RESPONSE TO REVIEWERS

Questions appear in green.

All changes to the master manuscript appear in **bold.**

Editor Decision: Reconsider after major revisions (27 Feb 2015) by Prof. Matthew Hipsey

Comments to the Author:

Dear Authors,

First my apologies for the delay in getting the reviewers comments in. I now have received two new sets of comments, which are copied below. In summary Rev 1 rated the revised manuscript as poor in terms of significance and quality, and outlined several reasons for this. Rev 2 (who had reviewed the earlier version) is more positive and saw the improvements, but also still raised concerns about the quality of the presentation and readability. For the paper to be published it is important that the concerns raised in the Rev1 comments below are adequately addressed. If you feel this is too onerous then we can cancel the review process so you can resubmit elsewhere (if the review process is cancelled in this way we can arrange credit of your fee for your next submission). However, I would hope that you are willing to undertake these revisions and further develop the paper to ensure the review fully covers the state of the art literature.

REVIEWER 1:

Overall, I think the review has been much improved after the previous reviewer comments. However, I think there and two key problems with this review, particularly in relation to the nitrogen cycle.

First, it does not really synthesise our understanding much beyond the classic texts of Kadlec and Wallace (Treatment Wetlands) and Reddy and DeLaune (Biogeochemistry of Wetlands), which are not even cited! I think the article should be more upfront about the existence of these works and specifically address how this review synthesizes the state of play beyond these texts. For example the discussion of HLR and HRT on nitrogen removal from lines 200 – 207 is well covered in Kadlec and Wallace.

Response: We are aware that there have been quite a number of excellent reviews around on wetlands biogeochemistry in general. However, we are dealing in this review with the specificities of constructed wetlands and not wetlands in general. This aspect should be taken into consideration in the reviewing of this paper. It emerges that we may know quite a number of aspects related to wetlands but not so much about constructed wetlands. To make this clearer we added a sentence in the introduction. The following lines have been added:

Line 55: While nutrient cycles have been addressed in previous books and review papers, we strongly suggest that *in situ* in transformation and fate of the transformation products with regard to pollution swapping requires further detailed examination.

Line 66: While there are excellent reviews and books available on the biogeochemistry of wetlands in general (Reddy and Delaune, 2008; Kadlec and Wallace, 2009), less is known about constructed wetlands (CWs), which provide much more controlled conditions (Vymazal and Kröpfelova, 2010).

Second, the article seems to have missed many recent relevant articles in the field, and many statements are vague, incorrect and not referenced. A few examples of this are given below.

Response: We have added many more references to the text and all those suggested by the reviewer.

Blackburn, T. H.: Methods for measuring rates of NH4+ turnover in anoxic marine sediments, using a 15N-NH4+ dilution technique, Appl. Environ. Microbiol., 37, 760-765, 1979.

Huygens, D., Trimmer, M., Rütting, T., Müller, C., Heppell, C. M., Lansdown, K., and Boeckx, P.: Biogeochemical N cycling in wetland ecosystems: 15N isotope techniques, in: Methods in biogeochemistry of wetlands, edited by: Reddy, K. R., Megonigal, J. P., and Delaune, R. D., Soil Science Society of America, 553-591, 2013.

Kadlec, R. H., and Wallace, S. D.: Treatment wetlands, CRC Press Taylor and Francis, 1000 pp., 2009.

Minett, D. A., Cook, P. L. M., Kessler, A. J., and Cavagnaro, T. R.: Root effects on the spatial and temporal dynamics of oxygen in sand-based laboratory-scale constructed biofilters, Ecological Engineering, 58, 414-422, 2013.

Müller, C., Laughlin, R. J., Spott, O., and Rütting, T.: A 15N tracing method to quantify N2O pathways from terrestrial ecosystems, European Geological Union General Assembly, Vienna, 27 April to 2 May 2014, 1907, 2014.

Payne, E. G. I., Fletcher, T. D., Cook, P. L. M., Deletic, A., and Hatt, B. E.: Processes and drivers of nitrogen removal in stormwater biofiltration, Critical Reviews in Environmental Science and Technology, 44, 796-846, 2014a.

Payne, E. G. I., Fletcher, T. D., Russell, D. G., Grace, M. R., Cavagnaro, T. R., Evrard, V., Deletic, A., Hatt, B. E., and Cook, P. L. M.: Temporary storage or permanent removal? The division of nitrogen between biotic assimilation and denitrification in stormwater biofiltration systems, PLoS ONE, 9, e90890, 2014b.

Reddy, K. R., and Delaune, R. D.: Biogeochemistry of Wetlands: Science and Applications, CRC Press, 800 pp., 2008.

Rütting, T., and Müller, C.: Process-specific analysis of nitrite dynamics in a permanent grassland soil by using a Monte Carlo sampling technique, Eur. J. Soil Sci., 59, 208-215, 2008.

Rütting, T., Boeckx, P., Müller, C., and Klemedtsson, L.: Assessment of the importance of dissimilatory nitrate reduction to ammonium for the terrestrial nitrogen cycle, Biogeosci., 8, 1779-1791, 10.5194/bgd-8-1169-2011, 2011.

Vymazal, J.: Removal of nutrients in various types of constructed wetlands, Sci. Tot. Environ., 380, 48-65, 2007.

Vymazal, J., and Kröpfelová, L.: Types of constructed wetlands for wastewater treatment in: Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow, Environmental Pollution Springer, Heidelberg, 121-202 2010.

A key open question is the relative amount of nitrogen denitrified compared to that assimilated by plants. A recent study has shown that the amount of nitrogen that is lost through denitrification relative to assimilation depends strongly on nitrogen loading

Response: ***The Payne et al study deals with stormwater biofiltation systems which are characterised by inundation and prolonged dry periods. This provides conditions quite different compared to constructed wetlands as reviewed in this paper. In particular during the drier periods it is expected that assimilation may be enhanced. This aspect has been added in lines 280***

The following line has been deleted from the ms: '*Impact of plant species on nutrient removal has not been reported clearly in the published literature, where species differences for one or more nutrient was observed, because of the problems with plant growth due to unpredicted environmental conditions, wastewater toxicity, and in many cases the reasons were not specified (Fraser et al., 2004; Solano et al., 2004; Haule et al., 2002).It has been replaced with:*

Line 161: Payne et al. (2014a) has discussed the role of plants in nutrient removal.

and Line 280: Competition for NO_3- may occur between denitrification and biotic assimilation. This is likely governed by the prevailing aerobic/anaerobic conditions and therefore dependent on the type of wetland. For instance in stormwater biofiltration systems, prolonged periods of inundation and dry periods may support bio-assimilation over denitrificaton (Payne et al., 2014a,b).

The two Payne et al. articles have been referenced.

