Nitrogen Dynamics and Associated Greenhouse Gases Emissions in Constructed Wetlands: A Review

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Abstract

Wastewater, derived from various sources, contains levels of pollutants that exceed water quality guidelines. Constructed wetlands (CWs) are a rising technology globally used to remove these pollutants (e.g. carbon- C and nitrogen- N) from wastewater. The N removal efficiency of CWs is very inconsistent and does not in itself reveal whether the removal processes are due to physical attenuation or whether the different species have been transformed to other reactive forms causing pollution swapping. There are many pathways by which the removed N can contribute to water and air pollution: accumulation and adsorption in soils, leaching to groundwater (nitrate-NO_3^- and ammonium-NH_4^+), emission to atmosphere via nitrous oxide- N_2O and ammonia and/or conversion to di-nitrogen (N_2) gas. The kinetics of these pathways/processes varies with CW management and therefore needs to be studied quantitatively, for their sustainable use. For example, the quality of groundwater underlying CWs with regards to the reactive N (Nr) species is largely unknown. Equally, there is a dearth of information on the extent of Nr accumulation in soils and discharge to surface waters and air. Moreover, CWs are rich in dissolved organic carbon (DOC) and produce substantial amounts of carbon dioxide (CO_2) and methane (CH_4). These dissolved C species discharge to ground and surface waters and are emitted to the atmosphere. The dynamics of dissolved N_2O, CO_2 and CH_4 in CWs is a key ‘‘loop hole’’ in global greenhouse gas budgets. In this review we provide an overview of the current knowledge and discussion about the dynamics of C and N in CWs and their likely impacts on aquatic and atmospheric environments. We recommend that the fate of various N species in CWs and their surface emissions and subsurface export need to be evaluated in a holistic way to better understand their potential for pollution swapping. Research on process based N removal and balancing of end products into reactive and benign forms is critical when assessing the environmental performance of CWs. Thus we strongly suggest further detailed examinations of *in situ* N transformations and the fate of such transformational products with respect to pollution swapping.

Keywords: Carbon, nitrogen, constructed wetlands, pollution swapping, nitrous oxide, methane

1. **Introduction**

Increasing anthropogenic loading of reactive N (Nr) along the nitrogen (N) cascade in the environment raises many critical concerns for human health, drinking water quality (Gray, 2008), coastal and marine water degradation causing eutrophication, as well as algal blooms and hypoxia (Conley et al., 2009; Rabalais et al., 2010). There are natural sinks for Nr along the N cascade

(Galloway et al, 2003; Tanner et al., 2005) but other sinks that are rehabilitated or artificial, may also be introduced and evaluated at key locations e.g. constructed wetlands (CWs) (Gold et al., 2013) or permeable reactive interceptors (Fenton et al., 2014). Many investigations target single contaminant remediation, whilst disregarding the reality of mixed contaminants entering and leaving such systems. They do not consider the dynamic 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 review focuses on CWs which are now an established technology used globally as a mitigation option for nutrient retention at the delivery end of the transfer continuum (Dunne, et al., 2005; Søvik et al., 2006; Tanner and Kadlec, 2013; Tanner and Sukias, 2011). They have been successfully used for treating domestic sewage, urban runoff and storm water, industrial and agricultural wastewater, and leachate (Scholz and Lee, 2005; Gill et al., 2014). In addition, CWs provide multi-faceted ecosystem services that include both socio-economic and ecological aspects (Turner et al., 2008; Milder et al., 2010; Lei et al., 2011). Although CWs have a proven potential for the removal of organic matter and N, studies have shown that removal efficiencies have been highly variable, due to inadequate observations of N transformations and their removal mechanisms (Seitzinger et al., 2002) and lack of improved adoption and placement (Gold et al., 2013). The removal efficiencies can vary with the types of CWs (i.e. surface flow and subsurface flow) and with types of plants grown. CWs are complex bioreactors which facilitate a number of physical, chemical and biological processes but often continue to be evaluated as a ‘black box’ in terms of process understanding (Langergraber, 2008). The removal efficiencies can vary with the types of CWs (i.e. surface flow and subsurface flow) and with types of plants grown.

