



Carbon and nitrogen gaseous fluxes from subsurface flow wetland buffer strips at mesocosm scale in East Africa



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ABSTRACT

This study investigated carbon (CH₄, CO₂) and nitrogen (N₂O) gaseous fluxes as finger prints for microbial wastewater treatment processes in vertical (VF) and horizontal (HF) subsurface flow mesocosms, planted with *Cyperus papyrus* and operated under batch hydraulic loading. The closed chamber method was used to measure gaseous emissions for 12 weeks (April–June 2014) in Kampala, Uganda. Organic matter (OM) (BOD₅ and COD) and inorganic nitrogen (NH₄⁺ and NO₃⁻) nutrient concentrations were monitored to estimate OM degradation rates and potential nitrification and denitrification. The highest mean CH₄ flux (mg CH₄–C m⁻² h⁻¹) was 38.3 ± 3.3 in unplanted HF compared to the lowest (3.3 ± 0.4) established in planted VF mesocosms. CO₂ fluxes (mg CO₂–C m⁻² h⁻¹) were significantly higher (*P* < 0.05) in planted mesocosms, with no significant difference (*P* > 0.05) between the planted HF (2213.5 ± 122.4) and VF (2272.8 ± 191.0) mesocosms. The high CO₂ flux was attributed to efficient degradation of the inflow organic carbon facilitated by sufficient oxygen supply especially in the planted mesocosms. Although N₂O flux was relatively higher in HF mesocosms, it did not vary significantly (*P* > 0.05) in all treatments. Generally the results indicated significant nitrification, especially in the planted mesocosms. However, high fluxes of N₂O comparable to other denitrifying CWs suggested potential for coupled nitrification and denitrification in these systems. Overall, compared to CH₄ and N₂O, CO₂ was found to be the most significant gaseous flux under induced aerobic conditions enhanced by use of *C. papyrus* plants and an intermittent loading regime.

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1. Introduction

Use of subsurface flow (SSF) constructed wetlands (CWs) regarding removal of organic matter (OM) and nitrogen (N) from

wastewater has attracted enormous research attention with varying degree of full scale application on a global scale (Kivaisi, 2001; Vymazal, 2007; Wu et al., 2014). The OM degradation from wastewater is essential in mitigating the effects of organic pollution (Metcalf and Eddy, 2004), especially oxygen depletion in receiving aquatic environments (Saeed and Sun, 2012). On the other hand, N removal is critical to abate the increasing eutrophication problem (Rast and Thornton, 1996; Srivastava et al., 2008) as observed in the East African region (Cózar et al., 2007; Machiwa, 2003; Nyenje et al., 2012).

The functioning of SSF CWs regarding OM and N transformation and removal processes has been associated with the dynamic interaction between macrophytes, substrate and microbial community established therein (Leto et al., 2013; Samsó and García, 2013;

Abbreviations: DO, dissolved oxygen; EF, emission factor; F, gas flux; HF, horizontal subsurface flow; VF, vertical subsurface flow; OM, organic matter; WBS, wetland buffer strip.

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Truu et al., 2009). Besides influencing a number of essential physical processes such as hydraulic retention, filtration and sedimentation (Kadlec and Wallace, 2009); macrophytes can enhance microbial activity through oxygen transfer into the rhizosphere, release of organic exudates and complex biofilm development (Salomo et al., 2009; Zhang et al., 2010a). In addition, high primary production of macrophytes throughout the annual cycle in warm tropical climates has been reported to enhance significant N uptake and carbon sequestration (Kansiime et al., 2007b; Kivaisi, 2001; Neue et al., 1997; Saunders et al., 2007). Consequently, various studies comparing planted and unplanted SSF CWs have generally indicated relatively higher efficiency of OM degradation and N elimination rates in planted systems (Abou-Elala et al., 2013; Canga et al., 2011; Saeed and Sun, 2012; Vymazal, 2007; Zhang et al., 2010b).

Microbial activity in SSF CWs is the main driver for OM degradation and nutrient removal processes including but not limited to N elimination as highlighted by various studies (Faulwetter et al., 2009; Saeed and Sun, 2012; Yeh et al., 2010). Microbial bioremediation of pollutants, including OM and N, is enzymatically driven, and its effectiveness is influenced by environmental conditions which enhance microbial activity (Dunn et al., 2014; Karigar and Rao, 2011; Salomo et al., 2009). Some of the key environmental conditions influencing microbial activity in SSF CWs include pH, temperature, oxygen availability hence redox potential, substrate structure and hydraulics, presence of plants and/or species richness, quantity and quality of carbon supply, nutrient levels, bioavailability of contaminants and presence of toxic compounds (Dunn et al., 2014; Faulwetter et al., 2009; Karigar and Rao, 2011; Salomo et al., 2009; Truu et al., 2009).

Various studies have provided significant evidence that the presence of macrophytes in CWs play a major role in enhancing enzyme activity (Kong et al., 2009; Zhang et al., 2010b) through excretion of exogenous enzymes in exudates and provision of aerobic conditions for biogeochemical processes (Faulwetter et al., 2009; Kong et al., 2009; Saeed and Sun, 2012). For example, Kong et al. (2009) found a significant positive correlation ($P > 0.001$) between enzyme activity of urease and cellulase with efficient removal of total nitrogen (TN) and chemical oxygen demand (COD), respectively, in CWs planted with *Cyperus flabelliformis*. Also, Zhang et al. (2010b) established that plant species richness enhanced enzyme activity (β -glucosidase and invertase for carbohydrates decomposition, urease for hydrolysis of urea, nitrate reductase for denitrification and Protease for protein degradation) coupled with significant nutrient retention and OM degradation in vertical sub-surface flow CWs.