Payne, E. G. I. and others 2014. Biotic assimilation or denitrification? The division of nitrogen between temporary storage and permanent removal in stormwater biofiltration systems. Plos One 9: e90890.

Payne, E. G. I., T. D. Fletcher, P. L. M. Cook, B. E. Hatt, and A. Deletic. 2014. Processes and drivers of nitrogen removal in water biofiltration. Critical Reviews in Environmental Science and Technology 44: 796-846.

Some of the synthesis on nitrogen cycling is vague and is not resolved for example the discussion on the effect of C:N ratio on denitrification and DNRA lines 188 – 191. Lines 284.

Response: Rather than the C/N ratio it is the C/NO_3^- ratio that governs the relative reduction of denitrification and DNRA. Above 12, DNRA is favoured. This information is added in line 280 – 307:

Competition for NO_3^- may occur between denitrification and biotic assimilation. This is likely governed by the prevailing aerobic/anaerobic conditions and therefore dependent on the type of wetland. For instance in stormwater biofiltration systems, prolonged periods of inundation and dry periods may support bio-assimilation over denitrificaton (Payne et al., 2014a,b). The conditions that favour the occurrence of either denitrification or DNRA are still in debate (Rütting et al., 2011). DNRA is thought to be favoured by a $C:NO_3^-$ ratio of >12 (Rütting et al., 2011) and

occurs at low levels of oxidation-reduction potential (Thayalakumaran et al., 2008). The differences between denitrification and DNRA may be due to the availability of organic matter, because DNRA is the favoured at a high C:NO_3^- ratio and denitrification is favoured when carbon supplies are limiting (Korom, 2002; Kelso et al., 1997). The fermentative bacteria that carry out DNRA are obligate anaerobes, and so cannot occupy all the niches that denitrifiers can (Buss et al., 2005). Takaya (2002) stated that a more reducing state favours DNRA over denitrification. Pett-Ridge et al. (2006) showed that DNRA is less sensitive to dissolved oxygen (DO) than denitrification. Fazzolari et al. (1998) showed that the effect of DO levels on DNRA is dependent on the C:NO_3^- ratio and C, rather than DO, is the main factor regulating NO_3^- partitioning between DNRA and denitrification. Significant DNRA may occur only at a C:NO_3^- ratio above 12 (Yin et al., 1998). Different numbers of electrons are required in the reduction of each NO_3^- molecule: five for denitrification and eight for DNRA. Therefore, more organic matter can be oxidized for each molecule of NO_3^- by DNRA than by denitrification. In addition, NO_3^- reduction is generally performed by fermentative bacteria that are not dependent on the presence of NO_3^- for growth under anaerobic conditions. So, DNRA bacteria may be favoured by NO_3^-- limited conditions (Laanbroek, 1990). Recent studies have suggested that DNRA may be an important process compared to denitrification in wetland sediments (Burgin and Hamilton, 2008). Van Oostrom and Russell (1994) found a 5% contribution of DNRA to the NO_3^- removal in CWs. Little is known about the eventual fate of the NO_3^- that is converted to NH_4^+ *via* DNRA pathways. In recent years, N cycling studies have increasingly investigated DNRA in various ecosystems to explore its importance in N cycling (Rütting et al., 2011), but controls on DNRA are relatively unknown (Burgin et al., 2013), DNRA being probably the least studied process of N transformation in wetlands (Vymazal, 2007). However, DNRA can be a significant pathway of NO_3^- reduction that impacts on the CW ecosystem services and so should therefore be evaluated

The idea of anoxic microsites is one that is used a lot with little evidence. The work of Minett et al offers some insights based on actual measurements within the rhizosphere.

Strong evidence of denitrification in microsites is quite accepted and has been proven in soil studies (Parkin, T. B.: Soil microsite as a source of denitrification variabilitiy, Soil Sci. Soc. Am. J., 51, 1194-1199, 1987). Denititrification hot spots are related to the patchy distribution of organic C and may be supplied via rhizodeposition with evidence for instance shown in the Minett et al study in a simplified sand bed systems. Thus, there is no reason why this should not happen in constructed wetlands where microsites of organic accumulation may develop. These are hotspots of high activity which may promote anoxic conditions due to high O_2 consumption which is not replenished quickly enough through limited diffusion to those sites.

Lines 270: The existence of microsites that support high activity and promote denitrification has been shown in soils (Parkin, 1987) and such conditions are also likely to occur in CWs where patchy distribution of organic material (e.g. particulate organic carbon) can occur (Hamersley and Howes, 2002), which may be supplied by rhizodepositions (Minett et al., 2013). Minett et al. (2013) found that simultaneous oxygenation of the rhizosphere, through radial oxygen loss, and enhanced oxygen consumption by the soil occurs in the area immediately surrounding the roots. Nitrate produced in the rooting zone can be taken up by plants or denitrified and/or converted back to NH_4^+ by DNRA.

Line 291 An important statement with no reference Line 301 No reference is provided,

Response: ***a suitable reference has been added. (Vymazal, 2007)***

I think the general paradigm is that DNRA is favoured at high organic matter loading or highly reducing conditions.

Response: ***It is favoured both at C/NO3- above 12 (outlined in the DNRA section) and it is a strict anoxic process. Details are provided in the manuscript.***

REVIEWER 2:

I have been reading over this review paper. It has undergone a very major improvement since my first read. And I very much like their new figure, which is a great summary of what the different wetland elements do to the different nutrient species. I am also impressed by their tables showing the range of emission rates for the different nutrients. Just the type of tables we frequently need and bemoan the fact no-one has compiled.

Overall I believe it is a very useful paper, and would be good for HESS to publish it.

It still requires a science editor to go through it. Still quite a few awkward sentences and phrases. Much improved from the last read, but still enough in there to be jarring for the reader. Mistakes with grammar, tenses etc.

So I guess I would say "very good job with the improvements", but corrections required, specifically with the English.

Response: The authors wish to thank the reviewer for positive feedback. The manuscript has undergone a complete edit and in some parts re-structuring with respect to the points made by the reviewer.

