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Graphical Abstract
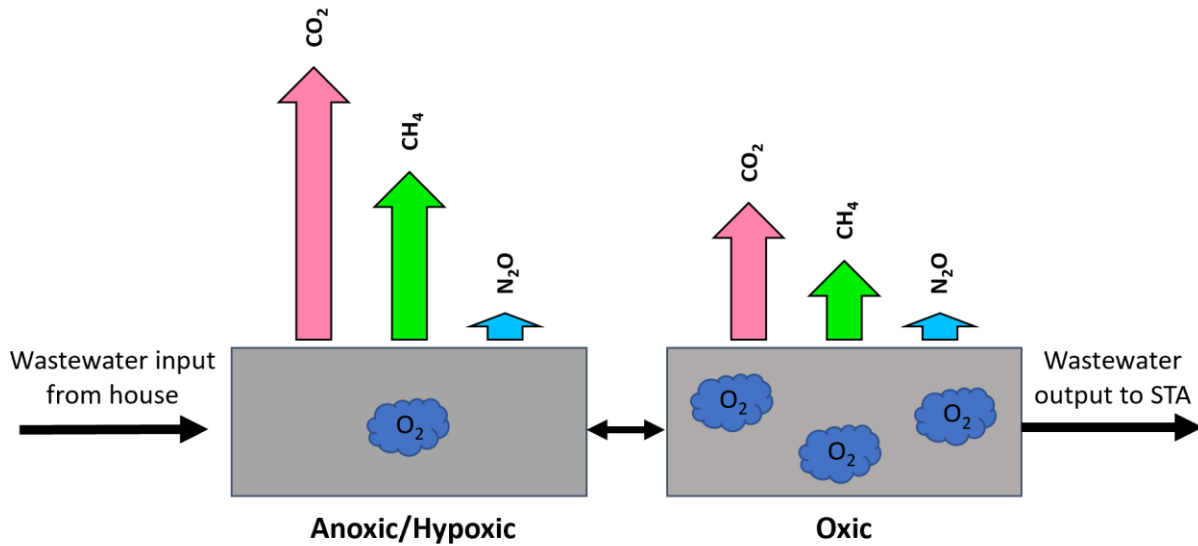
Abstract

Advanced onsite wastewater treatment systems (OWTS) designed to remove nitrogen from residential wastewater play an important role in protecting environmental and public health. Nevertheless, the microbial processes involved in treatment produce greenhouse gases (GHGs) that contribute to global climate change, including CO_2, CH_4, N_2O. We measured GHG emissions from 27 advanced N-removal OWTS in the towns of Jamestown and Charlestown, Rhode Island, USA, and assessed differences in flux based on OWTS technology, home occupancy (year-round *vs*. seasonal), and zone within the system (oxic *vs*. anoxic/hypoxic). We also investigated the relationship between flux and wastewater properties. Flux values for CO_2, CH_4, and N_2O ranged from -0.44 to 61.8, -0.0029 to 25.3, and -0.02 to 0.23 μmol GHG m^{-2} s^{-1}, respectively. CO_2 and N_2O flux varied among technologies, whereas occupancy pattern did not significantly impact any GHG fluxes. CO_2 and CH_4 – but not N_2O – flux was significantly higher in the anoxic/hypoxic zone than in the oxic zone. Greenhouse gas fluxes in the oxic zone were not related to any wastewater properties. CO_2 and CH_4 flux from the anoxic/hypoxic zone peaked at ~22-23°C, and was negatively correlated with dissolved oxygen levels, the latter

suggesting that CO_2 and CH_4 flux result primarily from anaerobic respiration. Ammonium concentration and CH_4 flux were positively correlated, likely due to inhibition of CH_4 oxidation by NH_4^+. N_2O flux in the anoxic/hypoxic zone was not correlated to any wastewater property. We estimate that advanced N-removal OWTS contribute 262 g CO_2 equivalents capita^{-1} day^{-1}, slightly lower than emissions from conventional OWTS. Our results suggest that technology influences CO_2 and N_2O flux and zone influences CO_2 and CH_4 flux, while occupancy pattern does not appear to impact GHG flux. Manipulating wastewater properties, such as temperature and dissolved oxygen, may help mitigate GHG emissions from these systems.

Introduction

Wastewater can be a major source of nitrogen (N) to groundwater and coastal waters. Decentralized, advanced N-removal onsite wastewater treatment systems (OWTS) are used to mitigate the impact of excess N from wastewater on receiving waters, which includes eutrophication, fish and shellfish kills, and threats to public health (Carpenter et al., 1998; Sohail & Adeloju, 2016; Ward et al., 2005). Advanced OWTS remove N by cycling wastewater through a treatment train that includes oxic and anoxic/hypoxic zones that promote successive nitrification (microbial oxidation of NH_4^+ to NO_3^-) and denitrification (microbial reduction of NO_3^- to N_2O and N_2). When denitrification proceeds to completion, NO_3^- is reduced to harmless N_2; however, incomplete denitrification produces N_2O, a potent greenhouse gas (GHG) with 265 times the global warming potential of CO_2 (Jones et al., 2013; Tomaszek and Czarnota, 2015; Wrage et al., 2001). Advanced N-removal OWTS also emit CO_2, which is produced by microbial respiration – including processes that remove organic matter – and methane (CH_4), which is produced by Archaea under anoxic conditions (Kong et al., 2016).

Greenhouse gas emissions from centralized wastewater treatment plants (WTPs) have been quantified (Cakir & Stenstrom, 2005; Foley et al., 2010; Kong et al., 2016; Parravicini et

al., 2016). Nationwide, the U.S. Environmental Protection Agency (USEPA) estimates that

centralized WTPs treating domestic wastewater in the U.S. contribute 9.2 and 4.9 million metric

tons of CO_2 equivalents of CH_4 and N_2O, respectively, to the atmosphere every year (USEPA,

2015). In 2010, domestic wastewater treatment at WTPs accounted for 1.4% of U.S. N_2O

emissions, and domestic and industrial wastewater treatment combined accounted for 2.4% of

CH_4 emissions (USEPA, 2015). Assessment of GHG emissions from WTPs typically excludes

CO_2 due to its biogenic origins (Doorn et al., 2006). However, recent studies have shown that

non-biogenic CO_2 emissions from various C-containing household and personal care products

can significantly contribute to GHG emissions from WTPs, and should be included in emissions

analysis (Griffith, et al., 2009; Law et al., 2013; Tseng et al., 2016).