Following the aforementioned evidence, technological innovations focused on optimizing environments which promote microbial mediated processes to enhance treatment efficiency have been prioritized (Saeed and Sun, 2012; Wu et al., 2014). More specifically, oxygen availability and its distribution as a function of hydraulics and hydrology (Kadlec and Wallace, 2009; Langergraber et al., 2009), in the treatment beds of horizontal flow (HF) and vertical flow (VF) SSF-CWs is of great interest, since it influences the prevalence of aerobic or anaerobic conditions (Maltais-Landry et al., 2009b; Wu et al., 2014), with significant implications to the development of microbial communities and dominant processes therein (Langergraber et al., 2009; Meng et al., 2014; Saeed and Sun, 2012; Samsó and García, 2013).

Whereas aerobic environments and processes are predominant in VF beds, HF systems are mainly anaerobic (Kadlec and Wallace, 2009; Langergraber et al., 2009; Vymazal, 2007). Therefore, in order to enhance aerobic microbial processes which are considered to be limiting regarding N transformation through nitrification (Faulwetter et al., 2009; Meng et al., 2014; Saeed and Sun, 2012), interventions such as; effluent recirculation, artificial aeration, tidal operation, drop aeration and the traditional use of macrophytes

have been tested and implemented (Vymazal, 2013; Wu et al., 2014). Moreover the current priority is focused on advancement in coupling aerobic-anaerobic environments to optimize N elimination through nitrification and denitrification processes (Ávila et al., 2013a,b; Langergraber et al., 2011; Meng et al., 2014; Vymazal, 2013).

Nitrification mainly proceeds in presence of oxygen under aerobic environments (Crites et al., 2006; Metcalf and Eddy, 2004), and forms the main mechanism for ammonium nitrogen ($\text{NH}_4^+\text{-N}$) removal from wastewater. This process is aided by a synergy of ammonium oxidizing bacteria (AOB, *Nitrosomonas* spp.) which converts ammonium (NH_4^+) to nitrite (NO_2^-) and nitrate oxidizing bacteria (NOB, *Nitrobacter* spp.) which completes the oxidation of NO_2^- to nitrate (NO_3^-) (Faulwetter et al., 2009; Saeed and Sun, 2012; Wu et al., 2014). On the other hand, denitrification is regarded as the main total N elimination mechanism in CWs (Kadlec and Wallace, 2009; Saeed and Sun, 2012; Vymazal, 2007). It generally proceeds under anaerobic conditions in which NO_3^- is converted to nitrogen gaseous products, mainly N_2 (nitrogen gas) and N_2O (nitrous oxide) (Faulwetter et al., 2009; Gutknecht et al., 2006; Huang et al., 2013; Metcalfe et al., 2007). Microbial studies indicate that the most common denitrifying microbial genera include; *Bacillus*, *Micrococcus* and *Pseudomonas* in soils and sediments whereas *Pseudomonas*, *Aeromonas* and *Vibrio* dominate aquatic environments (Faulwetter et al., 2009). In addition, NO_2^- and NH_4^+ can be converted to N_2 through the anammox reaction pathway (Kadlec and Wallace, 2009; Metcalf and Eddy, 2004; Saeed and Sun, 2012) facilitated by *Candidatus Brocadia anammoxidans* and *Candidatus Kuenenia stuttgartiensis* under anaerobic conditions (Faulwetter et al., 2009).

OM degradation also occurs in both aerobic and anaerobic environments facilitated by a consortium of microbial species through different biogeochemical pathways. Under aerobic environments, OM degradation by aerobic heterotrophic bacteria proceeds faster through oxidation to release carbon dioxide (CO_2) as one of the main by-products (Faulwetter et al., 2009; Saeed and Sun, 2012; Wu et al., 2014). On the contrary, anaerobic heterotrophic bacteria dominate anaerobic SSF beds (Faulwetter et al., 2009), and gradually degrade OM through coupled fermentation and methanogenesis processes to release methane (CH_4) as one of the main carbon gaseous by-products (Gutknecht et al., 2006; Liikanen et al., 2006; Mitsch et al., 2010).

The intensification of full scale SSF CWs performance has ultimately resulted into increased efficiency (Meng et al., 2014; Wu et al., 2014) regarding N and OM removal especially in Europe and North America (Canga et al., 2011; Vymazal, 2007; Wu et al., 2015), with only a few cases of progress reported in other regions such as Asia (Canga et al., 2011; Jin et al., 2014) and South America (Zurita et al., 2012). With the current global agenda toward low carbon development as a mechanism for climate change mitigation and adaptation (Mulugetta and Urban, 2010), CWs are vital wastewater treatment technological options especially in developing countries, since they are less energy intensive compared to the conventional technical systems (Langergraber, 2013; Saeed and Sun, 2012). Besides the limited external energy inputs, SSF CWs provide other complementarily benefits for low economies like East Africa. These include among others; the low investment and operational costs (Langergraber, 2013; Langergraber and Muellegger, 2005) and abatement of the increasing surface and ground water pollution especially in urban areas (Bateganya et al., 2015b; Katukiza et al., 2015; Kulabako et al., 2007; Nsubuga et al., 2004).

The aforementioned benefits notwithstanding, various studies have indicated that CWs can potentially be carbon sinks due to efficient sequestration mechanisms or sources with significant CO_2 and CH_4 emissions (de Klein and van der Werf, 2014; Mander et al., 2008) depending on the hydrological regime and ultimately

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