Carbon and Nitrogen Dynamics and Greenhouse Gas Emissions in Constructed Wetlands Treating Wastewaters: A Review

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Abstract

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

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

1. Introduction

Increasing anthropogenic loading of reactive nitrogen (Nr; all forms of nitrogen except di-nitrogen gas, N_2) 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, as well as algal blooms and hypoxia (Conley et al., 2009; Rabalais et al., 2010). **While there are**

excellent reviews and books available on the biogeochemistry of wetlands in general (Reddy and Delaune, 2008; Kadlec and Wallace, 2009), less is known about constructed wetlands (CWs), which provide much more controlled conditions (Vymazal and Kröpfelova, 2010). **C**onstructed wetlands (CWs) **are artificial sinks for Nr (Galloway et al, 2003; Tanner et al., 2005), and** have been successfully used for treating domestic sewage, urban runoff and storm water, industrial and agricultural wastewater, and leachate. Although CWs have a proven potential for organic **carbon (C)** and N removal, **with few exceptions, studies have not adequately delineated removal pathways. This has meant that reported removal efficiencies have been variable** (Seitzinger et al., 2002**), and appropriate design and management strategies have not been adopted.**

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

Constructed wetlands receive organic C from the influent wastewater and from fixation by the photosynthetic hydrophytes, which are incorporated into soil as organic C. Soil organic C undergoes the biogeochemical processes that regulate C accretion in soil and microbial

101 respiration, producing carbon dioxide (CO_2). Anaerobic mineralization of organic C by
102 methanogenic archaea can produce methane (CH_4) (Laanbroek, 2010; Ström et al., 2007; Søvik et
103 al., 2006; Pangala et al., 2010). Constructed wetlands can also contribute to the dissolved organic
104 carbon (DOC) load transfer to ground and surface waters, which may produce and exchange
105 substantial amounts of CO_2 and CH_4 with the atmosphere (Clair et al., 2002; Elberling et al.,
106 2011). Therefore, CWs can diminish the environmental benefits of wastewater treatment. The
107 dynamics of dissolved N_2O, CO_2 and CH_4 in CWs is a key knowledge gap in global greenhouse
108 gas budgets.
109
110 Surface emissions of GHG from CWs have been commonly measured by the closed chamber
111 method (Johansson et al., 2003, 2004; Mander et al., 2005, 2008), but have rarely been measured
112 by ebullition and diffusion methods (Søvik et al., 2006). The measured rates have shown high
113 spatial, temporal and diurnal variations due to the change in biogeochemistry of C and N and
114 plant-microbe-soil interaction over time and space. Surface emissions cannot explain the kinetics
115 of production and consumption rates of GHG, which we need to know in order to adopt better
116 management practices to mitigate emissions. In addition, subsurface export of dissolved nutrients
117 and GHG, an important pathway of nutrient loss (Riya et al., 2010), is frequently ignored. Mass
118 balance analysis of the different components of the N cycle and kinetics of their transformation
119 processes occurring within the treatment cells using the isotope-tracing ^{15}N technique can provide
120 mechanistic information for N transformation products (Lee et al., 2009; O'Luanaigh et al., 2010)
121 and may be used to start to answer such questions. Similarly, ^{14}C application and measurement of
122 C species (e.g. CO_2, CH_4, and DOC) may elucidate the C mineralization and CO_2 and CH_4
123 production and consumption. Used in combination, these methods may provide a comparative
124 analysis of the rates of C and N transformation processes and role of these processes in delivering
125 NO_3^-, NH_4^+ and DOC to ground/surface waters and N_2O, CO_2 and CH_4 to the atmosphere.
126
127 Past reviews on CWs, though very limited, summarise the performance of different types of CWs
128 on C and N removal (Vymazal, 2007) and surface emissions of GHG (Mander et al., 2014), but
129 have not discussed the mechanisms of nutrient removal and the fate of the nutrients delivered and
130 removed to and from CWs. Therefore, the objectives of this review are (i) to understand the
131 biogeochemical dynamics of C and N in CWs to (ii) better understand the fate of various C and N
132 species in a holistic manner, in addition to the conventional influent/effluent balance for nutrient
133 removal (iii) identify the research gaps that need to be addressed to optimise nutrient removal and

134 mitigate GHG emissions, and (iv) discuss emerging measurement techniques that may give
135 insights into the production and reduction of GHG.
136
137 ## 2. Removal Efficiency, Hydraulic Loading and Retention Time
138 In CWs, the efficiency of C and N removal is generally limited and highly variable over CW
139 types, plant types, seasons, climatic regions and management practices. On average, it appears
140 that 50 and 56% of the influent total nitrogen (TN) and total organic carbon (TOC), respectively,
141 can be removed, but the removal rates are very inconsistent. Mean (±standard error) TN
142 removals, obtained from the literature cited in this paper, ranged from 31.3 ± 6.3% in surface
143 flow (SF) CWs to 40.4 ± 4.4% in subsurface flow CWs, whereas TOC removal ranged from 18.8
144 ± 9.4% in SF CWs to 56.2 ± 9.5% in vertical subsurface flow CWs (Table 1 and Table 2). In
145 European systems, for example, typical removals of ammoniacal-N in long-term operation are
146 around 35%, but can be enhanced if some pre-treatment procedures are followed (Verhoeven, and
147 Meuleman, 1999; Luederitz et al., 2001). Generally, TN removal is higher in SF CWs than
148 subsurface flow (SSF) CWs (Table 1), but studies differ. For example, Van der Zaag et al. (2010)
149 showed higher N removal in SF CWs than SSF, but Søvik et al. (2006) and Gui et al. (2007)
150 showed the opposite. In SSF CWs, limited removal can be caused by a reduced environment that
151 enhances NH_4^+ accumulation and limits NH_4^+ oxidation. In SF CWs, denitrification rates can be
152 limited due to lack of NO_3^-. In vertical subsurface flow (VSSF) CWs, aeration can increase NH_4^+
153 oxidation to NO_3^-, which can be denitrified or converted to NH_4^+ by dissimilatory NO_3^- reduction
154 to **NH_4^+** (DNRA).
155
156 Plant species are important components of CWs, and affect C and N removals. Optimal species
157 selection for best removal is difficult because some species are efficient in removing one
158 pollutant but not the other (Bachand and Horne, 2000; Bojcevska and Tonderski, 2007; da Motta
159 Marques et al., 2000). In some studies there are no inter-species differences at all (Calheiros et
160 al., 2007). Mixed species perform better than monocultures to remove C and N pollutants because
161 they increase microbial biomass and diversity. **Payne et al. (2014a) has discussed the role of**
162 **plants in nutrient removal.** Plants regulate CWs hydrology (evaporation and transpiration) and
163 temperature (insulating CWs from seasonal temperature change, trapping falling and drifting
164 snow and heat loss of wind). Some species can create large surface area for microbial attachment
165 and enhance microbial diversity, but experimental evidence is still scarce.
166

Soil physicochemical properties are important factors controlling the purification capacity in CWs. Soils with high permeability enhance downward nutrient movement to groundwater. High cation exchange materials in soil enhance NH_4^+ fixation by the soil matrix. Microbial activities and growth depend on substrate C quality and C:N ratios, which affect nutrient removal. Better growth of heterotrophic microorganisms is a function of the wastewater C:N (Makino et al., 2003). Higher C:N ratios can enhance denitrification by providing electron donors for denitrifiers, but the opposite can increase nitrification. High C:N ratios can also encourage DNRA over denitrification. Yan et al. (2012) measured a high TN removal but low TOC removals at a C:N ratio 2.5:1, which indicates that removal of one parameter might lead to a problem with a different one. The uncertainty in the conditions for achievement of optimum removal suggests that the rates of C and N transformations and the fate of the removed nutrients within CWs should be investigated. However, to our knowledge, no study has provided a holistic evaluation of C and N attenuation and transformation.

