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Discussions

Carbon and nitrogen dynamics and greenhouse gases emissions in constructed wetlands: a review

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Carbon and nitrogen dynamics and greenhouse gases emissions

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Abstract

The nitrogen (N) removal efficiency of constructed wetlands (CWs) is very inconsistent and does not alone explain if the removed species are reduced by physical attenuation or if they are transformed to other reactive forms (pollution swapping). There are many pathways for the removed N to remain in the system: accumulation in the sediments, leaching to groundwater (nitrate-NO_3^- and ammonium-NH_4^+), emission to atmosphere via nitrous oxide- N_2O and ammonia and/or conversion to N_2 gas and adsorption to sediments. The kinetics of these pathways/processes varies with CWs management and therefore needs to be studied quantitatively for the sustainable use of CWs. 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 CO_2 and CH_4. These dissolved carbon (C) species drain out to ground and surface waters and emit to the atmosphere. The dynamics of dissolved N_2O, CO_2 and CH_4 in CWs is a key "missing piece" in our understanding of 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 suggest that the fate of various N species in CWs and their surface emissions and subsurface drainage fluxes need to be evaluated in a holistic way to better understand their potential for pollution swapping. Research on the process based N removal and balancing the end products into reactive and benign forms are critical to assess environmental impacts of CWs. Thus we strongly suggest that in situ N transformation and fate of the transformation products with regards to pollution swapping requires further detailed examination.

1 Introduction

Increasing anthropogenic loading of reactive N (N_r) along the N cascade in the environment raises many critical concerns for human health and 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 and artificial sinks for Nr along the N cascade (Galloway et al., 2003; Tanner et al., 2005). Constructed wetlands (CWs) are one such artificial sink along with others such as denitrifying bioreactors (Fenton et al., 2014). CWs are an emerging technology used globally (Dunne et al., 2005; Søvik et al., 2006; Tanner and Kadlec, 2013; Tanner and Sukias, 2011) as mitigation options for nutrient retention at the delivery end of the transfer continuum. 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 to wastewater treatment, applications of CWs have now been extended to greater ecosystem services that include some socio-economic and ecological aspects (Turner et al., 2008; Milder et al., 2010; Lei et al., 2011). Although CWs have a proven potential for organic substances and N removal, studies have shown that removal efficiencies have been highly variable, due to inadequate observations of N transformations and removal mechanisms (Seitzinger et al., 2002) and lack of improved adoption and placement (Gold et al., 2013). CWs are complex bioreactors, which facilitate a number of physical, chemical and biological processes but continue by and large a relative "black box" in terms of process understanding (Langergraber, 2008). Denitrification is the main mechanism in CWs to reduce nitrate (NO_3^{-}-N) but its intermediate product, N_2O, is a potent greenhouse gas, causing 6 % of anthropogenic global warming and the leading cause of stratospheric ozone depletion (Beaulieu et al., 2011). 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 effluents

or groundwater upon discharges to the surface waters. The importance of this N_2O has been increasing recently (IPCC, 2014). The IPCC (2006) has recognised the indirect N_2O emissions from CWs effluents that are discharged to aquatic environments, estimating a default emission factor (EF) of 0.005 ranging 0.0005–0.25, indicating large uncertainties, warranting further EF refinement. Production and reduction processes of N_2O in the environment are not yet fully understood (Butterbatch-Bahl et al., 2014). Dissimilatory NO_3^- reduction to ammonium (DNRA) occurring in wetland sediments, can contribute to NH_4^+ pollution to ground and surface waters although this is not a sink for Nr (Burgin et al., 2014). Even though in CWs the effluents have lower NH_4^+ concentrations than the influents, groundwater below CWs has been often reported to be contaminated with higher NH_4^+ than the effluents (Harrington et al., 2007; Dzakpasu et al., 2012). Therefore, the question has been raised as to which is the dominant process responsible for the higher concentration of NH_4^+ in the groundwater, leakage from the CWs cells or production in situ in groundwater by biological process.

Mass balance analysis on the different components and kinetics of the N transformation processes occurring within the treatment cells using the isotope-tracking ^{15}N technique, will provide mechanistic information for N transformations (Lee et al., 2009; O'Luanaigh et al., 2010). Huygens et al. (2013) step-by-step described two complementary stable isotope methods that can be used to study N cycle processes in CWs: (i) the isotope pairing and revised isotope pairing techniques, calculate total N_2 production by denitrification and anammox and (ii) isotope dilution and tracing techniques, assess gross N transformation in wetland soils. Denitrification in porewater samples can be measured by analysing samples for dissolved N_2 in membrane inlet mass spectrometer (MIMS; Kana et al., 1994) and N_2O in gas chromatograph (GC; Jahangir et al., 2012). However, this analysis cannot itself explain if the denitrification process has been in situ or if the gases have been transported from other soil zones to groundwater. A ^{15}N based method can 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 involves incubation of intact soil columns enriched

with $^{15}NH_4$ $^{14}NO_3$ and $^{14}NH_4$ $^{15}NO_3$ in the laboratory kept at the average temperature of the site (Huygens et al., 2013). The in situ denitrification, DNRA and C dynamics can be measured in groundwater below the CWs using the push-pull method (Addy et al., 2002; Jahangir et al., 2013; Burgin et al., 2014). These methods will help a com-
5 parative analysis of the N transformation processes in CWs to better understand their ecosystem level importance and managements.

CWs can either be sources or sinks of C (Whiting and Chanton, 2001) but only a few studies have considered CO_2 efflux from CWs (Mander et al., 2008). CWs are characterised by anaerobic conditions with low organic matter turnover (Augustin et al., 1998)
10 but they also release C back into the atmosphere (Clair et al., 2002). Wetlands can largely contribute to DOC load to ground and surface waters and can exchange substantial amounts of CO_2 (Clair et al., 2002) and CH_4 with the atmosphere (Clair et al., 2002; Elberling et al., 2011). CO_2 is lost by plant respiration and aerobic organic matter/peat decomposition, whilst CH_4 is lost by methanogenic activity during anaerobic
15 peat decomposition and DOC by steeping of soil organic matter in water. Thus, CWs resembling natural wetlands are characterised by anoxic conditions and so, emit CH_4 (Ström et al., 2007; Søvik et al., 2006; Pangala et al., 2010). CWs therefore have negative climatic impacts (Ström et al., 2007), enhance pollution swapping (Reay, 2004) and act as an active source of greenhouse gases (GHGs) as its byproducts (IPCC,
20 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 0.1–1.1 % of the total N removed in CWs. Estimation of GHG emissions from different CW management systems, especially in response to a changing climate, is poorly known. Consequently, construction and management of CWs require a much
25 more thorough understanding of C and N dynamics and their potential losses to both aquatic and atmospheric environments.