Denitrification is the main mechanism in CWs to reduce nitrate (NO_3^--N) but its intermediate product, nitrous oxide (N_2O), is a potent greenhouse gas (GHG), causing 6% of anthropogenic global warming as well as being the leading cause of stratospheric ozone depletion (Beaulieu et al., 2011). It is known that CWs significantly contribute to atmospheric N_2O emissions either directly to the atmosphere from the surface of the wetland as its byproduct (IPCC, 2014; Søvik et al., 2006; Ström et al., 2007; Elberling et al., 20011; van der Zaag et al., 2010) or indirectly via dissolved N_2O in the effluent or groundwater upon discharge to surface waters. The global importance of this N_2O emission has been increasingly recognised (IPCC, 2014). Indirect N_2O emissions from CWs effluents that discharge directly to aquatic environments have a default emission factor (EF) of 0.005 but with a wide range (0.0005-0.25). The wide range of EF is highlighting the large uncertainty, which warrants further refinement. Indeed, the production and reduction processes of N_2O in the environment are not yet fully understood (Butterbatch-Bahl et al., 2014).

In CWs soil organic N is degraded releasing ammoniacal-N which further undergoes transformation to other forms. Ammonium can accumulate in CWs due to limited oxidation of NH_4^+ in the reduced environment. For example, questions arise with respect to NH_4^+ concentrations in groundwater underlying the CWs (often higher in groundwater than the effluent e.g. Harrington et al., 2007; Dzakpasu et al., 2012). Dissimilatory NO_3^- reduction to ammonium (DNRA) occurring in wetland soils can contribute to ammonium (NH_4^+) pollution (Burgin et al., 2013). Mass balance analysis of the different components of the N cycle and kinetics of the N 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 therefore can be used to start to answer such questions. Huygens, et al. (2013) described two complementary stable isotope methods that can be used to determine N cycle processes in CWs: i) the isotope pairing and revised isotope pairing techniques which calculate total N_2 production by denitrification and anaerobic ammonium oxidation (anammox) and ii) isotope dilution and tracing techniques which assess gross N transformation in wetland soils. 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 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). However, this analysis cannot in itself explain whether the denitrification process has occurred *in situ* or if the gases have been transported from other soil zones to groundwater. A ^{15}N based method could be developed to measure *in situ* N cycle processes and to understand the movement (both upward and downward) of the N cycle products in wetland sediments. This would involve incubation of intact laboratory soil columns from the site in question enriched with $^{15}NH_4{}^{14}NO_3$ and $^{14}NH_4{}^{15}NO_3$ kept at average site temperature (Huygens et al., 2013). The *in situ* denitrification, DNRA and C dynamics of groundwater below the CWs can be elucidated in the field with shallow piezometers or boreholes using push-pull investigations (Addy et al., 2002; Jahangir et al., 2013; Burgin et al., 2013). These methods in combination will be able to provide a comparative analysis of the rates of N transformation processes occurring in CWs. For example, *in situ* quantification of the rates of NH_4^+ oxidation and denitrification in CWs will give insights into their role in delivering NO_3^- and or NH_4^+ to groundwater and N_2O to the atmosphere.

CWs can either be sources or sinks of C (Whiting and Chanton, 2001) but only a few studies have considered carbon dioxide (CO_2) efflux from CWs (Mander et al., 2008). CWs are characterised by anaerobic conditions with low organic matter turnover (Augustin et al., 1998) but they also release C back into the atmosphere (Clair et al., 2002). Wetlands can contribute to the dissolved organic carbon (DOC) load transfer to ground and surface waters and can exchange substantial amounts of CO_2 and methane (CH_4) with the atmosphere (Clair et al., 2002; Elberling et al., 2011). CO_2 is lost by plant respiration and aerobic organic matter decomposition, whilst CH_4 is lost by methanogenic activity during anaerobic decomposition of DOC. Methane is emitted from wetlands due to anoxic or suboxic conditions that occur in the soils (Ström et al., 2007; Søvik et al., 2006; Pangala et al., 2010). In addition to the positive aspects highlighted previously, CWs can have negative climatic impacts (Ström et al., 2007), enhance pollution swapping (Reay, 2004) and act as an active source of GHGs (IPCC, 2014; Clair et al., 2002; Mander et al., 2008; Mitsch and Gosselink, 2000). For example, van der Zaag et al. (2010) measured CH_4-C emissions as 0.2- 27% of the total C removed and N_2O emissions as 0.1- 1.16% of the total N removed in CWs. Estimation of GHG emissions from different CWs management systems, especially in response to a changing climate, is poorly known. Consequently, the improved understanding of integrated C and N dynamics is required to improve the CW management to optimise their environmental benefits.