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

Fluctuating hydraulic loading influences all biotic and abiotic processes in CWs. For example, if the groundwater table is lowered through changes in hydraulic loading, soil aeration can increase or decrease. Ammonification and nitrification rates increase with increased soil aeration and this enhances C utilization by bacteria and, therefore, can stimulate the removal of C and N. Investigation into the effects of fluctuating hydraulic loadings ("hydraulic pulsing") on C and N removals and their transformation products will provide information about the fate of the added nutrients in terms of their environmental benefits and/or pollution swapping potential. For example, if the dominant product is N_2, the system will be relatively benign in terms of its impact on the environment, but if it is NH_4^+, it can be fixed in the soils or transported to ground and surface waters connected to CWs if the cation exchange sites become saturated. Several authors have used a wide range of **HLRs** and **HRTs** to measure nutrient removal efficiency**,** but

experimental evidence **linking HLR and HRT to removal efficiency** is scarce (Toet et al., 2005). Luo et al. (2005) reported that low HLR results in incomplete denitrification, whereas Zhang et al. (2006) argued that low HLR increases NH_4^+ and chemical oxygen demand oxidation. The way in which the performance of a CW is assessed can lead to different conclusions regarding the removal of Nr. For future studies, evaluation of CWs in a holistic manner, which includes pollution swapping at different HLRs and HRTs, is important, particularly within the context of the changing hydrologic cycle in a changing climate. In addition, local legislative targets should be considered and weighting factors (e.g. the relative importance of, say, GHG over water quality targets) should be developed to evaluate the overall performance of CWs. In addition to the estimation of nutrient removal rates, investigation of the effect of HLR and HRT on the different forms of nutrients in the final effluent and their fate in the natural environment may help elucidate the pollution swapping potential of CWs.

3. Accumulation of C and N in CWs Soils

The soil in CWs is a major sink for C and N (Mustafa and Scholz, 2011). However, although data on the influent and effluent N concentrations are available, data on N accumulation (dissolved organic nitrogen (DON), TN, NH_4^+ or NO_3^--N) within the soil profile of various CWs are scarce. The wide range of N accumulation reported in the literature (e.g. 30-40%, Shamir et al., 2001; 39%, Harrington et al., 2007; 9%, Mander et al., 2008; 2.5%, Obarska-Pempkowiak and Gajewska, 2003) may be due to the variations in CW types and management strategies. The accumulated species of N are reactive unless they have been transformed to N_2 by biogeochemical processes. However, there is a dearth of information on the extent of Nr accumulation in soils and discharge to surface waters and air (Shamir et al., 2001). Accumulated organic N could be mineralised to NH_4^+ and NO_3^-, depending on the physico-chemical properties of soil. The Nr could be assimilated by plants and microbes, which are recycled in a soil-plant-soil continuum. Nitrogen spiralling occurs from NH_4^+ to organic N and back to NH_4^+ within the CW (O'Luanaigh et al., 2010). Typically, N accumulation has been found to decrease with soil depth (Shamir et al., 2001). In terms of the conventional input-output balance, these are considered as removed N, but may, in fact, remain in such a biogeochemically active system**.** In addition to N, organic C accumulation occurs in CW soils (Nguyen, 2000). As such, soils of 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. Estimation of the rates of nutrient accumulation in soils in various types of CWs under different management

234 systems is important. The stability of the accumulated C and N under changing climatic scenarios
235 also needs to be addressed to consider the long-term sustainability of CWs.
236

237 ## 4. C and N Dynamics and Greenhouse Gas Emissions

238 Increased nutrients input to the CWs increases the productivity of wetland ecosystems and the
239 production of GHG. As CWs are designed to remove pollutants in an anaerobic/suboxic
240 environment, they change the C and N biogeochemistry and contribute significantly to CH_4 and
241 N_2O emissions (Johansson et al., 2002, 2003; Mander et al., 2005, 2008; Stadmark and
242 Leonardson, 2005; Liikanen et al., 2006). Søvic et al. (2006) measured N_2O, CH_4 and CO_2
243 emissions in various CWs in different European countries, and suggested that the potential
244 atmospheric impacts of CWs should be examined as their development is increasing globally.
245 Management of CWs must consider the negative climatic aspects of increased emissions of GHG
246 in addition to their primary functions (Ström et al., 2007). Therefore, estimation of the
247 contribution of CWs to global warming is required. In this regard, measurement of spatial and
248 temporal variations (seasonal and diurnal) of GHG emissions is necessary to accurately estimate
249 CW-derived GHG emissions. A holistic assessment of ecologically engineered systems has been
250 outlined by Healy et al. (2011, 2014) and developed further by Fenton et al. (2014). Such
251 assessments can be applied in evaluating nutrient dynamics in CWs. Moreover, plant mediated
252 GHG emissions could be an important component of total emissions, but again research in this
253 area is very limited. Effective modelling or up-scaling of GHG emissions from watershed to
254 regional/national scales is important for the improvement of global GHG budgets. Such up-
255 scaling needs an accurate estimation of C and N inputs and outputs i.e., a balance coupled with
256 net GHG emissions, while considering all possible processes and pathways involved. A study of
257 the dynamics of C and N in CWs is crucial, as the forms of removed C and N are particularly
258 pertinent to their potential for pollution swapping, global warming and water pollution.
259

260 Processes involved in N removal and N transformations in wetlands include sedimentation of
261 particulates (Koskiaho, 2003); nitrification, denitrification and DNRA (Poach et al., 2003; Burgin
262 et al., 2013), microbial assimilation and plant uptake-release (Findlay et al., 2003), anammox and
263 deamox (DEnitrifying AMmonium OXidation). Constructed wetlands are complex systems that
264 facilitate aerobic and anaerobic microsites. Nitrification, denitrification and nitrifier
265 denitrification are the processes responsible for the production of N_2O. Depending on the
266 environmental conditions or management practices prevailing, a certain process will dominate

e.g. denitrification is the dominant process in SF CWs (Beaulieu et al., 2011), but nitrifier denitrification is dominant in VSSF CWs (Wunderlin et al., 2013). Generally, CWs are anaerobic but aquatic macrophytes can transport oxygen from the atmosphere to the rooting zone, where it can sustain nitrification. **The existence of microsites that support high activity and promote denitrification has been shown in soils (Parkin, 1987) and such conditions are also likely to occur in CWs where patchy distribution of organic material (e.g. particulate organic carbon) can occur (Hamersley and Howes, 2002), which may be supplied by rhizodepositions (Minett et al., 2013). Minett et al. (2013) found that simultaneous oxygenation of the rhizosphere, through radial oxygen loss, and enhanced oxygen consumption by the soil occurs in the area immediately surrounding the roots**. Nitrate produced in the rooting zone can be taken up by plants or denitrified and/or converted back to NH_4^+ by DNRA.