2 Physical and hydraulic properties of soils in CWs beds

The risk of ground/surface waters contamination from CWs depends on soil properties, water table and infiltration rates (Ham, 2002). Subsurface flow in CWs can transport the nutrient-rich water to ground/surface waters (Hathaway et al., 2010) and can endanger
5 groundwater quality if soil permeability is not managed properly (Dzakpasu et al., 2014; USEPA, 2000). Analysis of porewater nutrients and dissolved gases have indicated that physical properties of wetland soils and subsoils influence nutrient dynamics and gas diffusion (Elberling et al., 2011; Ström et al., 2007). Soil properties (e.g. soil texture, bulk density, compaction and permeability) in CWs beds vary with the type of wetlands
10 (e.g. surface flow or subsurface flow), materials used to fill the excavated bed (e.g. gravel, sand, local earth worked soils, clay liner or plastic liner) and site geology. Studies documenting leaching of dissolved nutrients from CWs to groundwater are scarce (Dzakpasu et al., 2012). The hydraulic properties of CW soils (e.g. residual and saturation water content) are normally heterogeneous but how they control nutrients and
15 gas migration to the underlying groundwater has not been reported. For example an assessment of the self-sealing and infiltrability of soil materials typically used as a cell liners for CWs is required (Dzakpasu et al., 2012, 2014). In addition, soil type and depth in CW beds can influence nutrient movement and delivery to ground/surface waters-e.g. deep soil layer with high cation exchange capacity (CEC) can increase NH_4^+ fix-
20 ation and N accumulation. Examples of the soil type and depth in CWs which have been used for studies into nutrient removal are shown in Table 1. For example, Harrington et al. (2007) developed integrated CWs that are underlain by 1.5 m of subsoil, with the upper 0.5 m local earth worked materials (Table 1). Conversely, Zhoua and Hosomi (2008) constructed a surface-flow wetland which was filled with typical paddy field
25 soil to an approximate depth of 0.60 m (not compacted) in Japan. Song et al. (2011) constructed two mesocosm-scale wetlands with a 0.6 m water depth where sediment was composed of two layers; the lower 0.3 m consisted of gravel and the upper 0.3 m was sand mixed with organic soil giving a mixed organic matter (OM) content of 11 %.

Despite such a good number of studies on nutrient removal in CWs, more targeted research on the effects of physical properties of soils and subsoils in CWs bed on the movement of nutrients to ground and surface waters are required. These investigations will give insights into a better understanding of the fate of nutrients in CWs which will improve management of CWs in connection to pollution swapping by drainage fluxes of nutrients and GHGs.

3 Removal efficiency, hydraulic loading and retention time

In CWs for wastewater treatment, the efficiency of OM removal is often satisfactory, but N removal is generally limited. A summary of influent composition and their removal efficiencies are given in Table 2. In European systems, for example, typical removal percentages of ammoniacal-N in long-term operation are only 35 % (Luederitz et al., 2001). Vymazal (2007) reported the removal rate of total N between 40 and 50 % with removed load ranging between 250 and 630 g N m^{-2} yr^{-1}. In subsurface flow CWs in Estonia, Mander et al. (2008) reported average N removal 46–48 % of the total N input. In Ireland, dairy soiled water (DSW) with BOD$_5$ 816, NH$_4^+$ 64, NO$_3^-$-N 2.6 and PO$_4^{3-}$ (MRP) 10 mg L^{-1} was treated to a mean effluent quality of BOD$_5$ 854, NH$_4^+$ 34, NO$_3^-$-N 6.4 and PO$_4^{3-}$ 4.3 mg L^{-1} (DEHLG, 2010). O'Luanaigh et al. (2010) in a subsurface CW showed 29 % N removal across a secondary treatment system (receiving septic tank effluent) and 41 % N removal across a tertiary treatment system (receiving secondary treated effluent) with little evidence of any seasonal change. However, to our knowledge, no study is available that identifies the various N species removed from the system with the extent of physical attenuation and transformations to other forms (N$_2$O, NH$_3$, NH$_4^+$ etc.) or transfer via leaching to groundwater below the CW systems are still largely unknown. Other research on the treatment of single house domestic wastewater in Ireland using CWs (Gill et al., 2012) has shown mean removal efficiencies of 235 mg L^{-1} down to 158 mg L^{-1} (Total-N) for one system and 136 mg L^{-1}

down to 51 mg L^{-1} for another system but again did not investigate the nature of N transformations.

The removal efficiency of pollutants in CWs has been found to depend on hydraulic loading rates (HLR) and hydraulic retention time (HRT) (Toet et al., 2005). However, contrasting results on the impacts of hydraulic loadings on nutrients removal efficiency are available, e.g. low HLR results in incomplete denitrification (Luo et al., 2005), increases NH$_4^+$ and COD removals by 25 and 11 %, respectively (Zhang et al., 2006). Several authors have used a wide range of hydraulic loading rates and retention time to measure nutrient removal efficiency but experimental evidence is still scarce (Toet et al., 2005). For example, Zhoua and Hosomi (2008) constructed a surface flow wetland with a HLR of 0.20 m^3 m^{-2} d^{-1} and average water depth of 0.056 m. Song et al. (2011) reported an average HRT of 2.4 days during wetland operation periods, being used for removing NO$_3^-$ and NH$_4^+$. They amended inflow water with 15.5 mg L^{-1} of NO$_3^-$ where N removal efficiency was 61 %. Dzakpasu et al. (2011) measured mean inflow rate of 104 m^3 d^{-1}, yielding average HLR of 7 mm d^{-1} in CWs treating domestic wastewater. Dunne et al. (2005) reported inflow rate of 5.9–8.6 m^3 d^{-1} with the corresponding outflow rates of 13.9 to 37.5 m^3 d^{-1} with the additional inflow from the rainfall.

So far, the nutrient removal efficiency estimation is based on the deduction of output from the input but the transformation kinetics of the removed nutrients is unknown. In a subsurface CW, O'Luanaigh et al. (2010) with ^{15}N isotope showed that N spiralling occurred between NH$_4^+$ to organic N and back to NH$_4^+$ within the CW. This implies that the NH$_4^+$ leaving the wetland were not the same NH$_4^+$ that had entered but been taken up into organic N and then broken down again into NH$_4^+$. 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. 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$_2$O. 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 to NH_4^+, it will be fixed in soils and after saturation of the cation exchange site there is potential for transport to ground and surface waters connected to CWs. High NH_4^+ concentrations in groundwater below the CW systems is evident in literature (Dizakpasu et al., 2014).

4 Accumulation of C and N in CWs soils

The soil in CWs has been proved to be a major sink for N. However, although data on the influent and effluent N concentrations are often explicitly available; data on the N accumulation (DON, TN, NH_4^+ or NO_3^--N) within the soil profile of various CWs are scarce. Shamir et al. (2001) measured 30–40 % N accumulation in soil of the total accumulated N in a clay-lined surface flow CWs system of which about 80 % of the total N accumulated was organic and the remainder was NH_4^+. The N accumulation decreased with soil depth, with 42–48 % in the upper 0.15 m and 20–24 % in deeper layers. In the top 0.15 m layer, mean values of total N and NH_4^+ concentrations were 685 and 156 mg N kg^{-1}. In the 15–30 cm layer, they were 505 and 151 mg N kg^{-1} and in the deeper layer (30–60 cm), they were 278 and 28 mg N kg^{-1}. Organic materials can penetrate into the deep soil layers via channels created by roots, holes of invertebrates and cracks with dissolved organic N transported with the percolating water. Harrington et al. (2007) estimated 155 kg N ha^{-1} to have entered the CWs from farmland over one year which contributed 60 kg N ha^{-1} to ground, 2.5 kg N ha^{-1} to surface water and remainder (90 kg N ha^{-1}) was assumed to have been taken up by vegetation or emitted to the atmosphere. In a gravel-bed CW, Nguyen (2000) measured 1.55, 1.50 and 0.4 % of TN and 17.5, 16.2 and 3.6 % of TC accumulation at surface, 0–10 and 10–40 cm depth, respectively for 5 years after the wetland establishment for treating farm wastewater. Over the 5 years, wastewater and plant residues contributed to 8.2 and 8.5 kg m^{-2} organic solids, respectively with sediments C : N ratios of 10.7, 10.3 and 9.1 at surface, 0–10 and 10–40 cm, respectively. From horizontal flow subsurface CWs, Mander et al. (2008) and Obarska-Pempkowiak and Gajewska (2003) estimated 9 and

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2.5 % N accumulation, respectively by soil matrix of the total N input. Application of ^{15}N tracer technique ($^{15}NO_3^-$) can give insight into the NH_4^+ fixation capacity of sediments below CWs.