Unlike WTPs, few studies have quantified GHG emissions from OWTS, which serve

nearly one quarter of households in the U.S. (U.S. Census Bureau, 2017). Diaz-Valbuena et al.

(2011) investigated CO_2, CH_4, and N_2O emissions from conventional OWTS – consisting of a

septic tank where gravity-separation of wastewater solids takes place, and the clarified effluent is

treated below ground using soil-based treatment – and found net emission of all three GHGs at

all study sites, with average values (g capita^{-1} day^{-1}) of 33.3 for CO_2, 11 for CH_4, and 0.005 for

N_2O. Truhlar et al. (2016) assessed CO_2, CH_4, and N_2O emissions from various components of a

conventional OWTS treatment train, including the roof vent (which releases gases generated in

the septic tank to the atmosphere) and the soil treatment area (STA). They also found net

emission of GHGs from all components, with 0.17 and 0.050 tonnes CO_2 equivalents capita^{-1}

year^{-1} from the roof vent and STA, respectively. Somlai-Haase et al. (2017) found that the STA

for a conventional OWTS emitted 15.0 kg more CO_2 per year than a similarly sized control area

that received no wastewater inputs, further highlighting the contribution of OWTS to global GHG emissions.

Emissions of N_2O from advanced N-removal OWTS were first quantified by Brannon et al. (2017). They compared emissions of N_2O from nine advanced N-removal OWTS – representing three different technologies – to emissions from a WTP with a biological N removal (BNR) component that also relies on successive nitrification and denitrification. They found that the flux of N_2O ranged from -4×10^{-3} to 3×10^{-1} µmol N_2O $m^{-2}\,s^{-1}$ and was typically higher for the WTP, followed by the three advanced N-removal treatment technologies. However, when emissions were normalized by treatment tank area and number of people served, the ranges of emissions for the WTP and the advanced N-removal technologies overlapped. Carbon dioxide equivalents – calculated based on N_2O emissions from the N-removal OWTS – ranged from 1.6 $\times 10^{-3}$ to 8×10^{-2} tonnes CO_2 equivalents capita^{-1} yr^{-1}.

As the world's population increases, the number of OWTS will also increase (Amador & Loomis, 2018). The relatively low cost of installation and limited maintenance required make them viable wastewater treatment options, especially in areas with low population densities. Advanced N-removal OWTS will be particularly critical in addressing eutrophication of coastal ecosystems and groundwater contamination. Because wastewater treatment produces GHGs, we need to consider whether their use shifts the pollution stream from ground and surface waters to the atmosphere. As such, we need to develop a better understanding of the magnitude and composition of GHG emissions from OWTS – including advanced N-removal systems – and the factors that control them.

We quantified the flux of CO_2, CH_4, and N_2O from five different advanced N-removal OWTS technologies in the towns of Charlestown and Jamestown, Rhode Island, USA:

SeptiTech® Series D, Orenco Advantex® AX20, Orenco Advantex® RX30, BioMicrobics MicroFAST®, and Norweco Singulair®. We measured GHG flux in the summer and fall of 2016, and summer and winter of 2018. Because differences in home occupancy pattern may drive differences in microbial activity and the GHGs produced by this activity, we assessed the relationship between home occupancy patterns and GHG emissions by sampling systems used year-round and systems only used during the summer season. GHG emissions result from microbial processes that respond to environmental conditions and availability of nutrients, electron acceptors, and organic C, and thus we assessed the relationship between gas emissions and effluent temperature, pH, and dissolved oxygen (DO), as well as effluent 5-day biochemical oxygen demand (BOD_5; a proxy for organic C), ammonium, nitrate, and total N concentration. We also quantified emissions per capita in terms of CO_2 equivalents to allow for comparison with other types of wastewater treatment.

Methods

Study Systems and Measurement Locations

We measured CO_2, CH_4, and N_2O emissions, and determined wastewater properties from a total of 27 advanced N-removal OWTS serving single-family homes in the towns of Jamestown and Charlestown, Rhode Island, USA. Sites were chosen in collaboration with the Rhode Island Department of Environmental Management (RIDEM) and the Office of Wastewater Management in each town. All the sites included in this study rely on well water for potable water and discharge effluent to a low-pressure dosed soil treatment area using a pump. Sites were selected based on adherence to these criteria, as well as the homeowners' willingness to participate in the study. Data from Jamestown sites were collected in June and October of 2016, and data from Charlestown sites were collected in July/August and December of 2018.

We sampled 9 systems in Jamestown (3 SeptiTech® Series D, 3 Orenco Advantex® AX20, and 3 BioMicrobics MicroFAST®), and 18 sites in Charlestown (6 Orenco Advantex® AX20, 6 Orenco Advantex® RX30, 3 BioMicrobics MicroFAST®, and 3 Norweco Singulair® (models 960 and DN)). All 9 systems sampled in 2016 served homes occupied year-round. Of the systems sampled in 2018, 11 served homes occupied year-round and 7 served seasonally-occupied homes (June to September). Sampling from systems occupied seasonally and year-round allowed us to examine differences due to home occupancy. We assessed occupancy pattern based on daily flow data obtained from each system, as well as homeowner self-identification (Ross et al., 2020a).

Although specific design configurations vary by technology, all of the systems in this study have an oxic zone for nitrification (referred to as H-OX) and an anoxic/hypoxic zone for denitrification (referred to as L-OX) (see Supplemental Materials for individual technology designs/descriptions). On every sampling event we measured the flux of GHG and obtained a sample of wastewater from the oxic (H-OX) and anoxic/hypoxic (L-OX) zones of the treatment train. To avoid impacting GHG emissions from disturbance of the wastewater, GHG flux measurements were made prior to wastewater sampling.