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

The conditions that favour the occurrence of either denitrification or DNRA are still in debate (Rütting et al., 2011). DNRA is thought to be favoured by a C:NO_3^- ratio of >12 (Rütting et al., 2011) and occurs at low levels of oxidation-reduction potential (Thayalakumaran et al., 2008). The differences between denitrification and DNRA may be due to the availability of organic matter, because DNRA is the favoured at a high C:NO_3^- ratio and denitrification is favoured when carbon supplies are limiting (Korom, 2002; Kelso et al., 1997). The fermentative bacteria that carry out DNRA are obligate anaerobes, and so cannot occupy all the niches that denitrifiers can (Buss et al., 2005). Takaya (2002) stated that a more reducing state favours DNRA over denitrification. Pett-Ridge et al. (2006) showed that DNRA is less sensitive to dissolved oxygen (DO) than denitrification. Fazzolari et al. (1998) showed that the effect of DO levels on DNRA is dependent on the C:NO_3^- ratio and C, rather than DO, is the main factor regulating NO_3^- partitioning between DNRA and denitrification. Significant DNRA may occur only at a C:NO_3^- ratio above 12 (Yin et al., 1998). Different numbers of electrons are required in the reduction of each NO_3^- molecule: five for denitrification and eight for DNRA. Therefore, more organic matter can be

oxidized for each molecule of NO_3^- by DNRA than by denitrification. In addition, NO_3^- reduction is generally performed by fermentative bacteria that are not dependent on the presence of NO_3^- for growth under anaerobic conditions. So, DNRA bacteria may be favoured by NO_3^-- limited conditions (Laanbroek, 1990). Recent studies have suggested that DNRA may be an important process compared to denitrification in wetland sediments (Burgin and Hamilton, 2008). Van Oostrom and Russell (1994) found a 5% contribution of DNRA to the NO_3^- removal in CWs. Little is known about the eventual fate of the NO_3^- that is converted to NH_4^+ *via* DNRA pathways. In recent years, N cycling studies have increasingly investigated DNRA in various ecosystems to explore its importance in N cycling (Rütting et al., 2011), but controls on DNRA are relatively unknown (Burgin et al., 2013), DNRA being probably the least studied process of N transformation in wetlands (Vymazal, 2007). However, DNRA can be a significant pathway of NO_3^- reduction that impacts on the CW ecosystem services and so should therefore be evaluated.

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

The various components of the C cycle include: fixation of C by photosysnthesis, respiration, fermentation, methanogenesis and CH_4 oxidation with reduction of sulphur, iron and NO_3^-. Anaerobic methane oxidation coupled with denitrification, a recently proposed pathway of the C cycle (á Norði and Thamdrup, 2014; Haroon et al., 2013; Islas-Lima et al., 2004), can reduce CH_4 emissions in CWs. The C removal processes are sedimentation, microbial assimilation, gaseous

335 emissions, dissolved C losses through water to ground and surface water bodies, and chemical
336 fixation (bonding with chemical ions). Net primary productivity of wetland hydrophytes varies
337 across CW type, season, climatic region and local environmental conditions. For example, results
338 can vary remarkably for CWs containing the same plant species in different geographical regions
339 (Brix et al., 2001). Carbon mineralization in sediments depends on the redox chemistry of soil,
340 availability and quality of C (labile or recalcitrant) and temperature. In CWs, C cycling is very
341 complex due to the changes in redox chemistry, which regulates production and consumption of
342 CO_2 and CH_4 (Brix et al., 2001). In low redox conditions with limited DO, methanogens can
343 consume DOC and thus it is conducive to CH_4 production. The C:N ratios of wastewater affect
344 microbial growth and development which, in turn, affect their response to C and N cycles and
345 GHG emissions. Previous research on the effects of C:N ratios on nutrient removal and GHG
346 emissions are limited. A few examples include Yan et al. (2012) and Zhao et al. (2014), who
347 measured lower CO_2 and CH_4 emissions at C:N ratios of between 2.5:1 and 5:1, but this lower
348 range of C:N ratios decreased TOC removal. Hence, investigation of the influence of C:N ratio
349 on nutrient removal efficiencies and GHG emissions across CW and management types is crucial.
350 In summer, oxygen diffusion to the topsoil can reduce methanogenesis and stimulate CH_4
351 oxidation (Grünfeld and Brix, 1999). However, an increase in temperature can decrease DO in
352 deeper subsoil layers, which can enhance CH_4 production. Only a limited number of studies have
353 considered CH_4 and CO_2 efflux from CWs (e.g. Mander et al., 2008). As in all biochemical
354 reactions, temperature increases C and N turnover in CWs, causing high variations in GHG
355 emissions in different regions (temperate/ tropical/ arctic). These variations need to be considered
356 while extrapolating GHG emissions for different types of CWs under different management
357 practices.
358
359 Emissions of GHG in CWs can vary across CW typologies e.g. surface flow or subsurface flow
360 (Van der Zaag et al., 2010). A summary of N_2O, and CO_2 and CH_4 were presented in Table 3 and
361 Table 4, respectively. Generally, CH_4 emissions are higher in SF CWs than in SSF CWs (Table
362 3), but may vary with season. Nitrous oxide and CO_2 emissions are higher in VSSF CWs than
363 HSSF and SF CWs. The N_2O emissions factors (EF; N_2O/TN input $\times 100$) ranged from 0.61 ±
364 0.21% in SF CWs to 1.01 ± 0.48% in VSSF CWs. The EF for CH_4 emissions ranged from 1.27 ±
365 0.31% in VSSF CWs to 16.8 ± 3.8% in SF CWs. The GHG from CWs can vary between
366 vegetated and non-vegetated systems (Table 5).
367