5 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 dissimilatory nitrate reduction to ammonium (DNRA) (Poach et al., 2003; Burgin et al., 2014), microbial assimilation and plant uptake and release (Findlay et al., 2003) and anaerobic ammonium oxidation (anammox). All of these pathways emit Nr to the environment. Müller et al. (2014) developed a ^{15}N tracing model which was 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. Anammox can remove NO_2^- and NH_4^+ as benign N (N_2) in CWs as it is a hypoxic environment. The DEAMOX (DEnitrifying AMmonium OXidation) 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). In CWs denitrification has been estimated to be a significant N removal process but actual quantification data are scarce. Mander et al. (2008) estimated 19 % of the total N input removed by denitrification in horizontal subsurface flow CWs. In a horizontal subsurface flow CW, Obarska-Pempkowiak and Gajewska (2003) estimated 14 % N removal of the total N input by plant biomass and soil matrix and assumed that the rest (86 %) was lost by denitrification. In denitrification NO_3^- is used as a terminal electron acceptor to produce N_2 or N_2O (Starr and Gillham, 1993). Hence study on these pathways in CWs will give insights into an improved N management towards lowering Nr in the environment.

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Similarly, C transformations involve respiration, fermentation, methanogenesis, CH_4 oxidation and abiotic reduction of S, Fe and NO_3^- with DOC consumption. Anerobic methane oxidation coupled with denitrification, a recently proposed pathway of C cycle (á Norði and Thamdrup, 2014; Haroon et al., 2013; Islas-Lima et al., 2004), can reduce

5   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 bindings. The CWs, converted from agricultural land, create an anaerobic/suboxic environment and hence change the C and N dynamics and contribute significantly to CH_4 and N_2O emissions (Johansson et al., 2002, 2003; Mander

10   et al., 2005, 2008; Stadmark and Leonardson, 2005; Liikanen et al., 2006). As CWs are used for wastewater treatment, their hydrological, chemical and microbial processes will likely be changed from the processes occurring in more natural wetlands. Increased nutrients and organics inputs will increase the productivity of wetland ecosystems and increase the production of GHGs. Conceptual models of the C and N cycle processes

15   in CWs, including the source of C and N, production of DOC, CO_2, CH_4, NH_4^+, NO_3^-, N_2O and N_2 are presented in Figs. 1 and 2. Søvic et al. (2006) measured N_2O, CH_4 and CO_2 emissions in various CWs in European countries and suggested that the potential atmospheric impacts of CWs should be examined as their development is increasing globally. The authors estimated the mean global warming potential of N_2O and

20   CH_4 from CWs ranged from 5.7–26 and 0.83–5.1 g CO_2 equivalents $m^{-2} d^{-1}$ in summer and winter, respectively with generally higher emissions in vegetated CWs than the nonvegetated ones. Conversely, Johansson et al. (2003) measured higher N_2O emissions in ponds without plants than in ponds with plants in a CW system treating municipal wastewater in Sweden. Van der Zaag et al. (2010) reported significantly

25   higher emissions of N_2O and CH_4 in surface flow CWs than in subsurface flow CWs. From a Swedish wetland, constructed to treat sewage wastewater, Ström et al. (2007) measured CO_2, CH_4 and N_2O emissions equal to 90 tonnes of CO_2 equivalents annually. The authors recommended that CWs management processes must consider the negative climatic aspects of increased emissions of GHGs in addition to their primary

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functions. Vegetation and their 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). Emergent plant 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 GHG between rooting zone and atmosphere (Yavitt and Knapp, 1998). Therefore, the assessment of GHG emissions in various types of CWs (surface flow, subsurface flow; vertical and horizontal), under different management systems (vegetated, nonvegetated, plant species composition) and usage (municipal wastewater, 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.

6   Surface emissions vs. subsurface drainage fluxes of C and N

Porewater dissolved gases in wetland soils and subsoils can be exchanged to the atmosphere via vascular plants (transported from within the rooting zone) and groundwater (upon discharge to the surface waters). In wetlands, transport of subsurface soil gases occurs both via diffusive transport in the pores and through the vascular plants (Elberling et al., 2011). In addition to measuring the surface emissions, Ström et al. (2007) also measured 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 drainage fluxes will help estimate a better GHG balance in national and global contexts. 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 measurement of subsurface GHG production and net GHG emissions in this ecosystem in a climate change context. It is important

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to characterise soils and subsoils 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 are presented schematically in Figs. 3 and 4.
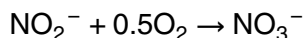
7 Hydrogeochemistry below CWs

CWs can be designed with or without a clay liner at the base or a compacted soil bed which leads to vast differences in permeabilities between layers and thus promotes very different interactions with the underlying groundwater (Dzakpasu et al., 2012, 2014). Groundwater hydrogeochemistry below CWs can therefore provide a unique insight into this interaction between nutrient rich water vertically discharged from CWs into the more regional groundwater flow beneath from upgradient, which is still unknown. Hydrogeochemical studies on the spatial and temporal scales should explain the effects of precipitation on nutrient removal by dilution as well in situ nutrient turnover. The local site hydrology has greater impacts on the pollutant removal by physical attenuation and by biochemical transformations. Despite conditions within CWs being saturated throughout the year, changes in water level 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 organic matter decomposition, increases CH_4 oxidation and CO_2 emissions. Highly contrasting results on gas emissions with fluctuating water levels are reported and the controlling mechanisms are unclear (Elberling et al., 2011). Effective CW management thus 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 drainage fluxes 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). Harrington et al. (2007) measured the concentrations of water quality parameters of groundwater below CWs of NH_4^+-N 4.0, NO_3^--N 0.2 and MRP < 0.01 mg L^{-1} where in the effluents they were 0.5, 1.5 and 0.5 mg L^{-1}, respectively. However, data on groundwater chemistry below the CWs appear to have been very limited so far, which made its contributions to the ecology of agricultural and urban ecosystems, where they are being used, very inconclusive.