2. Removal Efficiency, Hydraulic Loading and Retention Time

In CWs used for wastewater treatment, the efficiency of OM removal is often satisfactory, but N removal is generally limited. The limited removal can be caused by the typically reduced environment that enhances NH_4^+ accumulation and limits NH_4^+ oxidation, in addition to the lower denitrification rates due to lack of NO_3^- and N recycling by vegetation. On average, it appears that 50% of the influent N can be removed but the removal rates are very inconsistent. A summary of influent composition and their removal efficiencies is given in Table 1. In European systems, for example, typical removal percentages of ammoniacal-N in long-term operation are c. 35% but can be enhanced if some pre-treatment procedures are followed (Verhoeven, and Meuleman, 1999; Luederitz et al., 2001). O'Luanaigh et al. (2010) in a subsurface CW showed 41% N removal across a tertiary treatment system (receiving secondary treated effluent) with little evidence of any seasonal change. In a single house domestic wastewater treatment using CWs, Gill et al. (2012) has shown mean removal efficiencies of 33-36% of total N (TN) but did not investigate the N cycling processes. To enhance such a low removal, the nature of N transformations and the fate of the removed N within the CWs should be investigated. However, to our knowledge, no study is available that provides a holistic evaluation of N attenuation and transformations to other reactive N as losses to the atmosphere (N_2O, NH_3, N_2) and receiving surface waters or groundwater (NO_3^-, NH_4^+).

The removal efficiency of pollutants in CWs depends on hydraulic loading rates (HLR) and hydraulic retention time (HRT) (Toet et al., 2005). 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 contaminants. Therefore, to have the least amount of Nr delivery to the receiving waters or to the atmosphere, CWs need to be optimally designed with respect to HLR and HRT. Investigation into the effects of fluctuating hydraulic loadings (hydraulic pulsing) on N removal efficiency and its transformation products will provide information about the potential of pollution swapping for NO_3^-, NO_2^-, NH_3, NH_4^+ and N_2O. If the dominant product is N_2, the system will be more benign to the environment than many of the other potential end products. If N is transformed into NH_4^+ it should be fixed in the soils and subsoils but there is also the potential for transport to ground and surface waters connected to CWs if the cation exchange sites become saturated. However, contrasting results on the impacts of hydraulic loadings on nutrients removal efficiency are available in literature. 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 (COD) removals by 25 and 11%, respectively. Several authors have used a wide range of HLR and HRT to measure nutrient removal efficiency but experimental evidence is still scarce (Toet et al., 2005). For example, Zhou and Hosomi (2008) constructed a surface flow wetland with a HLR of 0.20 $m^3\ m^{-2}\ d^{-1}$ and an average water depth of 0.056 m. The authors estimated the N removal efficiency in terms of a comparison between N inputs and outputs but did not quantify how this rate corresponded to the different forms of the removed N. Similarly, Song et al. (2011) reported an average HRT of 2.4 days during wetland operation periods for the removal of NO_3^- and NH_4^+ without documented design optima or pollution swapping. The way in which a CW performance is assessed can lead to different conclusions regards removal of Nr. For future studies, evaluation of systems in a holistic manner, which include pollution swapping at different HLRs and HRTs is important, particularly within the context of the changing hydrological cycle in a changing climate. Also local legislative targets should be considered and weighting factors (are GHGs more important than water quality targets) developed for different species within the system to come up with overall balances for the CW. Thus far, estimates of nutrient removal efficiencies are based on the subtraction of the concentrations of nutrients in the effluent from the influent, but the transformation kinetics of the removed nutrients are unknown.

A holistic assessment of ecologically engineered systems has been thoroughly outlined in Healy et al. (2011) and developed further in Fenton et al. (2014). Such assessments can be applied in evaluating nutrient dynamics in CWs. In a subsurface CW, O'Luanaigh et al. (2010) used ^{15}N isotopes to show that N spiralling occurred between NH_4^+ to organic N and back to NH_4^+ within the CW. So, studying the dynamics of N in CW systems is crucial because the forms of removed N are particularly of concern with respect to their potential for pollution swapping; global warming and water pollution. In addition to the estimation of nutrient removal rates, investigations into the effect of HLR and HRT on the different forms of nutrients in the final effluents and their fate in the natural environment are needed to address the pollution swapping potential of CWs. In addition, 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) is required to understand their environmental consequences.