Greenhouse Gas Flux Measurements

At each study site CO_2, CH_4, and N_2O flux measurements were made using a closed chamber connected to a Gas Concentration Analyzer (Model G2518; Picarro, Inc., Santa Clara, CA), which uses cavity ring-down spectroscopy to measure GHG concentration every 2 seconds, providing real-time flux measurements in the field (Crosson, 2008). To measure gas flux, we placed an open-bottom cylindrical PVC chamber (0.13-m internal dia., 0.40-m length) on the

water so that the bottom of the chamber was submerged 7.5 cm below the water surface (Brannon et al., 2017). Air temperature inside the chamber was measured every 10 seconds using a Hobo® data logger (Onset, Bourne, MA). To maintain a consistent submersion depth, the chamber was equipped with a stabilizing bar that rested across the top of the system's access riser. The chamber was deployed for 5 to 10 minutes at each site, providing ample time for gas emissions to travel between the system and the gas analyzer (Brannon et al., 2017). The concentration of each gas was plotted against time and the data fitted using a linear regression as part of the flux calculation. An example of the increase of GHG concentrations over time can be found in the Supplemental Materials. We used R statistical software to determine the statistical significance of each gas flux value (R Core Team, 2017), as described in Brannon et al. (2017). GHG flux values below the detection limits of the instrument, and those with a p value > 0.05 for the slope of the linear regression, were assigned a value of zero. Fluxes above the upper detection limits of the instrument were excluded from our analysis. We calculated per capita CO_2 equivalents (CO_2e) as described in Brannon et al. (2017) using global warming potential values published by the IPCC (2014).

Wastewater Analysis

Effluent collected from each zone was transferred to a clear, 1-L plastic bottle. Part of the sample was used for *in situ* determination of temperature, pH and dissolved oxygen concentration (DO), and the remainder was stored in the dark at 4^oC and transported to the laboratory for analysis (within 8 h of sampling). A Hanna Instruments HI9828 Multiparameter Meter (Woonsocket, RI) was used to determine effluent temperature, pH, and DO. Upon arrival at the laboratory, effluent was analyzed for BOD_5 with an OxiTop BOD_5 measurement system

(WTW, Weilheim, Germany). Ammonium, nitrate, and total N were analyzed using standard laboratory colorimetric analysis (Ross et al., 2018). Values below the detection limit of a method were assigned a value of zero. A summary of wastewater properties for each zone can be found in the Supplemental Materials (Table S1).

Statistical Analyses

We used R (R Core Team, 2017) to perform a one-way ANOVA on ranks to examine statistical differences in GHG fluxes among technologies, and a Mann-Whitney U test to assess flux differences between zones (L-OX *vs*. H-OX) and between home occupancy patterns (seasonal *vs*. year-round). Dunn's test was used for ANOVA post-hoc analysis. In data represented by box and whisker plots, the solid line in the middle of each box represents the median and the edges of each box represents the first and third quartiles. Box whiskers extend $1.5 \times$ the inter-quartile range beyond the edges of the box and the dots represent outliers beyond $1.5 \times$ the inter-quartile range.

We used SigmaPlot v11.0 and Microsoft Excel to carry out linear and nonlinear regression analyses to assess the relationship between wastewater parameters and GHG fluxes. We added 1 as necessary to flux, DO, and nitrate measurements to eliminate negative and zero values, which preclude the use of some nonlinear regression models. We used a *p* value of less than or equal to 0.05 as a measure of statistical significance.

Results and Discussion

Carbon dioxide flux

Across all technologies, zones, occupancy patterns, and sampling events, CO_2 flux ranged from -0.44 to 61.8 µmol CO_2 m^{-2} s^{-1} ($n = 78$) (Fig. 1). The Advantex AX20 systems

produced the highest median CO_2 flux (μmol CO_2 m^{-2} s^{-1}) (range of -0.23 to 61.8; $n = 24$),

followed by Norweco Singulair (range of -0.44 to 20.42; $n = 6$), Advantex RX30 (range of 0 to

33.64; $n = 19$), BioMicrobics MicroFAST (range of 0.1 to 10.62; $n = 18$), and SeptiTech (range

of 0 to 5.32; $n = 11$). Negative CO_2 flux values were observed in two systems, likely due to the

alkaline pH of effluent (8.51 and 7.38) in these systems, which promotes the dissolution of

atmospheric CO_2 in water (Renforth, 2019).

The flux of CO_2 varied significantly by technology, with AX20 systems producing

significantly higher CO_2 emissions than FAST systems, suggesting that system design – which

differs considerably between AX20 and FAST systems (Supplemental Materials) – may

influence CO_2 emissions. FAST systems rely on fixed activated sludge for advanced effluent

treatment and are socially-dosed, while AX20 systems utilize a textile filter and are time-dosed.

Variations in system dosing mechanism and treatment train design may drive differences in the

microbial activity responsible for CO_2 emissions.

When values for all technologies were considered, CO_2 emissions were significantly

higher in L-OX (anoxic/hypoxic zone) than in H-OX (oxic zone) (Fig. 2). L-OX typically

contains significantly higher BOD$_5$ concentrations than H-OX, which can fuel anaerobic

respiration in L-OX, where anoxic/hypoxic conditions prevail. The lower amount of organic

substrate available for aerobic respiration in H-OX may result in lower CO_2 emissions.

When we considered values across all technologies, CO_2 flux did not differ based on

home occupancy pattern (Fig. 3). Studies by Ross et al. (2020) found that home occupancy

pattern did not impact treatment performance (Ross et al., 2020a) or microbial community

structure or composition (Ross et al., 2020b). These results suggest that the seasonally-used

systems' microbial communities are robust enough to endure months of not receiving wastewater inputs without compromising their biological activity, as evidenced by similar fluxes of CO_2.

Methane Flux

Across all technologies, zones, home occupancy patterns, and sampling events, CH_4 flux ranged from -0.0029 to 25.3 µmol CH_4 m^{-2} s^{-1} ($n = 79$) (Fig. 1). The Advantex RX30 systems produced the highest median CH_4 flux (µmol CH_4 m^{-2} s^{-1}) (0 to 25.3: $n = 19$), followed by Norweco Singulair (0 to 13.4; $n = 6$), Advantex AX20 (0 to 24.7; $n = 25$), SeptiTech (0 to 2.78; $n = 11$), and Biomicrobics MicroFAST (0 to 8.42; $n = 18$). Flux did not differ significantly among technologies or between occupancy patterns (Fig. 3), but L-OX consistently produced significantly higher CH_4 fluxes than H-OX (Fig. 2). Yan et al. (2014) investigated GHG emissions from WTPs employing various methods of wastewater treatment. They found that for all methods assessed, CH_4 emissions were significantly higher in the "grit tank" of the treatment trains, which serves a similar purpose as that of the anoxic/hypoxic zone of advanced N-removal OWTS (L-OX), than those from the oxic zones.