8 Major N transformation processes in CWs

8.1 Ammonification

Ammonification is the biological conversion of organic N to ammonia. The N in soils is present mostly within organic compounds that make it insoluble and unavailable for use by higher plants. Much of the N is present as amine groups (R–NH_2), in proteins or as part of humic compounds. Soil microorganisms, in particular exoenzmyes deaminate these to simpler amino-acids (monomers), then to NH_4^+. Decomposition processes in the wetlands can convert a significant part of the organic N to NH_4^+ (Mayo et al., 2004). The ammonification reaction is represented by:

$$R\text{-}NH_2 + H_2O + H^+ \rightarrow R\text{-}OH + NH_4^+$$
$$R\text{-}NH_2 + H_2 \rightarrow NH_4^+ + Energy$$

In wetlands soils organic N is degraded in both aerobic and anaerobic zones, releasing ammoniacal-N. However, this multi-step process depends on the environmental and soil mineralogy factors which determine the rate of N mineralization in the soil and thus the amount mineralized over time. In wetlands, soil temperature, aeration, moisture content, pH, C/N ratio, available nutrients and soil structure have a strong effect on

N mineralization reactions due to their effect on the activities of soil microbes involved in mineralization (Lee et al., 2009).

8.2 Nitrification

Nitrification is a chemolithoautotrophic oxidation of ammonia (NH_3) to NO_2^- to NO_3^-. Nitrification can also produce N_2O. This process is carried out by a small group of microbes e.g., *Nitrosomonas, Nitrospira, Nitrosococcus* convert NH_3 to NO_2^-, and *Nitrobacter* convert NO_2^- to NO_3^-. These bacterial genera use ammonia or NO_2^- as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. Nitrification followed by denitrification is believed to be the major pathway for NH_4^+ removal in wetlands (Vymazal, 2007; Gersberg et al., 1985). This process occurs strictly under aerobic conditions (Lee et al., 2009), but many other factors control the whole process: temperature, pH, alkalinity, inorganic C source, moisture, microbial abundance, NH_4^+ and DO. However, anaerobic nitrification in CWs has been reported to occur in aerated microzones adjacent to root and rhizomes (O'Luanaigh et al., 2010). Hence, study on the extent of nitrification in CWs beds is crucial to know the fate of organic N delivered from influents and plant litters. The nitrification process is enhanced by temperature (Cookson et al., 2002) and is curtailed below pH 6 (Paul and Clark, 1989), and inhibited at greater than pH 8 (Whitehead, 1995). In this process $3.16\,\mathrm{mg\,O_2\,(mg\,NH_4^+\text{-}N)^{-1}}$ is oxidized and $1.11\,\mathrm{mg\,O_2\,(mg\,NO_2^-)^{-1}}$ is oxidized (Lee at el., 2009), therefore increased levels of DO increase nitrification occurring in wetlands. Alkalinity is needed for nitrification as $7.07\,\mathrm{mg\,CaCO_3\,(mg\,NH_4^+\text{-}N)^{-1}}$ is oxidized (Ahn, 2006). In the nitrification process, for each NH_4^+ molecule $2H^+$ are produced and thus the pH drops. Therefore, liming can be helpful for neutralizing the acidity developed in nitrification process (Ahn, 2006). The whole reaction can be shown as follows:

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+$$
$$NO_2^- + 0.5O_2 \rightarrow NO_3^-$$

Or

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ + \text{Energy};$$
$$2NO_2^- + O_2 \rightarrow 2NO_3^- + \text{Energy}$$
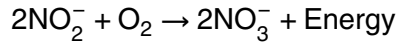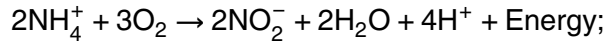
8.3 Denitrification

Denitrification is a microbially mediated multi-step process which converts NO_3^- to di-nitrogen (N_2) in low oxygen environments. The intermediate products of denitrification are NO_2^-, nitric oxide (NO) and N_2O. Nitrous oxide is an obligate intermediate product of denitrification which is a potent GHG, but the stable end product of denitrification is environmentally benign. In CWs denitrification has been estimated to be a significant N removal process but actual quantification data are scarce. In denitrification NO_3^- is used as a terminal electron acceptor to produce N_2 or N_2O (Starr and Gillham, 1993). This process is carried out by heterotrophs (*Pseudomonas, Micrococcus, Achromobactor* and *Bacillus*) and autotrophs. Heterotrophs are microbes that need organic substrates to obtain their carbon source for growth and evolution, and get energy from organic matter. In contrast, autotrophs utilize inorganic substances as an energy source and CO_2 as a carbon source (Rijn et al., 2006). In wastewater treatment systems heterotrophic denitrification process is mainly targeted and used even though autotrophic denitrification be important (Kim et al., 2004). In wetlands this process can reduce 60–95 % of total N removal. Denitrification occurs under anaerobic/anoxic conditions (because DO can suppress the enzyme systems required for this process) where electron donors (organic C) are available. In CWs, microsites with steep gradients of DO can be established, which allow nitrification and denitrification to occur in sequence, in very close proximity to each other (Lee et al., 2009). The denitrification rate is influenced by nitrate concentration, microbial flora, type and quality of organic carbon source, hydroperiods, plant residues, DO, redox potential, soil moisture, temperature, pH, presence of denitrifiers, soil type, water level, and the presence of overlying water (Sirivedhin and Gray, 2006; Golterman, 2004).

Denitrification process can be represented as below:

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow N_2O \rightarrow N_2$$
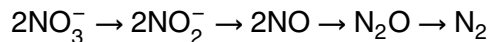
Biological denitrification can best be described by the following reaction:

$$4NO_3^- + 5CH_2O + 4H^+ \rightarrow 2N_2 + 5CO_2 + 7H_2O$$
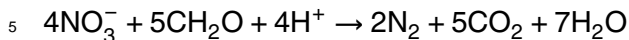
The NO_3^- reduction reaction can be written as a half-equation that illustrates the role of electron (e^-) transfer in the process and is non-specific to the electron donor (Tesoriero et al., 2000):

$$2NO_3^- + 12H^+ + 10e^- \rightarrow N_2 + 6H_2O$$

$$NO_3^- + 1.25(CH_2O) \rightarrow 0.5N_2 + 0.75H_2O + 1.25CO_2 + OH$$

$$NO_3^- + (CH_2O) \rightarrow 0.5N_2O + 0.5H_2O + CO_2 + OH^-$$
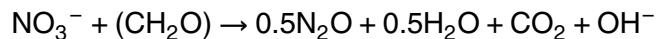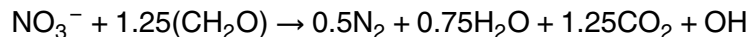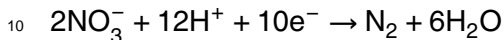
8.4 Dissimilatory NO_3^- reduction to ammonium (DNRA)

The DNRA is an anaerobic process where NO_3^- is transformed to NH_4^+ which can remain in that form only until it has contact with an aerobic environment (Tesoriero et al., 2000), after which it is oxidized to NO_3^- (Thayalakumaran et al., 2008). The DNRA reaction can be shown as below (Robertson et al., 1996):

$$2H^+ + NO_3^- + 2CH_2O \rightarrow NH_4^+ + 2CO_2 + H_2O$$
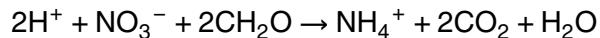
The conditions that favour the occurrence of either denitrification or DNRA are still in debate (Rütting et al., 2011). DNRA is thought to be favoured by a high C/N ratio and occurs at low level of Eh (Thayalakumaran et al., 2008). The differences between denitrification and DNRA may be due to the availability of organic matter, because DNRA is the favoured at high C/N ratio and denitrification is favoured when carbon supplies are limiting (Korom, 1992; Kelso et al., 1997). The fermentative bacteria which carry