3. Accumulation of C and N in CWs Soils

The soil in CWs is a major sink for N. Data on the N accumulation (dissolved organic N- DON, TN, NH_4^+ or NO_3^--N) within the soil profile of various CWs are scarce as most studies focus on N balances between influent and effluent N loads. The accumulation of N could be a substantial part of the total N added to CWs; 30-40% (Shamir et al., 2001), 39% (Harrington et al., 2007), 9% (Mander et al., 2008) and 2.5% (Obarska-Pempkowiak and Gajewska, 2003). The wider range of N accumulation could be due to variations in CW types and their management. The accumulated species of N are rather reactive unless they are transformed to N_2 by biogeochemical processes. For example, Shamir et al. (2001) measured about 80% of the total accumulated N as organic and the remainder as NH_4^+. The organic N could be mineralised to NH_4^+ and NO_3^-, reactive forms of N, depending on the physico-chemical properties of soil. The Nr could be assimilated by plants and microbes living in CWs, which are recycled in a soil-plant-soil continuum. Typically, N accumulation has been found to decrease with soil depth: for example, Shamir et al. (2001) reported mean NH_4^+ concentrations in 0-0.15, 0.15-0.30 and 0.30-0.60 cm depths of 156, 151, and 28 mg N kg^{-1}, respectively. However, dissolved nutrients can be preferentially leached down into deeper soil layers via different pathways e.g. root channels. In terms of the input-output balance, these are considered as removed N, but they remain in such a biogeochemically active system. In addition to N, organic C accumulation occurs in CW soils. In a gravel-bed CW, Nguyen (2000) measured 17.5, 16.2 and 3.6% of total C (TC) accumulation at surface, 0-10 and 10-40 cm depth. As such, CWs represent organic C and Nr rich systems where the products of the continuously occurring biogeochemical processes can be transported to fresh waters and to the atmosphere. The NO_3^- could be denitrified to N_2O or N_2 and NH_4^+ could be mineralised to NO_3^- or fixed in soil matrix. Estimation of the reaction kinetics of these processes is required to accurately measure the fate of the added nutrients in CWs. With methodological advancement, measurement of these processes is now possible. For example, application of ^{15}N tracer technique ($^{15}NO_3^-$) can give insights into the NH_4^+ oxidation and fixation capacity of sediments below CWs. Estimating the rates of nutrient accumulation in soil and subsoils and their *in situ* transformation kinetics in various types of CWs is important. Elucidating the fate of these added nutrients will help to reduce their potential for pollution swapping which has been ignored so far in managing CWs. 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

Processes involved in N removal and N transformations in wetlands include sedimentation of particulates (Koskiaho, 2003); nitrification, denitrification and DNRA (Poach et al., 2003; Burgin et al., 2013), microbial assimilation and plant uptake-release (Findlay et al., 2003), anammox and DEAMOX (DEnitrifying AMmonium OXidation). Müller et al. (2014) developed a ^{15}N tracing model, which is able to identify four different pathways of NO_2^- reduction to N_2O: i) reduction of NO_2^- associated with nitrification, ii) reduction of NO_2^- associated with denitrification, iii) reduction of NO_2^- associated with organic N oxidation, and iv) co-denitrification, a hybrid reaction where one N atom in NO_2^- originates from organic N and the other from NO_2^- reduction via denitrification. Most of these pathways transfer Nr (mainly NH_4^+ and N_2O) to the environment, however, some pathways can convert Nr to N_2 (e.g. denitrification, anammox and DEAMOX). During denitrification, NO_3^- is used as a terminal electron acceptor to produce N_2 or N_2O (Starr and Gillham, 1993). Anammox can remove NO_2^- and NH_4^+ as N_2 in CWs when the existing environment is hypoxic. The DEAMOX can remove NO_3^- and NH_4^+ as N_2 where NO_3^- is converted to NO_2^- by autotrophic denitrification with sulphide (Kalyuznyi et al., 2006). Denitrification has been estimated to be a significant N removal process but actual quantification data are scarce. Limited 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.