Nitrous Oxide Flux

Across all technologies, zones, home occupancy patterns, and sampling events, N_2O flux ranged from -0.02 to 0.23 µmol N_2O m^{-2} s^{-1} ($n = 78$; Fig. 1). Seventeen N_2O flux values were either statistically insignificant or below the instrument's detection limit, and were thus assigned a value of 0. The Norweco Singulair systems produced the highest median N_2O flux (µmol N_2O m^{-2} s^{-1}) (range of -0.01 to 0.1; $n = 6$), and exhibited far more variability than the other technologies, all of which had a median N_2O flux of 0: Advantex AX20 ($n = 24$) had a range of -

0.01 to 0.07; Biomicrobics MicroFAST (n = 18) had a range of -0.02 to 0.23; Advantex RX30 (n = 19) had a range of 0 to 0.03; and the range for SeptiTech (n = 11) was 0 to 0.01. The variability observed in flux measurements from Norweco systems may be due to intermittent on/off cycles of aeration to facilitate successive nitrification and denitrification, and when we sampled within that cycle.

Nitrous oxide flux varied significantly by technology, with RX30 systems producing significantly higher N_2O emissions than SeptiTech systems, suggesting that system design, which differs considerably between these two technologies (Supplemental Materials), may influence N_2O flux. Although both technologies utilize textile filters for advanced effluent treatment, they differ in the type of media material used, as well as in wastewater dosing mechanism. As was the case for differences in dosing mechanism between AX20 and FAST systems (which differed significantly in their CO_2 emissions), RX30 systems are time-dosed while SeptiTech systems are socially-dosed. The porous nature of the textile material and the time dosing employed by RX30 systems may enhance N2O production by denitrification and/or nitrification.

System zone did not significantly impact N_2O flux (Fig. 2) indicateing that processes that produce N_2O, such as nitrification and incomplete denitrification, take place to the same extent in both zones. Wigginton et al. (2020) found that the structure and composition of communities of ammonia oxidizing and denitrifying bacteria in these two zones in nine advanced N-removal OWTS did not differ significantly, suggesting a similar potential for N_2O production via these processes. As was the case for the other two gases, N_2O flux was not influenced by home occupancy pattern, mirroring the fact that the structure and composition of the microbial communities of seasonal and year-round systems were similar (Fig. 3).

Our values are comparable to those reported by Brannon et al. (2017) of -0.004 to 0.3 μmol N_2O m^{-2} s^{-1}, which included nine of the same advanced N-removal OWTS sampled in this study. Negative N_2O fluxes were reported from a BNR wastewater treatment plant by Brannon et al. (2017), and net N_2O consumption has been observed in soil and aquatic environments (Beaulieu et al., 2015; Chapuis-lardy et al., 2007; Soued et al., 2016). The final step of denitrification consumes N_2O as it is reduced to N_2, which takes place under hypoxic and anoxic conditions when nitrate is available as the terminal electron acceptor (Chapuis-Lardy et al., 2007). Because complete denitrification increases with decreased DO, the amount of N_2O produced from incomplete denitrification is largely dependent on DO. Six of the 7 negative N_2O values came from L-OX, the anoxic/hypoxic zone, suggesting that DO concentration played an important role in controlling N_2O emissions from OWTS.

Relationship between GHG flux and wastewater properties

Understanding the relationships between effluent properties and GHG flux may be useful in controlling GHG emissions from advanced N-removal OWTS while also maintaining effective treatment. To this end, we performed regression analyses between wastewater properties and GHG flux using data across technologies, occupancy patterns, and sampling events, allowing us to examine the relationship between wastewater properties and flux at a broader scale. Because there were clear differences between zones in wastewater properties and GHG flux values, we performed separate analyses for L-OX and H-OX.

No relationships were found between any wastewater properties and GHG flux in H-OX, the oxic zone. H-OX contains little organic matter and produced very low CO_2 and CH_4 fluxes in comparison with L-OX. In addition, we found no significant relationships between N_2O flux

and any wastewater property. N_2O flux values were typically very low, which likely made it difficult to identify a relationship if one exists.

The flux of CO_2 and CH_4 in the anoxic/hypoxic zone was correlated with a number of wastewater properties (Fig. 4). There was a positive linear relationship between the BOD_5 concentration and both CO_2 and CH_4 flux (Fig. 4), in line with the expectation that organic C in wastewater serves as substrate for heterotrophic microbial respiration. The relationship between flux of CO_2 and CH_4 and wastewater temperature was similar for both gases (Fig. 4). The flux increased with increasing temperature, peaking at 22-23°C, and declining at higher temperatures, following a typical response of microbial activity to temperature (Apple et al., 2006).

There was an inverse relationship – best described by a negative power function – between DO levels and the flux of CH_4 and CO_2 (Fig. 4). This indicates that, as expected, emissions of these two gases in the anoxic/hypoxic zone result from anaerobic respiration processes, including methanogenesis. Acetoclastic methanogenesis contributes significantly to anaerobic respiration in anoxic wastewater treatment environments (Qiao et al., 2015). The process produces equimolar amounts of CH_4 and CO_2. Linear regression analysis comparing CH_4 flux with CO_2 flux in L-OX confirmed this 1:1 relationship (slope = 1.2; intercept = 3.5; R^2 = 0.32), suggesting that acetoclastic methanogenesis is an important driver of CH_4 and CO_2 emissions. Anaerobic respiration processes are sensitive to the introduction of O_2, resulting in inhibition at DO levels greater than 2 mg/L (John, 1977; Hernandez & Rowe, 1988; Vaquer-Sunyer & Duarte, 2008). The presence of O_2 interferes with methanogenesis through at least two mechanisms: (i) by favoring aerobic metabolic processes, which have a more favorable energy yield than CO_2, and (ii) through toxic effects on methanogenic Archaea, which are obligate anaerobes (Mer et al., 2001).