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out DNRA are obligate anaerobes (Hill, 1996) and so cannot occupy all the niches that denitrifiers can (Buss et al., 2005). Takaya (2002) stated that more reducing state favours DNRA over denitrification. Pett-Ridge et al. (2006) showed that DNRA is less sensitive to O_2 level than denitrification. Fazzolari et al. (1998) showed that effect of O_2 levels on DNRA is dependent on the C/NO_3^- ratio and C rather than O_2 levels is the main factor regulating NO_3^- partitioning between DNRA and denitrification. Significant DNRA 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: 5 for denitrification and 8 for DNRA. Therefore, more organic matter can be oxidized for each molecule of NO_3^- by DNRA than 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. So, DNRA bacteria may be favoured by NO_3^- limited conditions (Laanbroek, 1990). Recent studies have suggested that DNRA can be important process compared to denitrification in wetland sediments (Burgin and Hamilton, 2008). Van Oostrom and Russell (1994) found a 5 % contribution of DNRA to the NO_3^- removal in constructed wetland. 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., 2014), 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 which impacts on the CWs ecosystem services and so should therefore be evaluated.

8.5 Immobilisation

This process is opposing mineralisation and describes the conversion of Nr (NH_4^+ and NO_3^- formed by mineralization and nitrification) into organic forms and ultimately biomass. The extent of immobilization is determined by the amount and quality of C and N in soils and sediments, but in groundwater, dissolved C and N is generally

low. Mineralization, nitrification and immobilization occur simultaneously resulting in the transformations of N from organic to inorganic forms and vice versa. Therefore a net mineralization term is generally used to determine the amount of NO_3^- and NH_4^+ readily available to be fed from soil to groundwater, and then groundwater to receptors.

$$NO_3^- + 2e^- \rightarrow NO_2^- + 6e^- \rightarrow NH_4^+$$

$$NH_4^+ + R\text{-}OH \rightarrow R\text{-}NH_2 + H_2O + H^+$$
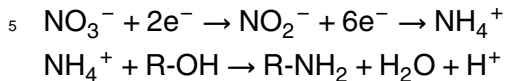
8.6 Anammox

Anaerobic ammonium oxidation (Anammox) is a chemolithoautotrophic process by which NH_4^+ is combined with NO_2^- under anaerobic conditions, producing N_2 (Kuenen, 2008). The NO_2^- is derived from the incomplete reduction of NO_3^-, possibly by denitrifying bacteria, and anammox therefore contributes to permanent NO_3^- removal. Ammonium is autographically oxidised to N_2 while NO_2^- is used as an electron acceptor under anaerobic conditions. It is a relatively recently discovered N transformation pathway (Jetten et al., 1999) and so has opened up a new avenue in the study of N cycling. Since the process has no demand a C source, it can result in cost saving from a wastewater treatment perspective as well as removing the risk of insufficient conversion of organic substances (Ward, 2003). The process was discovered in a wastewater treatment system in the 1990s, and since then, studies have shown it to occur in anoxic wastewater, oxygen depleted zones of the ocean, temperate shelf sediments, sea ice, and cold Arctic shelf sediments (Rysgaard and Glud, 2004; Rysgaard et al., 2004). It has also recently been reported in one freshwater ecosystem (Schubert et al., 2006). Scientists still know relatively little about the bacteria that carry out anammox, and no pure cultures exist (Strous et al., 2006). Thus, anammox may be most important in ecosystems with limited labile carbon or an excess of N relative to carbon inputs. This may include substantial parts of the pelagic ocean and continental shelves (Dalsgaard et al., 2005). In Lake Tanganyika, Schubert et al. (2006) found that 7–13 % of the N_2 production was derived by anammox. Recent studies in a saturated septic tank

plume in groundwater have indicated that the anammox process is a dominant reaction (Robertson et al., 2012). The anammox reaction can be shown as (van de Graaf et al., 1995):

$$5NH_4^+ + 3NO_3^- \rightarrow 4N_2 + 9H_2O + 2H^+$$
$$NO_2^- + NH_4^+ \rightarrow N_2 + 2H_2O$$
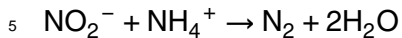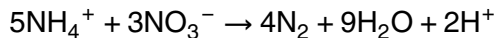
Studies on the anammox process in CWs have not been reported so far. However CWs, being a suitable ecosystems for anammox, can have a large contribution of N removal by anammox provided that they have sufficient NO_2^- and NH_4^+ input or in situ production of these two forms of reactive N. The CWs with fluctuating water level or surface soils with high O_2 diffusion can contribute to NO_2^- production and thus to anammox when this NO_2^- reach anaerbic zone.

8.7 DEAMOX

The DEAMOX combines anammox with autotrophic denitrifying conditions using sulphide as an electron donor for producing NO_2^- from NO_3^- and thus remove NO_3^- and NH_4^+ as N_2 (Kalyuznyi et al., 2006). The whole reaction can be shown with the below partial reactions (Kalyuznyi et al., 2006):

$$DEAMOX : 4NO_3^- + HS^- \rightarrow 4NO_2^- + SO_4^{2-}$$
$$Anammox : NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$$
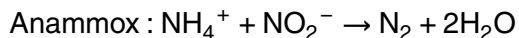
The CWs are organic N and SO_4^{2-} rich environment where both combined reactions of DEAMOX and anammox can remove Nr as a benign N (N_2) and reduce pollution swapping potential of the system. In situ studies of these pathways are therefore crucial in CWs ecosystem for improved ecosystem services of the system.

8.8 Ammonium adsorption

In this physical process, NH_4^+ is adsorbed from soil solution to clays, humic substances, detritus, inorganic sediments or soil through cation exchange reaction or can be fixed in clay lattice. Generally at equilibrium, a fixed amount of NH_4^+ will be adsorbed to the exchange sites but it will be loosely bound and can be released in response to changing water chemistry. If the NH_4^+ concentration is reduced in the solution, some NH_4^+ will be desorbed to regain the equilibrium with a new concentration. If the NH_4^+ concentration is increased in the soil solution, the NH_4^+ adsorption will increase. The rate and extent of these reactions are influenced by several factors such as: the nature and amount of clays, alternate wetting and drying and the nature and amount of organic matter (Savant and DeDatta, 1982). In wetlands, periodic wetting and drying can cause oxidation of the adsorbed NH_4^+ to NO_3^-. Where large amount of clay is used in wetland bed, adsorption can be important in wastewater NO_3^- removal. These processes can be measured in CWs by the simultaneous measurements of NH_4^+ in solution/porewater and in the soil matrix.

9 Methodological developments

To improve the ecosystem services and to minimize the pollution swapping of CWs study on N cycling processes is crucial. 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; Müeller et al., 2014). Isotope technique relies on the introduction of a known amount of ^{15}N in the CWs system and quantifying N concentrations and isotopic compositions through different N pools after incubation for a specific period. Laboratory methods involve collection of intact soil/sediment core and incubation in the laboratory but in situ techniques involved application of ^{15}N solution in situ in the CWs soils. However, in situ stable isotope techniques may be more appropriate 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 described elsewhere (Huygens et al., 2013). Here an example of a laboratory and an in situ ^{15}N based experimentation methods were outlined.