In addition, the two other processes that can remove Nr from the CWs (anammox and DEAMOX) are not well understood and so it is crucial to identify which of the processes are occurring in a specific type of CW and at what rate they occur. Once a process that provides N_2 as the end product is determined in a specific system then the CW management could be directed towards enhancement of that process. Hence, quantifying the rates of these processes under various CW types is required for improved N management towards lowering Nr in the environment. There is large uncertainty about the processes involved and their magnitude. Many studies focus on the mass balance approach and use the difference as the rate of N attenuation. N_2O emissions from CWs have been reported to be high (EF 0.005; IPCC, 2014) but there is large uncertainty around these values due to the limited scope of the research that has been carried out to quantify such processes. The enhanced reduction of N_2O to N_2 needs further elucidation.

Similarly, C transformations involve respiration, fermentation, methanogenesis, CH_4 oxidation and reduction of S, Fe and NO_3^-. Anerobic 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). As CWs are designed to remove pollutants in an anaerobic/suboxic environment, they change the C and N dynamics and contribute significantly to CH_4 and N_2O emissions (Johansson et al., 2002, 2003; Mander et al., 2005, 2008; Stadmark and Leonardson, 2005; Liikanen et al., 2006). The hydrological, chemical and microbial processes of CWs are likely to be different from the processes occurring in more natural wetlands, because they receive nutrient rich waters from various sources. Increased nutrients and organics inputs will increase the productivity of wetland ecosystems and increase the production of GHGs. 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. Ström et al. (2007) recommended that CW management processes must consider the negative climatic aspects of increased emissions of GHGs in addition to their primary functions. Therefore, estimation of the contribution of CWs to global warming is required. Modelling or up-scaling of GHG emissions at national and regional scales to a global scale is important to improve global GHG budgets. The use of CWs to improve water quality is likely to increase to meet policy drivers such as the European Union Water Framework Directive and so the uncertainty of GHG budgets needs to be reduced to prevent pollution swapping.

In this regard, measurement of spatial and temporal variations (seasonal and diurnal) of GHG emissions is required. Moreover, plant mediated GHG emissions could be an important component of total emissions but research in this area is very limited. The GHG from CWs can vary with vegetated and non-vegetated systems. Vegetation and its composition affect the nutrient dynamics and the production, consumption and transport of greenhouse gases and hence their exchange between wetlands and atmosphere (Ström et al., 2003, 2005; Søvic et al., 2006; Johansson et al., 2003). Emergent plants can transport atmospheric O_2 to rooting zone and contribute to C and N dynamics in wetland soils e.g. N_2O and CO_2 production and CH_4 consumption (Brix, 1997). Vascular plants can exchange GHGs between the rooting zone and atmosphere (Yavitt and Knapp, 1998). Emissions of N_2O and CH_4 in CWs can vary across CW typologies e.g. surface flow or subsurface flow (Van der Zaag et al., 2010). Therefore, the assessment of GHG emissions in various types of CW (surface flow, subsurface flow; vertical and horizontal), under different management systems (vegetated, nonvegetated, plant species composition) and usage (municipal waste water, agricultural runoff, landfill leachate) is necessary in light of the national and global GHG budgets and mitigation of GHG emissions. In addition, such measurements will help scientists, environmental managers and policy makers to adopt environmental friendly construction and management of CWs. Assessment of the reactive versus the benign forms of C and N transformation products in various types of CWs will give insights into their environmental friendly design and management.

5. Surface Emissions vs. Subsurface Export of C and N

Dissolved GHGs in porewater in wetland soils and subsoils can be emitted to the atmosphere by transpiration of vascular plants (from within the rooting zone) and via groundwater, upon discharge to the surface waters. Dissolved GHGs in groundwater can flow towards surface waters by advective transport and or by dispersion of groundwater. The GHGs produced in soils/subsoils in CWs can also be emitted to the atmosphere by ebullition and diffusion. Elberling et al. (2011) reported that in wetlands, the transport of subsurface soil gases occurs both via diffusive transport in the pores and through the vascular plants. In addition to measuring the surface emissions, Ström et al. (2007) also measured a considerable quantity of CH_4 in porewater and found a significant correlation ($p = 0.021$) between the surface emissions and porewater CH_4 concentrations in vegetated wetlands. Measuring porewater GHGs 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) linked subsurface gas concentrations in wetlands to the surface fluxes using a diffusion model which has demonstrated the need for future studies on subsurface GHG production, consumption and net GHG emissions in CWs ecosystem in a climate change context. It is important to characterise soils and subsoils physical and hydraulic properties and to assess their potential to percolate dissolved nutrients and gases to the underlying groundwater. To our knowledge, this 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 GHGs. 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