We also observed an inverse relationship between CO_2 and CH_4 flux and nitrate concentration (Fig. 4). Others have reported negative effects of N on CO_2 and CH_4 flux. For example, the flux of CO_2 from agricultural soil decreases in response to amendments with inorganic N (Ramirez et al., 2010), and Anderson et al. (2019) found that nitrate addition to soil amended with anoxic septic tank effluent lowered the flux of both CO_2 and CH_4. Nitrate is potentially toxic to some methanogens (Bollag & Czlonkowski, 1973; Klüber & Conrad, 1998), and its favorable energy yield as a terminal electron acceptor relative to CO_2 can suppress methanogenesis. The inverse relationship between nitrate concentration and the flux of CO_2 and CH_4 in the anoxic/hypoxic zone may also reflect the intermittent introduction of aerated wastewater with a high concentration of DO from the oxic zone via recirculation.

Methane flux increased linearly with ammonium concentration, but there was no clear relationship between ammonium level and CO_2 flux (Fig. 4). The flux of CH_4 is the net result of gross methane production by methanogenic Archaea and gross consumption by methanotrophic bacteria. Ammonium is a competitive inhibitor of methane monooxygenase, the enzyme used by methanotrophs to oxidize CH_4 (Norton et al., 2008; O'Neill & Wilktnson, 1977; Schnell & King, 1994). Thus, as the concentration of NH_4^+ in wastewater increases, we would expect greater inhibition of CH_4 oxidation and an increase in CH_4 flux, assuming gross rates of methanogenesis remain constant.

The apparent response of CH_4 and CO_2 emissions to wastewater properties suggests that changes in some of these properties may be used to control the flux of these gases. For example, maintaining system temperature below 22-23°C – through the use of insulation around and over treatment compartments – may help limit CH_4 and CO_2 flux, provided the temperature is not so low that it interferes with performance. Increasing DO levels in wastewater – by changing

recirculation ratios and/or mechanical aeration – could help also help mitigate CO_2 and CH_4 emissions from these systems. This would also increase nitrate levels, which may also curb emissions of these gases. However, the effluent would have to maintain oxygen levels low enough to allow for denitrification to take place to maintain effective N removal. Limited introduction of oxygen into the anoxic/hypoxic zone may also promote ammonia oxidation, reducing its inhibitory effect on methane oxidation. In contrast, although emissions increase with increasing concentration of BOD_5, the latter is a function of homeowner lifestyle and cannot be controlled by changes in system operation. Manipulation of BOD_5 levels is thus not a practical management strategy for limiting CH_4 or CO_2 emissions.

CO_2 Equivalents and Comparisons to Other Systems

Greenhouse gases differ in global warming potential (GWP) due to differences in their residence time and reactivity in the atmosphere. According to the Intergovernmental Panel on Climate Change's Fifth Assessment (2014), CH_4 and N_2O have 28 and 265 times the GWP of CO_2, respectively. Converting GHG flux values into CO_2 equivalents (CO_2e) accounts for these differences in GWP and allows us to assess the relative impact of individual gas fluxes on the atmosphere, and to compare emissions from advanced N-removal OWTS those from other wastewater treatment technologies. We calculated per capita emissions (CO_2e; Brannon et al., 2017)f or each gas and for the sum of the three gases based on: (i) the average flux from L-OX and H-OX at each system across all sampling events, (ii) the cross-sectional area of the tank, and (iii) the assumption that each system serves a household of 3 people (Amador et al., 2018). Advanced N-removal OWTS contributed a median of 40, 176, and 9 g CO_2e capita^{-1} day^{-1} for CO_2, CH_4, and N_2O, respectively (Fig. 5), with methane emissions accounting for a the bulk of

the impact of these systems on the atmosphere. The median sum of emissions from advanced N-removal systems based on our data is 262 g CO_2e capita^{-1} day^{-1} (Fig. 5). This is slightly below the range of values reported by Diaz-Valbuena et al. (2011) of 277 g CO_2e capita^{-1} day^{-1} for a conventional OWTS, and by Truhlar et al. (2016), who found that the roof vent of a conventional OWTS, which releases gases produced in the septic tank, produced an average of 469 g CO_2e capita^{-1} day^{-1}. Our systems produced more CH_4 (176 CO_2e capita^{-1} day^{-1}) and less N_2O (9 CO_2e capita^{-1} day^{-1}) when compared with values for centralized WTPs in the U.S of 99 and 52 CO_2e capita^{-1} day^{-1}, for CH_4 and N_2O, respectively (USEPA, 2015). Higher emissions of CH_4 from OWTS are not surprising, since organic C, which is a major driver behind CH_4 emissions, is present in higher concentrations in OWTS than in centralized WTPs, where organic C inputs are diluted by large volumes of water with low organic C content (Amador & Loomis, 2018).

Our results indicate that, despite the enhanced microbial activity facilitated by increased aeration and system design, advanced N-removal OWTS do not have higher total GHG emissions than conventional OWTS. Advanced N-removal OWTS may have an even lower impact than conventional systems on the atmosphere if we consider emissions from the soil treatment area (STA). Final effluent from advanced OWTS has a much lower organic C concentration than septic tank effluent from a conventional system (Amador and Loomis, 2018). This translates into lower amounts of substrate and lower CO_2 emissions from heterotrophic microbial processes when effluent is dispersed to the STA. A lower concentration of organic compounds such as acetate – the substrate for acetoclastic methanogenesis – in effluent from advanced N-removal OWTS may also result in lower CH_4 emissions from the STA.

Conclusions

Advanced N-removal OWTS rely on microbial processes for wastewater treatment, and thus inevitably emit GHGs. Our study provides the first comprehensive assessment of GHG emissions from advanced N-removal OWTS. We observed differences in CO_2 and N_2O, but not CH_4, flux based on technology, suggesting that system design and/or dosing mechanism may influence emissions of these gases. Home occupancy did not significantly impact the flux of any of the three gases measured, suggesting that daily GHG emissions are not affected by seasonal and year-round usage patterns and associated differences in the volume of wastewater inputs. The flux of CO_2 and CH_4 – but not N_2O – was significantly higher in the anoxic/hypoxic zone than in the oxic zone. The flux of both gases – but not N_2O – in the anoxic/hypoxic zone was related to wastewater properties, including BOD_5 concentration, temperature, DO and inorganic N levels, providing an opportunity to mitigate GHG emissions through manipulation of some of these properties. N_2O fluxes from advanced N-removal OWTS appear to be minimal, accounting for a small fraction of total CO_2e from these systems.