9.1 Laboratory incubation of soil column to measure N cycle processes and gaseous emissions

To study the N dynamics in the constructed wetland mirror labelling approaches of laboratory incubations; one set with ^{15}NH$_4$^{14}NO$_3$ labelling and another with ^{14}NH$_4$^{15}NO$_3$ labelling are recommended. Intact soil column: 50 cm height with aerobic, mixed (anaerobic–aerobic interface) and anaerobic soil layers can be incubated with suitable ^{15}N labels over for long time periods (e.g. 30 days). Soil column needs to be equipped for wastewater addition, ^{15}N solution injection and leachate collection (Fig. 5a). For each ^{15}N experiment one set of soil columns will be incubated for long time (e.g. 30 days) and equipped with a headspace cap, gas sampling tube in three specified depths inserted from the site ports, and a tube at the bottom to collect the leachate (Fig. 5b). While labelling with ^{15}N in one depth the two other depths should be injected with ^{14}N to keep the total NH$_4^+$ + NO$_3^-$ similar along the depths. The ^{15}N solution should be injected carefully to make a homogeneous distribution of the solution using disposable syringes. During the incubation, dirty water of known N concentrations should be added daily through the top of the column at the same rate as applied in the wetland site. Typically, destructive soil sampling should then be done on day 0, 1, 5, 10 and 30/60 (depending on how quick the movement occurs) of incubation with appropriate replications. Parameters to be analysed are total C and N, NH$_4^+$, NO$_3^-$, ^{15}NH$_4^+$, ^{15}NO$_3^-$, ^{15}N$_2$O, ^{15}N$_2$, CO$_2$, and CH$_4$. This will provide additional information also on the amount of fixed NH$_4^+$ in soil.

The quantification of simultaneously occuring N transformation rates rely on the analysis with appropriat ^{15}N tracing models. Development in the recent years in ^{15}N tracing

techniques are 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 investiation of gross N dynamics in sediments (Blackburn, 1979) should be combined with latest ^{15}N tracing techniques where all N transformation rates are included that are important wetlands and under anoxic condition (Huygens et al., 2013). Thus, current models might have to consider processes such as ANAMMOX and or^{-1} DEAMOX and should then be tested for CWs environments under various conditions.

9.2 In situ push-pull method to quantify N cycle processes in CWs

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 extended to apply to CWs soils and sediments to investigate in situ C and N dynamics. The push-pull method comprises two steps: (1) the push-pull pre-test, injection of previously collected groundwater amended with a conservative tracer into a single piezometer, followed by the extraction of the groundwater mixture from the same piezometer after an incubation period to gain insights into balancing high recovery of the plume with sufficient time in situ for microbial denitrification to occur at detectable levels; and (2) the NO_3^- push-pull test, injection of previously collected groundwater amended with conservative tracers and ^{15}N-enriched NO_3^-, followed by the extraction of the injected solution after an incubation period (determined in the push-pull pre-test) and analyses of $^{15}N_2O$ and $^{15}N_2$ produced via microbial denitrification. In both push-pulls, groundwater solutions are injected into a well and then pulled from the same well after incubating for a pre-defined period. Before preparing the solution, pumped groundwater is stored in a cold room at $4\,°C$ for a maximum of one week. To adjust the dissolved oxygen (DO) back to ambient conditions, the groundwater solution are bubbled with a noble trace gas, sulphur hexafluoride (SF_6) while the DO concentration

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are monitored using a DO probe. The carboy with amended solution is capped and its headspace is filled with the SF_6 gas. The total amount of aquifer materials covered by the solution is calculated using the Eq. (1) below:

$$Mt = \left[\frac{(Vt - Vg)}{\text{Porosity of aquifer}} \right] \times Bd \tag{1}$$

where Mt is the total mass of aquifer material (kg), Vt is the total volume of solution (m^3), Vg is the volume of gravel pack (m^3), and Bd is the bulk density ($kg\,m^{-3}$). Parameters to be analysed are $^{15}N_2O$, $^{15}N_2$, $^{15}NO_3^-$, and $^{15}NH_4^+$. In this method, simultaneous measurements of dissolved organic carbon (DOC) and gases (CO_2 and CH_4) will provide insights into the C consumption and transformation associated with the N transformations.

10 Conclusions and recommendations

A holistic assessment of C and N dynamics in CWs is needed to fully understand their removal and transport to underlying groundwater, nearby surface waters and emissions to atmosphere. The potential of CWs for pollution swapping must be considered rather than only estimating the nutrient removal percentage, which only yields very uncertain insights about the fate of nutrients. Generally agricultural or domestic systems have mixed contaminant scenarios that enter to the CWs but some time mitigation measures are designed to remove single contaminants (e.g., NO_3^- from artificial bioreactors). However transformation processes from either scenario can develop a mixed contaminant environment. Therefore the way of removal efficiencies are documented must change to consider pollution swapping. Leakages of nutrients and gases from CWs to groundwater can only be elucidated through techniques itemised herein and a better understanding of the transformation processes along a vertical gradient in CWs into the underlying aquifer. The reactive vs. the benign forms of the N transformation products should be evaluated in various CWs. Understanding of how N removal occurs and

how losses of N and associated gases impact water and air quality is required. Denitrification, DEAMOX and anaerobic ammonium oxidations (anammox) are the processes which remove N to its benign form (N_2) and all other processes produce only reactive forms of N. Contribution to N removal by these processes in various CWs is necessary to know for their environment friendly design and management. The sources and fate of NH_4^+ in groundwater below CWs are unknown. The CWs have the potential to produce N_2O, DON, DOC, CO_2 and CH_4. It is clear that data on C and N dynamics in various CWs systems are thus crucial to make a solid input-output balance of C and N in such a rising and engineered ecosystem. Estimation of GHGs emissions under different management systems is critical which will result in much more rigorous estimates of emission factors.

Managing wetting and drying spells (pulsing hydrology) in CWs can help NH_4^+ oxidation and then denitrification to the benign N_2 because the wetland soils have highly reduced environment with sufficient DOC for denitrification or anammox to occur. However, 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 (Müeller and Bünemann, 2014). Wastewater contaminated with high NH_4^+ concentration can first be transported through an aerobic cell to convert NH_4^+ to NO_3^- which can then be transported to wet cells for denitrification or anammox. Further reducing the saturated hydraulic conductivity below the wetland bed will help reduce nutrients leaching to groundwater below the CWs cells. Selection of species is important to increase nutrients removal, sequester more C and decrease greenhouse gas emissions. Plant species selection still requires more research across types of CWs and countries. More research is still needed to be done on the impacts of hydraulic retention time on nutrients dynamics and removal. Subsurface drainage fluxes of nutrients and greenhouse gases to groundwater and

subsequently to surface water bodies through leachates, preferential flow paths by dead roots and holes of burrowing invertebrates should be accounted in CWs management. Nutrients accumulation or fixation in soils and their in situ transformation rates will give insight into

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. the fate of nutrients over time in a changing climate.