Aquatic plants play an important role in GHG production and transport to the atmosphere by releasing GHG through their interconnected internal gas lacunas (Laanbroek, 2010). Emergent plants can transport atmospheric oxygen to the rooting zone and contribute to increased N_2O and CO_2 production and CH_4 consumption (Brix, 1997). Vascular plants can exchange GHG between the rooting zone and atmosphere (Yavitt and Knapp, 1998). Vegetation and its composition affect the nutrient dynamics and the production, consumption and transport of GHG and hence their exchange between wetlands and atmosphere (Ström et al., 2003, 2005; Søvic et al., 2006; Johansson et al., 2003). They can also affect the biogeochemistry of CWs due to the differences in their growth and development, longevity, root systems, root density, root depth and microbial ecology in the rhizosphere. As some plant litter decomposes, organic matter with lignocellulose and humic compounds may be released that are more or less labile or stable in nature than others. Release of low molecular weight organic matter that is labile in nature is more likely to produce GHGs than stable forms. For example, *Z. latifolia* showed higher nutrient removal and CH_4 fluxes than *P. australis* (Inamori et al., 2007). The *Z. lotifolia* root system is shallow and the activity of methanotrophs is primarily confined to the top soil. The root systems of *P. australis* are deeper, which is more favourable for the oxidisation of CH_4. A fluctuating water table in CWs has significant impacts on GHG dynamics. Pulsing hydrologic regimes decreases CH_4 but increases N_2O emissions. In aerobic and anaerobic conditions caused by pulsing hydrology, incomplete nitrification and denitrification increase N_2O emissions. However, the effects of pulsing hydrologic regimes on GHG emissions are contradictory. For example, intermittent hydrologic regimes decrease both N_2O (Sha et al., 2011) and CH_4 emissions (Song et al., 2010). Highly contrasting results on gas emissions with fluctuating water levels have been reported and the controlling mechanisms are unclear (Elberling et al., 2011).

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

5. Surface Emissions vs Subsurface Export of C and N

Dissolved GHG produced in soils and subsoils can be emitted to atmosphere by transpiration of vascular plants (from within the rooting zone), ebullition and diffusion from soils. Elberling et al.

(2011) reported that in wetlands, the transport of gases through subsoil occurs both *via* diffusive transport in the pores and through the vascular plants. Surface emissions of GHG from CWs are well recognised and have been commonly measured by chamber methods. The GHG produced in CWs can also be transported to the groundwater with the percolating water and emitted to the atmosphere upon discharge to surface waters. It can also flow towards surface waters by advective transport and/or by dispersion of groundwater. Dissolved nutrients can be preferentially leached down into deeper soil layers and groundwater *via* different pathways (e.g. root channels). The Nr delivered to groundwater can be transformed *in situ* to other reactive or benign forms. Hence, quantification of such Nr loadings to groundwater and their *in situ* consumption (e.g. N_2O to N_2 or CH_4 to CO_2) is necessary to understand their environmental consequences. In addition, DON, NO_3^- and NH_4^+ and DOC delivered to surface waters can undergo biochemical reactions and produce N_2O, CO_2 and CH_4 in streams and estuaries. Ström et al. (2007) measured a considerable quantity of CH_4 in porewater and found a correlation between the surface emissions and porewater CH_4 concentrations in vegetated wetlands. Measuring only the surface emissions of GHG can omit substantial quantities of GHG released from CWs. For example, Riya et al. (2010) measured emissions of CH_4 and N_2O accounting for 2.9 and 87% of the total emissions. Measuring porewater GHG and linking these to the surface emissions and subsurface export to groundwater below CWs will help to estimate a better GHG balance from both a national and global context. Elberling et al. (2011) linked subsurface gas concentrations in wetlands to the surface fluxes using a diffusion model. This demonstrates the need for future studies on subsurface GHG production, consumption and net GHG emissions in CWs within a climate change context.

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

6. **Hydrogeochemistry below CWs**

Constructed wetlands can be designed with or without a clay liner or a compacted soil bed at the base, which can lead to large differences in permeability of the underlying layers. The variation in permeability of a CW soil bed will affect solute, nutrient and GHG flows, and their interactions with the underlying groundwater (Dzakpasu et al., 2012; 2014). Groundwater hydrogeochemistry below CWs can therefore provide a unique insight into such interactions. An example of such interactions would be between nutrient-rich water discharging from CW cells mixing with laterally moving regional groundwater. It should be noted that groundwater can also discharge into CWs depending on the hydraulic gradients. This means that fully screened, multi-level piezometers or boreholes should be installed at such sites to elucidate groundwater flow direction, hydraulic gradients and conductivities. Such monitoring networks allow water samples to be collected and the sources of nutrients in groundwater bodies below CWs to be identified. The local site hydrology (precipitation, groundwater table fluctuations and evapotranspiration) has a large impact on the pollutant removal. Hydrogeochemical studies at an accurate spatial and temporal resolution should explain the effects of precipitation on nutrient removal by dilution as well *in situ* nutrient turnover. Effective CW management requires an understanding of the effects of wetland hydrology on the physical and biochemical attenuation of nutrients in order to assess their impacts on the surface emissions and subsurface export of nutrients and GHG. Data on the species of N in groundwater below the CWs are required to provide an in-depth understanding of wetland ecosystem services, particularly if CWs have the potential to leak pollutants down into the groundwater (Dzakpasu et al., 2014). Higher NH_4^+ concentrations in groundwater below the CWs than the effluent are often reported (Harrington et al., 2007; Dzakpasu et al., 2012). Therefore, questions arise with respect to NH_4^+ concentrations in groundwater below the CWs if they have been transported from CWs. Linking geochemistry of groundwater below CWs to site hydrology, water table fluctuations and soil/subsoil physico-chemical properties is required to elucidate the major environmental drivers of C and N removal, and/or pollution swapping. The quality of groundwater underlying CWs with regards to the Nr species is largely unknown.

7. **Methodological Developments**

To improve the ecosystem services and to minimize the pollution swapping of CWs, **quantification of** N cycling is crucial. Measurement of GHG using the closed chamber method is widely used, but has large uncertainty in estimating the diurnal variability due to internal changes in temperature and physical access to the chambers over a 24-h time period. Gas ebullition and

diffusion measurements are quite challenging in CWs covered by vegetation, because of the difficulties in estimation of gas transfer velocity. Application of the eddy covariance method is not appropriate for most CWs, as it requires a large surface area (> several ha) to avoid contribution of surrounding area and complication of GHG foot printing. A combination of chamber, ebullition and diffusion methods in a single system could minimise the uncertainly in GHG estimation. Methane ebullition measurement was found to be similar to surface emissions by the chamber method, but N_2O and CO_2 ebullition measurements were lower than the surface emissions (Søvik et al., 2006).

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

wetlands (Addy et al., 2002; Kellogg et al., 2005) and in deep groundwater in arable/grassland (Jahangir et al., 2013).