Daily per capita CO_2e for advanced N-removal OWTS were slightly lower than those for conventional OWTS. It appears that, despite the enhanced biological N removal that takes place in advanced OWTS, their contribution to atmospheric pollution is not different from that for systems that remove considerably less N from wastewater.
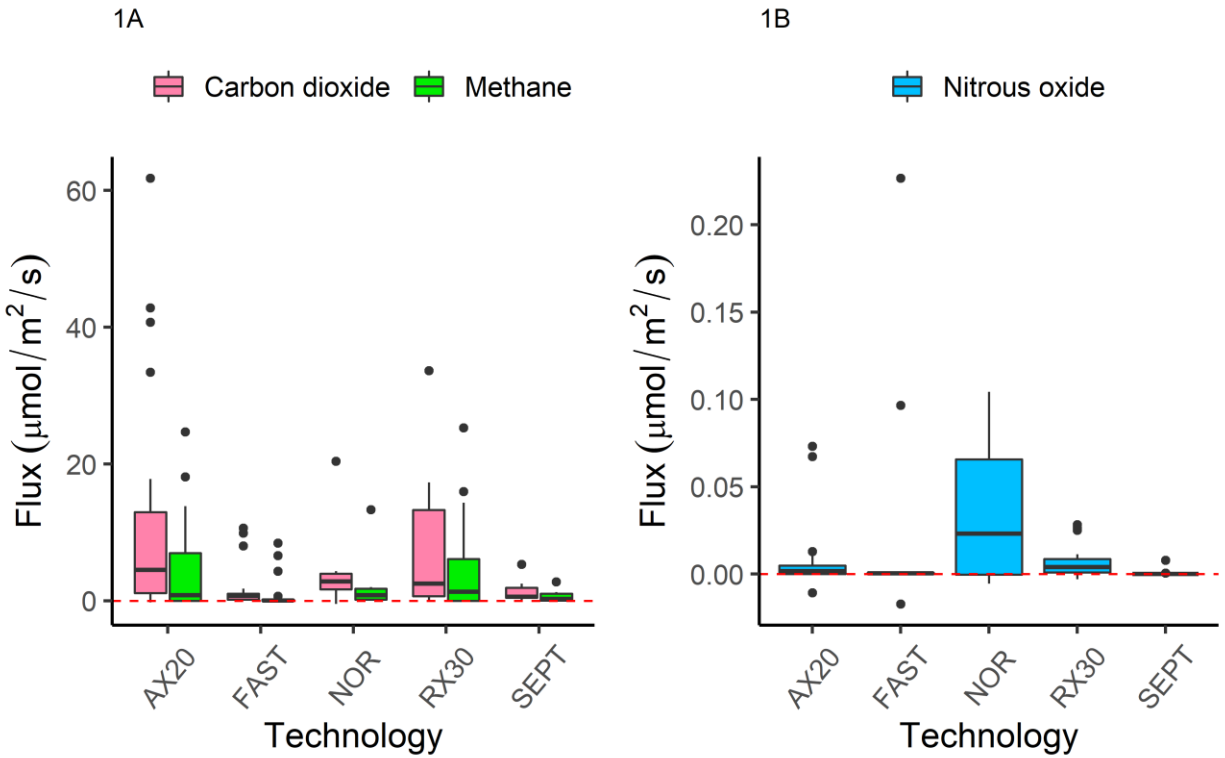
Figures