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Table 1. Summary of soil depth and hydraulic properties used in CW beds available in the past research.

Type of CWs	Sand (%)	Silt (%)	Clay (%)	Bulk density ($g\,cm^{-3}$)	k_s ($m\,s^{-1}$)	Depth of soil (cm)	References
Surface					1.0×10^{-8}	50	Carty et al. (2008)
Surface					9.8×10^{-9}	50	Dzakpasu et al. (2011)
Surface					1.0×10^{-8}	50	Dunne et al. (2005)
Surface	25	49	26	1.38	1×10^{-8}	50	Harrington et al. (2007)
Surface				0.43		30	Song et al. (2011)
Subsurface						80–90	Picek et al. (2007)
Subsurface	72.8	17.0	6.3	1.79		100	Zhoua and Hosomi (2008)

Table 2. 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	5.6	12.9			45–62	Vymazal (2007)
SF	2.6	64	816	10	> 90	DEHLG (2010)[a]
SF	6.4	34	854	4.3	> 90	DEHLG (2010)[b]
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)

SF – surface flow; SSF – subsurface flow; TN – total N;
[a] soiled water;
[b] wastewater.
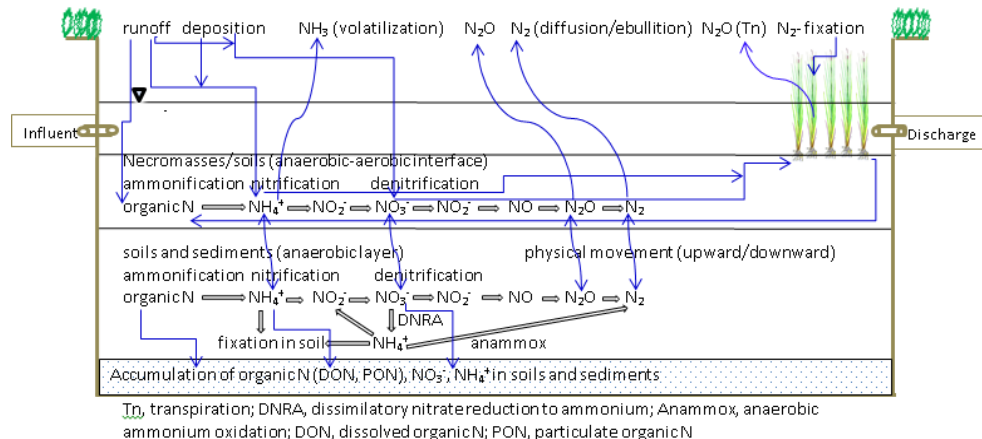
Figure 1. A schematic of N cycle in constructed wetlands showing interactions among different N species.

Figure 2. A schematic of C cycle in constructed wetlands showing interactions among different C compounds.

Carbon and nitrogen dynamics and greenhouse gases emissions

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N input: DON, NH$_4^+$, NO$_3^-$ through wastewater, runoff, atmospheric deposition and biological fixation

N output 1: NH$_3$ and N$_2$O emissions through diffusion, ebullition and transport by vascular plants

N output 2: DON, NH$_4^+$, NO$_3^-$, NO$_2^-$, N$_2$O, N$_2$ discharge to surface water bodies

N dynamics

DON:
- Ammonification (produces NH$_4^+$)
- Leaching with percolating water
- Oxidation (produces NO$_2^-$, N$_2$O)

NH$_4^+$:
- Autotrophic nitrification (produces NO$_2^-$, NO$_3^-$, N$_2$O)
- Heterotrophic nitrification (produces NO$_2^-$, NO$_3^-$, N$_2$O)
- Volatilization (produces NH$_3$)
- Assimilation by plants
- Fixation by soil matrix
- Anammox (produces N$_2$)
- Leaching with percolating water

NO$_3^-$:
- Denitrification (produces NO$_2^-$, N$_2$O, N$_2$)
- DNRA (produces NH$_4^+$)
- Assimilation by plants
- Anammox (produces N$_2$)
- Leaching with percolating water

NO$_2^-$:
- Denitrification (produces N$_2$O, N$_2$)
- Co-denitrification (produces N$_2$O, N$_2$)
- Nitrification (produces NO$_3^-$, N$_2$O)
- Anammox (produces N$_2$)
- Leaching with percolating water

N$_2$O:
- Denitrification (produces N$_2$)
- Leaching with percolating water

N output 3: DON, NH$_4^+$, NO$_3^-$, NO$_2^-$, N$_2$O, N$_2$ transport to groundwater and subsequently to surface water bodies by leachates by preferential flow paths and
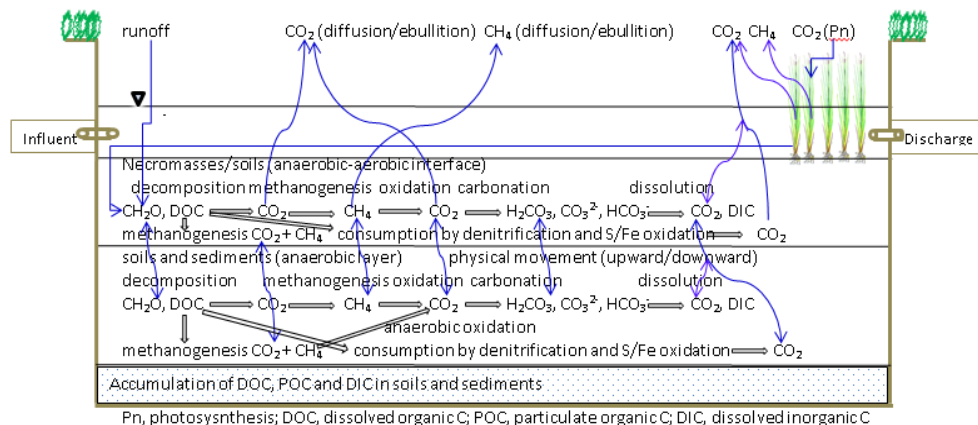
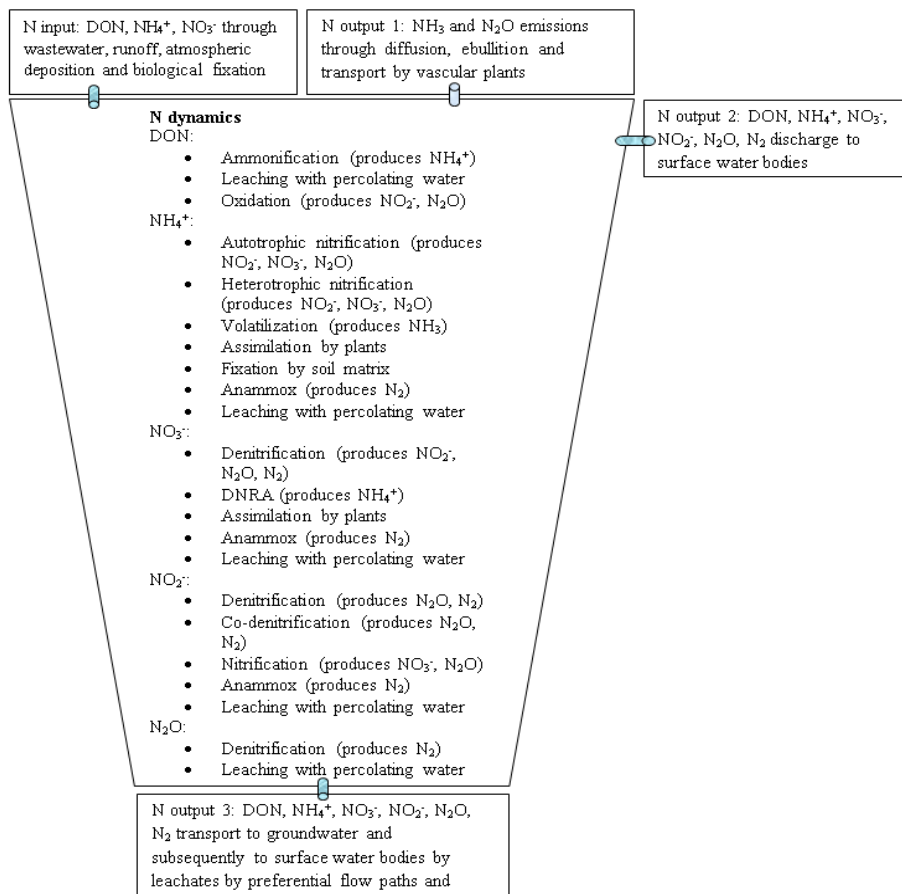**Figure 3.** Conceptual model showing fate of N species and their flows between CWs and the aquatic and atmospheric environments.

