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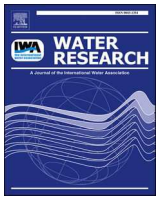

Greenhouse gas emission and denitrification kinetics of woodchip bioreactors treating onsite wastewater

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ABSTRACT

The accurate evaluation of denitrification rate and greenhouse gas (GHG) emission in field-scale woodchip bioreactors for onsite wastewater treatment are problematic due to inevitably varied environmental conditions and underestimated GHG production with limited analysis of dissolved gas in field samples. To address these problems, batch incubation experiments were conducted with controlled conditions to precisely evaluate the denitrification kinetics and N_2O and CH_4 emission of both gaseous and dissolved phases in fresh (6 months) and aged (5 years) woodchip bioreactors treating onsite wastewater at high (1–3 mg L^{-1}) and no (0 mg L^{-1}) dissolved oxygen (DO) levels. NO_3^- removal rate decreased from 37.5–119.0 g NO_3^--N $m^{-3}d^{-1}$ at no DO to 8.8–16.6 g NO_3^--N $m^{-3}d^{-1}$ at high DO (1–3 mg L^{-1}) due to the growth suppression of NO_2^- reducing microorganisms (37–55 % lower *nirS*+*nirK* abundance). However, the presence of high DO increased N_2O emission level from 5.6–6.9 mg N_2O—N m^{-3} at no DO to 179.5–273.6 mg N_2O—N m^{-3}) due to the enhanced growth of NO reducing microorganisms (1–7 times higher *norB* levels) and the decreased abundance of N_2O reducing microorganisms (53–75 % lower *nosZ* abundance). On the other hand, increased DO level negatively correlated with CH_4 production (1.0–3.9 g CH_4-C $m^{-3}d^{-1}$) in fresh woodchips, while showed insignificant impact on CH_4 production (0.1–1.4 g CH_4-C $m^{-3}d^{-1}$) in aged woodchips. Woodchip age increase (5 years) negatively impacted the NO_3^- removal rate (75–85 % lower than fresh woodchips) and CH_4 production rate (>3 times lower than fresh woodchips), probably due to the reduced biomass density of NO_2^- reducing microorganisms (52–58 % lower *nirS*+*nirK* abundance) and methanogens (95–98 % lower *mcrA* levels). The incubation results suggested that long hydraulic retention time (>2–5 days) and anaerobic/anoxic condition are preferred for the optimal NO_3^- removal and low N_2O emission potential of woodchip bioreactors treating onsite wastewater.

1. Introduction

In the United States, around 20 % of households are served by a conventional onsite wastewater treatment system (OWTS), which comprises a septic tank followed by a leaching field/pool (Capps et al., 2020; Chen et al., 2022a). The conventional OWTSs can effectively capture suspended solids in the raw wastewater and break down the organic nitrogen such as urea and amino acids to ammonium (NH_4^+) via microbe-driven enzymatic hydrolysis process (Lusk et al., 2017). The discharge of conventional OWTS effluents containing high concentrations of NH_4^+-N (40–70 mg L^{-1}) and dissolved organic nitrogen (DON, 2–10 mg L^{-1}) may cause eutrophication in a nearby waterbody and threaten the aquatic ecosystem balance and drinking water quality since these systems can only provide a limited level (10–40 %) of nitrogen (N) removal (Capps et al., 2020; Chen et al., 2022a, 2024). To reduce N loading in the aquatic environment, various advanced OWTSs such as constructed wetlands (CWs) and recirculating sand filters (RSFs) have been developed (Christopherson et al., 2005; Feng et al., 2020; Ross et al., 2020). These systems showed prominent NH_4^+-N removal performance, however, their nitrate (NO_3^-) removal performances were limited by the lack of a stable carbon source for denitrification (Behrends et al., 2007; Christopherson et al., 2005; Feng et al., 2020). Woodchip is a

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slow-releasing carbon source which has been extensively applied in the denitrification subunit of advanced OWTSs, stormwater bioretention systems, and agricultural drainage bioreactors (Chen et al., 2022b; Christianson et al., 2020; Ghane et al., 2018; Gobler et al., 2021; Israel et al., 2023). Previous studies have demonstrated that woodchip bioreactors could achieve over 70 % NO_3^- removal over 9-year treatment of agricultural wastewater with minimum maintenance (Christianson et al., 2020; Robertson, 2010).