Figure 1. Carbon dioxide, methane, and nitrous oxide fluxes from different advanced N-removal OWTS technologies across all home occupancy patterns, sampling dates, and system zones (L-OX and H-OX). AX20 = Orenco Advantex® AX20 (9 systems, $n = 30$); FAST = BioMicrobics MicroFAST® (6 systems, $n = 19$); NOR = Norweco Singulair® (3 systems, $n = 6$); RX30 = Orenco Advantex® RX30 (6 systems, $n = 20$); SEPT = SeptiTech® Series D (3 systems, $n = 12$).
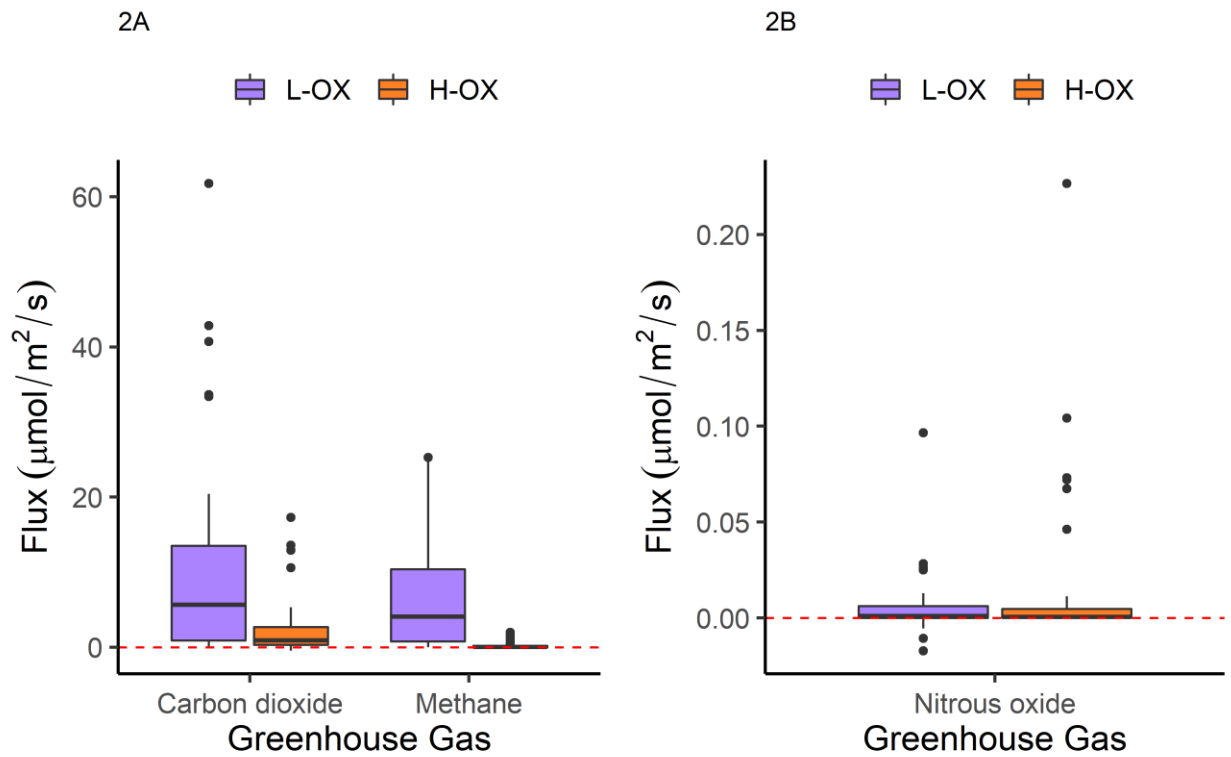
Figure 2. Carbon dioxide, methane, and nitrous oxide fluxes from the anoxic/hypoxic zone (L-OX, n = 25-36) and the oxic zone (H-OX, n = 42-43) across technologies, home occupancy patterns, and sampling dates.
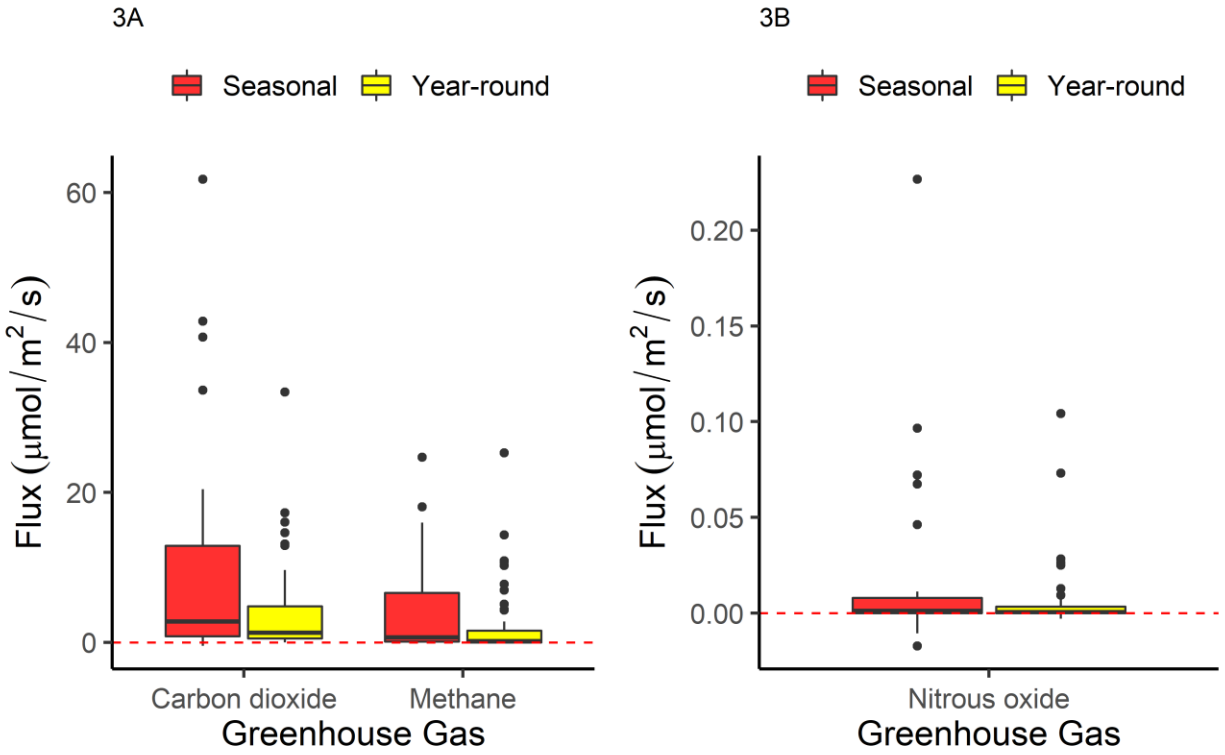