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

8. **Conclusions and Recommendations**

The transformational processes on a mixture of contaminants within and below CWs can cause pollution swapping. A holistic assessment of C and N dynamics in CWs is needed to fully understand their removal, transport and impact on water quality and emissions to atmosphere. Mixed contaminants entering CWs and those formed within and underneath CWs during transformational processes must be considered in future studies. The overall balance of these constituents will 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 the use of multi-disciplinary techniques both *in* and *ex situ* to fully characterise all pathways of C and N loss. At this time we cannot suggest any design optima in terms of nutrient removal and GHG mitigation because empirical information are not yet abundant. To do this, transformation kinetics of C and N and net GHG emissions through all possible pathways are required to provide a holistic assessment. However, a combination of various types of CW and plant types could provide higher removals

534 and lower GHG emissions. A conceptual model highlighting the current state of knowledge in
535 this area and the research gaps is presented in Figure 1.
536
537 Subsurface export of nutrients and GHG to groundwater should be accounted for in CW
538 management. Reducing the saturated hydraulic conductivity below the wetland bed will help
539 reduce nutrients leaching to groundwater. The reactive versus the benign forms of the N
540 transformation products should be evaluated. Data on when, where and the rates at which
541 denitrification, deamox and anammox occur in CWs are needed, as well as identification of the
542 key factors that control such processes. The provenance of NH_4^+ in groundwater below CW cells
543 and its impact on down-gradient receptors needs further elucidation. Constructed wetlands have
544 the potential to produce N_2O, DON, DOC, dissolved inorganic C (DIC), CO_2 and CH_4, which
545 may be exported to fresh waters *via* groundwater and degassed upon discharge to surface waters.
546 Moreover, the DOC and DIC transferred to the fresh water sediments (rivers and lakes) can
547 produce GHG that, in turn, emit to atmosphere. The amount of C and N exported from terrestrial
548 ecosystems *via* the subsurface pathway to fresh waters has been the missing piece of our
549 understanding of global C and N budgets. It is clear that data on the various C and N species,
550 along with the GHG emissions, are crucial to make a robust input-output balance of C and N in
551 CWs. Spatial and temporal variations of GHG emissions in CWs under different management
552 systems are also critical to get much more rigorous estimates of emission factors. These data will
553 reduce the existing uncertainties in global C and N budgets.
554
555 Managing wetting and drying spells (pulsing hydrology) in CWs can enhance NH_4^+ removal.
556 Similarly, oxidation of organic C will increase CO_2 production and, in anaerobic conditions, may
557 be reduced to CH_4. This requires more research into the C and N cycle processes over the wetting
558 and drying spells, which is now possible with the advancement in $^{14}C/^{15}N$ tracing and modelling
559 techniques. The selection of appropriate plant species is important to optimise nutrient removal,
560 sequester C and decrease GHG emissions, but more research is needed across species and
561 geographical locations. Further research is also needed to investigate the impacts of hydraulic
562 retention time on nutrient dynamics. Rates of nutrient accumulation or fixation in soils and their
563 *in situ* transformation in CWs need to be quantified to evaluate their contribution to C
564 sequestration and GHG emissions.
565
566 **Acknowledgements**

The research was funded by Irish Research Council and Department of Agriculture, Food and Marine in Association with The University of Dublin, Trinity College

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 Mineral N (mg N L^{-1}), total nitrogen (TN, mg N L^{-1}) and TN removal (%) in various CWs treating wastewater; average (± standard error, SE) are presented for TN removal; n.a – data not available

CW type	Treatment	N input (mg N L^{-1})			N output (mg N L^{-1})			N removal (%)			References
		TN	NH_4^+	NO_3^-	TN	NH_4^+	NO_3^-	TN	NH_4^+	NO_3^-	
SF_Finland	Municipal	1.4 ± 150	0.03 ± 5.8	0.3 ± 95	1.1 ± 48	0.01 ± 3.0	0.02 ± 6.7	21.4	66.7	93.3	Søvik et al., 2006
SF_Finland	Agril. runoff	66.1 ± 1.9	63.5 ± 1.3	0.7 ± 0.13	64.7 ± 1.7	61.2 ± 1.7	0.3 ± 0.09	2.1	3.6	57.1	Søvik et al., 2006
SF_Norway	Municipal	43.4 ± 3.6	41.5 ± 3.0	0.0 ± 0.0	36.7 ± 2.7	32.6 ± 1.9	0.9 ± 0.4	15.4	21.4	-800	Søvik et al., 2006
SF	Municipal	n.a	4.5	15.5	n.a	n.a	n.a	61	n.a	n.a	Song et al., 2011
SF	Domestic	n.a	40	5	n.a	n.a	n.a	97-98	n.a	n.a	Dzakpasu et al., 2011
SF	Various	n.a	39	4.4	n.a	n.a	n.a	39-48	n.a	n.a	Vymazal, 2007
SF	Municipal	n.a	36		n.a	n.a	n.a	39	n.a	n.a	Vymazal, 2010
SF	Municipal	n.a	196	<2	n.a	n.a	n.a	35	n.a	n.a	Shamir et al., 2001
SF	various	n.a	80	<1	n.a	n.a	n.a	>90	n.a	n.a	Harrington et al., 2007
SF	Municipal	n.a	0.95	1.54	n.a	n.a	n.a	45	n.a	n.a	Toet et al., 2005
SF	Dairy washout	227	n.a	n.a	n.a	n.a	n.a	40	n.a	n.a	Van der Zaag et al., 2010
All SF								31.3 ± 6.3			
HSSF_Estonia	Municipal	96.5 ± 3.0	83.9± 2.7	0.2 ± 0.02	46.2 ±1.5	36.2 ± 1.4	5.9 ± 0.65	52.1	56.9	-2850	Søvik et al., 2006
HSSF_Norway	Municipal	53.4 ± 4.3	38.4 ± 7.7	14.1 ± 7.5	45.0 ± 4.1	43.1 ± 4.7	1.0 ± 0.8	15.7	-12.2	92.9	Søvik et al., 2006
HSSF	Dairy washout	306 ±101*	n.a	n.a	177 ± 58*	n.a	n.a	42.2	n.a	n.a	Van der Zaag et al., 2010
HSSF	Domestic	n.a	74.9	3.9	n.a	n.a	n.a	29	n.a	n.a	O'Luanaigh et al., 2010
HSSF	Domestic	87						46-48			Mander et al., 2008
HSSF	Dairy washout	227						28			Van der Zaag et al., 2010
HSSF	Milk parlour	112	22	n.a	24	11	n.a	78	50	n.a	Kato et al., 2006
HSSF	Agriculture	67	40	0.85	27	11	1.1	47	39	-29	Vymazal and Kröpfelova, 2010
HSSF	Industry	124	65	8.5	103	31	7.4	20	20	8	Vymazal and Kröpfelova, 2010
HSSF	Landfill	157	149	1.5	147	98	1.3	30	33	31	Vymazal and Kröpfelova, 2010
HSSF	Municipal	43	24	2	24	14	1.2	40	30	33	Vymazal and Kröpfelova, 2010
All HSSF								40.4 ± 4.4			
VSSF_Estonia	Municipal	50.9 ± 9.2	35.7 ± 6.2	1.1 ± 0.32	43.1 ± 7.6	31.7 ± 5.5	1.7 ± 0.84	15.3	11.2	-54.5	Søvik et al., 2006
VSSF_Norway	Municipal	52.6 ± 5.2	49.6 ± 4.0	0.0 ± 0.0	47.8 ± 6.9	21.4 ± 6.9	25.5 ± 1.3	9.1	56.9	-25400	Søvik et al., 2006
VSSF	Municipal	41.0 ± 0.5	n.a	n.a	20.7 ± 0.8	n.a	n.a	49.3 ± 1.8	n.a	n.a	Yan et al., 2012
VSSF	Municipal	46 ± 13	n.a	n.a	n.a	n.a	n.a	74 ± 3	n.a	n.a	Zhao et al., 2014
All VSSF								37.0 ± 10.9			