CWs 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 gradients. This necessitates that multiple multi-level piezometers or boreholes are installed at such sites to elucidate groundwater flow direction, hydraulic gradients and hydraulic permeability. Such monitoring networks allow water samples to be taken and then sources of nutrients in the groundwater body below CWs can be identified. For example, natural abundance of N ($\delta^{15}N$) and oxygen ($\delta^{18}O$) can be used to identify the sources of NO_3- and infer transformational processes responsible for concentrations in groundwater (Baily et al., 2011). The local site hydrology (precipitation, groundwater table fluctuations and evapotranspiration) has greater impacts on the pollutant removal by physical attenuation and by biochemical transformations. Hydrogeochemical studies on an accurate spatial and temporal resolution should explain the effects of precipitation on nutrient removal by dilution as well in situ nutrient turnover. Despite conditions within CWs being saturated throughout the year, changes in the water table elevation affect the oxygen (O_2) concentrations in CWs which is key parameter for the biogeochemistry of soils and subsoils. For example, lowering the water level increases the O_2 concentrations and accelerates soil OM decomposition, thereby increasing CH_4 oxidation and CO_2 emissions. Highly contrasting results on gas emissions with fluctuating water levels have been reported and the controlling mechanisms are unclear (Elberling et al., 2011). 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 GHGs. 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 CW systems have the potential to leak pollutants down into the groundwater (Dzakpasu et al., 2014). A study on a clay lined CW system reported that the nutrient content in the underlying groundwater to be NH_4^+-N 4.0 mg L^{-1}, NO_3^--N 0.2 mg $L^{-1}$and molybdenum reactive phosphorus (MRP) <0.01mg L^{-1}. The elevated NH_4^+-N indicates transport of nutrient from the CW to groundwater through leakage or *in situ* N transformations such as DNRA. However, the impact of CWs on the local underlying groundwater quality has been seldom assessed. Linking geochemistry of groundwater below CWs to site hydrology, water table fluctuations and soil/subsoils physico-chemical properties is required to elucidate the major environmental drivers of C and N removal and or pollution swapping.

7. Methodological Developments

Quantification of the occurrence and magnitude of N transformations and hydrochemical properties is crucial to improve the assessment of CW ecosystem services and to minimize their potential for pollution swapping. With the recent advancement of isotope pairing and dilution techniques, single or simultaneously occurring N transformation processes in CWs 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 ^{15}N into the CW system and then quantification of N concentrations and isotopic compositions through different N pools after incubation for a specific period. Laboratory methods involve collection of intact soil/sediment cores, with subsequent incubation in the laboratory. The *in situ* field techniques involve release of ^{15}N solution *in situ* in the CWs soils. The *in situ* stable isotope techniques may be an appropriate tool for the determination of simultaneously occurring N transformation processes in wetland soils (Huygens et al, 2013).

Details of the application of isotope pairing/ revised isotope pairing techniques and isotope dilution/ tracing techniques have been presented elsewhere (Huygens et al., 2013). In brief, 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 occuring N transformation rates rely on the analysis with appropriate ^{15}N tracing models. Development in the recent years in ^{15}N tracing techniques is now available, which are able to identify process specific NO_2^- pools (Rütting and Müller, 2008), pathways 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) should be combined with the latest ^{15}N tracing techniques where all N transformation rates are included that are important in wetlands and under anoxic condition (Huygens et al., 2013). Thus, current models should consider processes such as anammox and/or DEAMOX and then be tested in CW environments under various conditions. 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). This method can be used in CW sites using piezometers or boreholes screened at different intervals to investigate *in situ* C and N dynamics. In this method the parameters of importance to be analysed are $^{15}N_2O$, $^{15}N_2$, $^{15}NO_3^-$, and $^{15}NH_4^+$. 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. Occurrence of C and N cycling processes are controlled by the environment which 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, 2013). Molecular approaches can be important tools to identifying and quantifying the genes that code for enzymes 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.

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 the 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 decide on whether a CW is a pollution source or a sink. This will necessitate a higher degree of multi-level spatial and temporal monitoring and use of multi-disciplinary techniques both *in* and *ex situ* to fully characterise all pathways of C and N loss.