Figure 3. Carbon dioxide, methane, and nitrous oxide fluxes from systems used seasonally ($n =$ 28-29) and year-round ($n =$ 49-50) across all technologies, system zones, and sampling dates.
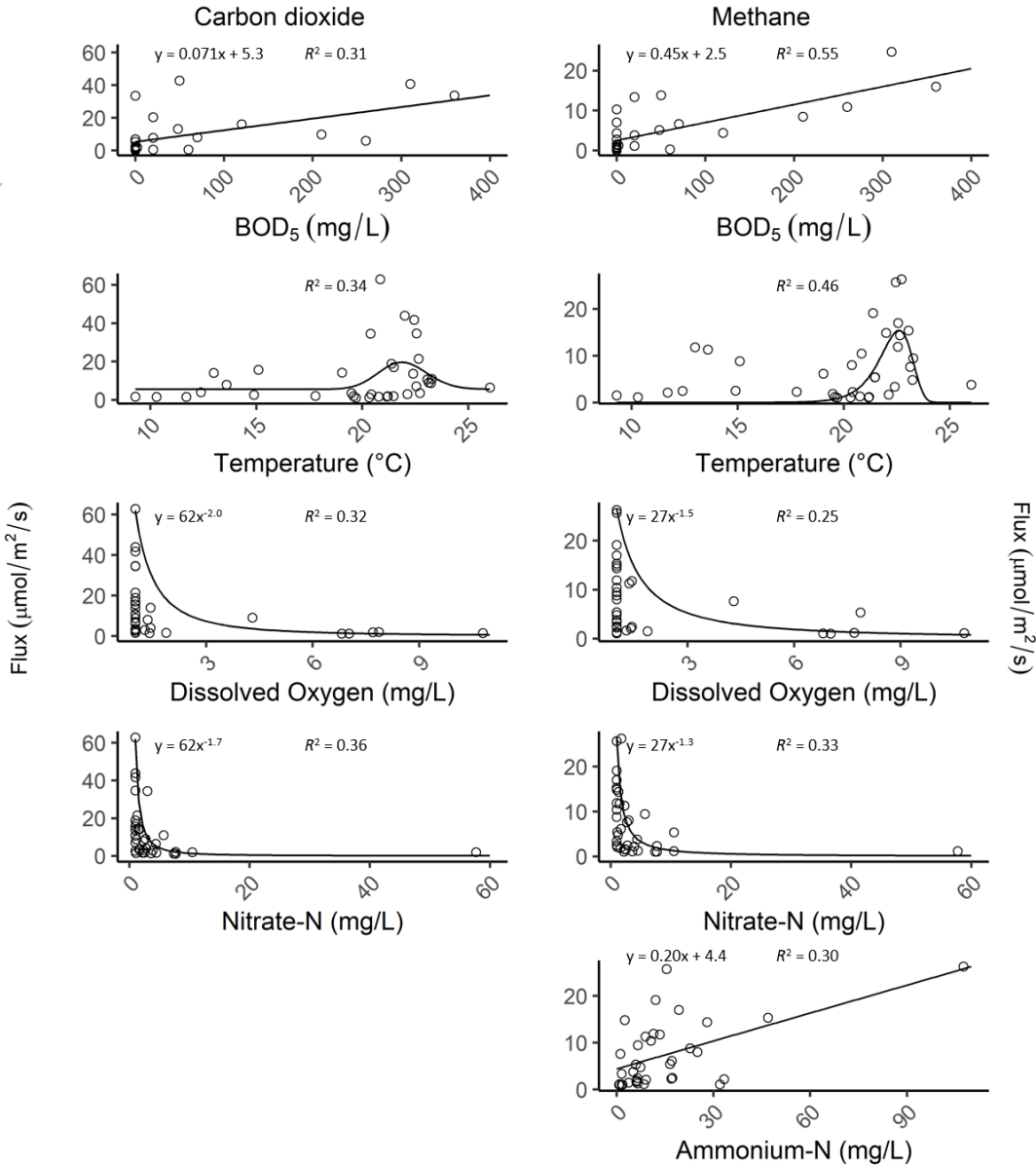
Figure 4. Relationship between wastewater properties and CH_4 and CO_2 fluxes in the anoxic/hypoxic zone (L-OX) of advanced N-removal OWTS. Best-fit line is shown in black. The regression parameters and equations for the temperature regressions can be found in the Supplemental Materials.
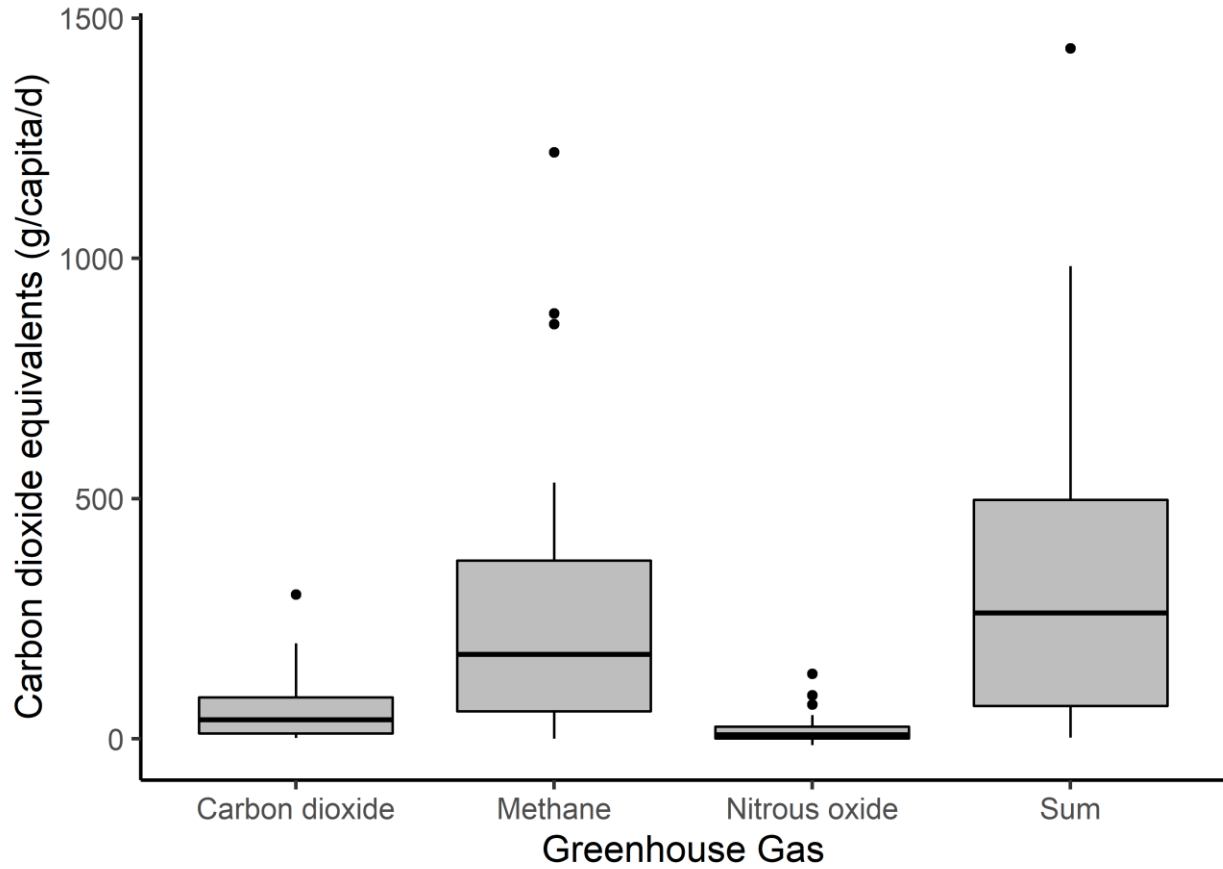
Figure 5. Carbon dioxide equivalent emissions ($n = 85$) of carbon dioxide, methane, and nitrous oxide, and sum of all GHGs across all technologies, home occupancy patterns, sampling dates, and system zones.

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