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

Table 2 Total organic C (TOC) removal (%) in various CWs treating wastewater; average (± standard error, SE) are presented for TOC removal; n.a - data not available

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

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

Table 3 Nitrous oxide (N_2O) emissions (mg N m^{-2} d^{-1}); N_2 emissions (mg N m^{-2} d^{-1}) and N_2O emission factor (N_2O/TN input*100) in various type of CWs; mean (± SE) was presented for N_2O emission factor; n.a - data not available

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

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

Table 4 Carbon dioxide (CO_2, mg C m^{-2} d^{-1}), CH_4 (mg C m^{-2} d^{-1}) and CH_4 emission factor (CH_4-C/TOC input*100) in various type of CWs; mean (± SE) was presented for CH_4 emission factor; n.a - data not available

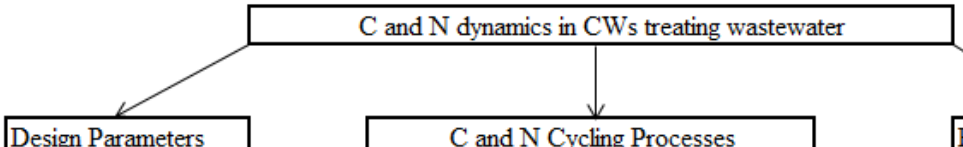
CWs type	Treatment	CO_2 emissions (mg C m^{-2} d^{-1})	CH_4 emissions (mg C m^{-2} d^{-1})	CH_4/ TC (%)	References
SF	Municipal	n.a	5.4	n.a	Tai et al., 2002
SF	Domestic	0.19	n.a	26	Gui et al., 2007
SF	Domestic	1.13	n.a	16	Liu et al., 2009
SF	Agril. drainage	n.a	0.88	31	Wild et al., 2002
SF	Dairy wash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

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

Table 5 Nitrous oxide (N_2O, mg N m^{-2} d^{-1}), CO_2 and CH_4 emissions (mg C m^{-2} d^{-1}) in various type of CWs under different plant types; n.a - data not available

CW type	Wastewater type	Plant type	N_2O (mg N m^{-2} d^{-1})	CH_4 (mg C m^{-2} d^{-1})	CO_2 (mg m^{-2} d^{-1})	Reference
HSF	Secondary	No plant	3.79±2.64	163±209		Johansson et al.,
	treated municipal	*Typha lotifolia*	2.64±4.09	109±185	n.a	2003; Johansson et
		Phalaris arundinacea	3.79±3.44	212±151	n.a	al., 2004
		Glyceria maxima	0.76±1.01	112±178	n.a	
		Lemna minor	1.45±1.18	450±182	n.a	
		Spirogyra sp.	0.98±1.25	107±135	n.a	
HSF	Sewage	No plant	-0.26±2.53	-4.76±61.8	4.32±0.73	Ström et al., 2007
	Treatment water	*Typha atifolia*	4.94±2.00	225±47.7	25.3±4.08	
		Phragmites australis	7.80±2.53	333±76.6	25.1±4.74	
		Juncus effusus	3.87±1.86	489±46.3	26.1±3.00	
HSSF	Domestic	No plant	0.04±0.02	87±6.3	80±6.3	Maltais-Landry et al.,
		Phragmites	0.06±0.03	50±7.5	200±35	2009
		Typha	0.03±0.01	28±3.0	235±32	
		Phalaris	0.01±0.01	45±6.0	195±31	
VSSF	Municipal	*Phragmites australis*	15±3.9	110±35	8400±2100	Søvik et al., 2006
VSSF	Municipal	*Phragmites australis*	264	384		Mander et al., 2005

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



	N_2O	CH_4	CO_2	C-cycling	N-cycling	Fate of C&N
CW type						
SF	decreases	increases	decreases	?	?	?
HSSF	decreases	increases	decreases	?	?	?
VSSF	increases	decreases	increases	?	?	?
Vegetation Type						
Shallow rooted	?	increases	?	?	?	?
Deep rooted	?	decreases	?	?	?	?
Wastewater quality						
C:N ratio	decreases	?	?	?	?	?
NH4+ pollution	decreases	?	?	?	?	?
NO3- pollution	increases	?	?	?	?	?
TOC pollution	?	increases	increases	?	?	?
Soil properties	?	?	?	?	?	?
HLR	increases	?	?	?	?	?
HRT	decreases	?	?	?	?	?
Hydologic pulsing	increases	increase	?	?	?	?

Effects of combination of surface and subsurface flow CWs on C and N cycling processes
Measurement of C and N cycle processes and estimation of their individual rate kinetics in the combined CW system
Effects of CW type and management on microbial community structure and functional gene abundance and diversity in CW soil and water

Effects of vegetation type on the fractionation of SOC (labile vs. stable), nutrient removal and GHG emissions
Investigate *in situ* C, N transformations and GHG production and consumption in soil, subsoil and water column of CWs

Comparative study of denitrification and DNRA in various C:N ratios in different CW types and management regimes
Investigation of soil infiltration rates and their contribution to dissolved C, N and GHG losses to groundwater below Cws
Impact of combined engineering of CW types on NH_4+ oxidation, TN removal and N_2O reduction to N_2.
Towards a complete C, N and GHG balance in Cws by integrating all possible pathways of C and N transformation and movement
Link groundwater quality to CW hydrogeochemistry under various CW types and management regimes

Investigate the impact of various HLR and HRT on C and N transformation and GHG emissions
Investigate the impact of hydrologic pulsing on C and N removal and GHG emissions across CW type and management regime.

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

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