	?	?	?
Soil properties	?	?	?	?	?	?
HLR	increases	?	?	?	?	?
HRT	decreases	?	?	?	?	?
Hydrologic pulsing	increases	increase	?	?	?	?

Research needed:

- Effects of combination of surface and subsurface flow CWs on C and N cycling processes. Measurement of C and N cycle processes and estimation of their individual rate kinetics in the combined CW system. Effects of CW type and management on microbial community structure and functional gene abundance and diversity in CW soil and water
- Effects of vegetation type on the fractionation of SOC (labile vs. stable), nutrient removal and GHG emissions. Investigate in situ C, N transformations and GHG production and consumption in soil, subsoil and water column of CWs
- Comparative study of denitrification and DNRA in various C:N ratios in different CW types and management regimes. Investigation of soil infiltration rates and their contribution to dissolved C, N and GHG losses to groundwater below Cws. Impact of combined engineering of CW types on NH_4+ oxidation, TN removal and N_2O reduction to N_2. Towards a complete C, N and GHG balance in Cws by integrating all possible pathways of C and N transformation and movement. Link groundwater quality to CW hydrogeochemistry under various CW types and management regimes
- Investigate the impact of various HLR and HRT on C and N transformation and GHG emissions. Investigate the impact of hydrologic pulsing on C and N removal and GHG emissions across CW type and management regime.

Figure 1. Conceptual model showing the current state of knowledge of C and N dynamics in constructed wetlands treating wastewater and the specific experimental work that needs to be undertaken in the future; SF – surface flow; HSSF – horizontal subsurface flow; VSSF – vertical subsurface flow; HLR – hydraulic loading rate; HTR – hydraulic retention time; ? – not known or very little known.

GHG emissions. A conceptual model highlighting the current state of knowledge in this area and the research gaps is presented in Fig. 1.

Subsurface export of nutrients and GHG to groundwater should be accounted for in CW management. Reducing the saturated hydraulic conductivity below the wetland bed will help reduce nutrients leaching to groundwater. The reactive versus the benign forms of the N transformation products should be evaluated. Data on when, where, and the rates at which denitrification, deamox, and anammox occur in CWs are needed, as well as identification of the key factors that control such processes. The provenance of NH_4^+ in groundwater below CW cells and its impact on down-gradient receptors needs further elucidation. Constructed wetlands have the potential to produce N_2O, DON, DOC, dissolved inorganic C (DIC), CO_2, and CH_4, which may be exported to fresh waters via groundwater and degassed upon discharge to surface waters. Moreover, the DOC and DIC transferred to the fresh water sediments (rivers and lakes) can produce GHG that, in turn, emit to the atmosphere. The amount of C and N exported from terrestrial ecosystems *via* the subsurface pathway to fresh waters has been the missing piece of our understanding of global C and N budgets. It is clear that data on the various C and N species, along with the GHG emissions, are crucial to make a robust input–output balance of C and N in CWs. Spatial and temporal variations of GHG emissions in CWs under different management systems are also critical to get much more rigorous estimates of emission factors. These data will reduce the existing uncertainties in global C and N budgets.

Managing wetting and drying spells (pulsing hydrology) in CWs can enhance NH_4^+ removal. Similarly, oxidation of organic C will increase CO_2 production and, in anaerobic conditions, may be reduced to CH_4. This requires more research into the C and N cycle processes over the wetting and drying spells, which is now possible with the advancement in $^{14}C/^{15}N$-tracing and modelling techniques. The selection of appropriate plant species is important to optimize nutrient removal, sequester C, and decrease GHG emissions, but more research is needed across species and geographical locations. Further research is also needed to investigate the impacts of hydraulic retention time on nutrient dynamics. Rates of nutrient accumulation or fixation in soils and their in situ transformation in CWs need to be quantified to evaluate their contribution to C sequestration and GHG emissions.

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