C input: Organic matter through wastewater, runoff, and photosynthesis by hydrophytes

C output 1: CO_2 and CH_4 emissions through diffusion, ebullition, and transport by vascular plants

C output 2: DOC, DIC, CO_2, CH_4 discharge to surface water bodies

C dynamics

CO_2:
- Photosynthesis (CH_2O/DOC)
- Methanogenesis (Hydrogenotrophic: $CO_2 + 4H_2 = CH_4 + 2H_2$)
- Carbonation (produces H_2CO_3, HCO_3^-, CO_3^{2-})
- Leaching with percolating water

H_2CO_3, HCO_3^-, CO_3^{2-}:
- Dissolution and inter-changes (produces H_2CO_3, HCO_3^-, CO_3^2, CO_2)
- Leaching with percolating water

DOC:
- Decomposition (CO_2)
- Methanogenesis (Acetotrophic: $CH_3COOH = CO_2 + CH_4$)
- Consumption by denitrification/ sulphate oxidation (produces CO_2)
- Leaching with percolating water

CH_4:
- Oxidation (produces CO_2)
- Leaching with percolating water

C output 3: DOC, DIC, CO_2 and CH_4 transport to groundwater and subsequently to surface water bodies by leachates by preferential flow paths and diffusion
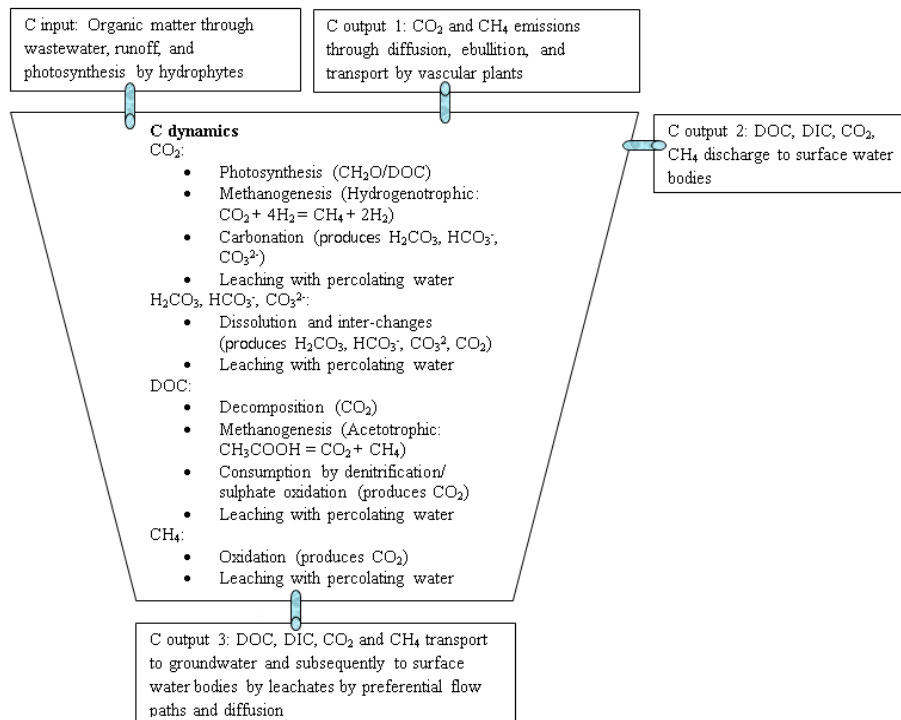
Figure 4. Conceptual model showing fate of C compounds and their flows between CWs and the aquatic and atmospheric environments.
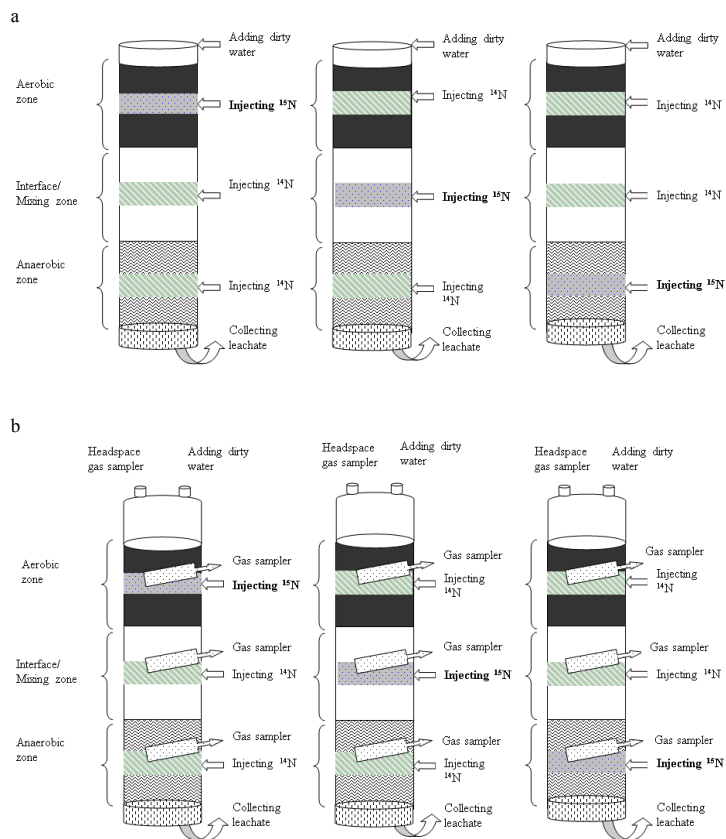
Figure 5. (a) Diagram showing sediments incubation and ^{15}N labelling facilities for total and ^{15}N NO_3^- and NH_4^+ pools analysis in soil samples. **(b)** Diagram showing sediments incubation and gas sampling facilities for N_2O, N_2, CO_2, CH_4 and O_2 analysis after ^{15}N labelling