Leakages of nutrients and gases from CWs to groundwater can only be elucidated through the techniques itemised herein and a better understanding of the transformation processes along a vertical geochemical gradient from the CW into the underlying aquifer. The reactive versus the benign forms of the N transformation products should be evaluated in various CWs. An understanding of how N removal occurs and how losses of N and associated gases impact water and air quality is required. Denitrification, DEAMOX and anammox are the processes which remove N to its benign form (N_2) and all other processes produce only reactive forms of N. Data on when, where and at what rates denitrification, DEAMOX and anammox occur in CWs are needed as well as what are the key factors that control such processes. The provenance of NH_4^+ in groundwater below CW cells needs clarification and its impact on down gradient receptors.

This review shows that CWs have the potential to produce N_2O, DON, DOC, DIC, CO_2 and CH_4 and that the GHGs produced in CWs can be emitted to the atmosphere. They can also 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 GHGs that in turn get emitted to the atmosphere. Therefore, it is important to know the concentrations of dissolved C and N species and the rates of production and consumption of GHGs in groundwater below CWs. The amount of C and N exported from terrestrial ecosystem 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 GHGs in various CWs systems are thus crucial to make a robust input-output balance of C and N in such a rising and engineered ecosystem. Spatial and temporal variation in N_2O emissions in CWs under different management systems is critical to get much more rigorous estimates of N_2O emission factors. These data will bring down the existing uncertainties in global C and N budgets.

Managing wetting and drying spells (pulsing hydrology) in CWs can enhance mineralisation of organic N and oxidation of NH_4^+ to NO_2^-/NO_3^- and then denitrification/anammox could transform these species to benign N_2. This requires more research into the N cycle processes over the wetting drying spells which is now possible with the advancement in ^{15}N tracing and modelling techniques. With the recent advancement of isotope pairing and dilution techniques single or simultaneously occurring N transformation processes can be quantified. The isotope based techniques can also be extended to other elements e.g., a ^{33}P tracing model has been developed recently to study phosphorous cycle in soil (Müller and Bünemann, 2014). Further reducing the saturated hydraulic conductivity below the wetland bed will help reduce nutrients leaching to groundwater below the CWs cells. The selection of plant species is important to increase nutrients removal, sequester more C and decrease greenhouse gas emissions which is an area that still requires more research across types of CWs and countries. More research is also still needed to be done on the impacts of hydraulic retention time on nutrients dynamics and removal. Subsurface export of nutrients and GHGs to groundwater through leachates, preferential flow paths created by dead roots and holes of burrowing invertebrates and subsequent transport to surface water bodies should be accounted in CW management. Rates of nutrient accumulation or fixation in soils and their *in situ* transformation in CWs need to be quantified to know their contribution to C sequestration fate in the environment. Estimation of GHG production in CWs, their consumption and emissions to the atmosphere in a changing climate is urgent. The ecosystems services from CW will make them an attractive option for water pollution mitigation while providing an important aquatic habitat, but their integrated impact on environmental quality needs to be addressed in a holistic manner to avoid unwanted side effects.

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Author’s Contributions

The first author, M.M.R. Jahangir has reviewed articles in the relevant area, analysed results and identified knowledge gaps and prepared the draft paper. All co-authors were directly involved in preparation of the paper and edited the paper for its improvement.

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Table 1 Summary of influent composition and their removal efficiencies being reported by several authors

CWs type	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	BOD_5 (mg L^{-1})	MRP (mg L^{-1})	TN Removal (%)	References
SF	15.5	4.5	1.5 (DOC)	0.2	61	Song et al., 2011
SF	5	40			97- 98	Dzakpasu et al., 2011
SF	4.4	39			39-48	Vymazal, 2007
SF		36	170	9.6	39	Vymazal, 2010
SF	2.6	64	816	10	>90	DEHLG, 2010*
SF	6.4	34	854	4.3	>90	DEHLG, 2010**
SF	<2	196	277		35	Shamir et al., 2001
SF	<1.0	80	1200	25	>90	Harrington et al., 2007
SF	1.54	0.95	32 (COD)	1.43	45	Toet et al., 2005
SSF	3.9	74.9	514 (COD)	11.1	29	O’Luanaigh et al., 2010
SSF		87 (TN)	167		46-48	Mander et al., 2008

SF-surface flow; SSF-subsurafce flow; NO_3^-- nitrate; NH_4^+- ammonium; BOD- biological oxygen demand; DOC- dissolved organic carbon; MRP- molybdenum reactive phosphorus; TN-total N; *soiled water; **wastewater