Methane (CH_4) and nitrous oxide (N_2O) are the second and third most abundant anthropogenic greenhouse gas (GHG). The unit mass of CH_4 and N_2O showed 28 and 265 times greater global warming potential than carbon dioxide (CO_2) (Davis et al., 2019; He and Löffler, 2024). While GHG emission has been extensively reported in municipal wastewater treatment plants (WWTPs) (Tallec et al., 2008; Wang et al., 2011), few studies were conducted to monitor GHG emission from OWTSs especially for woodchip bioreactors treating onsite wastewater. The U.S. Environmental Protection Agency (USEPA) estimated 3.0 Tg CH_4 $year^{-1}$ emission from septic systems which accounted for 10.4 % of the global CH_4 production from domestic wastewater (Huynh et al., 2021). Previous studies also reported that OWTSs may show higher normalized N_2O and CH_4 emission rate (60–200 mg N_2O $capita^{-1}d^{-1}$ and 8–11 g CH_4 $capita^{-1}d^{-1}$) than municipal WWTPs (2–5 mg N_2O $capita^{-1}d^{-1}$ and 0.1–6 g CH_4 $capita^{-1}d^{-1}$) (Brannon et al., 2017; Diaz-Valbuena et al., 2011; Fernández-Baca et al., 2018; Truhlar et al., 2016; Wang et al., 2011; Yan et al., 2019; Zhao et al., 2019). In addition, our previous study demonstrated that the woodchip denitrification subunit of a continuous flow bioreactor (CFB) was the major source (>88 %) of N_2O emission during onsite wastewater treatment (Chen et al., 2022b). In agricultural drainage and stormwater treatment, up to 6.7 g CH_4-C $m^{-3}d^{-1}$ and 478.4 mg N_2O—N $m^{-3}d^{-1}$ were also generated from woodchip bioreactors (David et al., 2016; Davis et al., 2019; Elgood et al., 2010). N_2O was mainly produced from incomplete denitrification and CH_4 was generated via methanogenesis at anaerobic conditions (El-Fadel and Massoud, 2001; Kampschreur et al., 2009). The overall emission potential of GHG from woodchip bioreactors may be underestimated since most studies only monitored dissolved GHG levels in influent and effluent water samples, while 16 % N_2O and 58 % CH_4 generated from woodchip bioreactors were partitioned into gas filled voids and finally released to the atmosphere (McGuire and Reid, 2019).

Woodchip age played an important role in controlling NO_3^- removal and GHG production from woodchip bioreactors. Although field installations of woodchip bioreactors have demonstrated efficient NO_3^- removal (70–90 %) from agricultural drainage, the NO_3^- removal rate decreased significantly (25–60 %) over long-term operation (5–9 years), due to the consumption of labile carbon by various reduction/oxidation processes in aged woodchips that limited carbon availability for denitrification (Robertson, 2010). A 5–11 % decrease in total carbon concentration was reported in aged woodchips after 4 to 9 years treatment of agricultural wastewater (Ghane et al., 2018; Moorman et al., 2010). In addition, the carbon-limiting condition may also result in the change of GHG emission pattern. In WWTPs, 140–920 % greater emission of N_2O and 135–385 % less production of CH_4 were reported when influent carbon to nitrogen ratio (C:N) decreased from 4.5–16.0 to 2.6–4.0 (Kishida et al., 2004; Zhang et al., 2023). In most woodchip bioreactors treating agricultural drainage, significant emission of N_2O (0–478.4 mg N_2O—N $m^{-3}d^{-1}$) and low production of CH_4 (<0.1 g CH_4 $m^{-3}d^{-1}$) were observed due to low C:N ratio (2–5) in agricultural wastewater (Audet et al., 2021; Christianson et al., 2020; Davis et al., 2019; Elgood et al., 2010; Ghane et al., 2018; Lavrnić et al., 2020; Vymazal and Březinová, 2018; Warneke et al., 2011; White et al., 2022). However, considering the higher C:N ratio (5–15) reported in the onsite domestic wastewater, the N_2O and CH_4 emission in woodchip-based OWTS may show different patterns which have not been evaluated in previous studies (Chen et al., 2022b; Gobler et al., 2021; Moorman et al., 2010).

Dissolved oxygen (DO) was reported to be another important factor controlling denitrification performance and GHG emission in wastewater treatment process (Hocaoglu et al., 2011; Kampschreur et al., 2009; Wang et al., 2011). DO was positively correlated with N_2O emission in the denitrification unit of WWTPs since N_2O reductase was more sensitive to O_2 (Kampschreur et al., 2009). In a batch anaerobic reactor, the increased DO level (from 0.5 to 1 mg L^{-1}) resulted in an increase of N_2O emission by 60–150 % (Von Schulthess et al., 1994). High DO levels can also inhibit NO_3^- removal and CH_4 emission in wastewater treatment process because denitrifying microorganisms preferably use oxygen (O_2) rather than NO_3^- as an electron acceptor and methanogens are restrict anaerobic microorganisms (Hocaoglu et al., 2011; Wang et al., 2011). Ceased denitrification was reported in WWTPs when DO was increased to 1–4 mg L^{-1} and 35 % lower production of CH_4 was observed in an up-flow anaerobic sludge bed reactor treating municipal wastewater with increased DO loading from 0.03 to 0.4 g O_2 $L^{-1}d^{-1}$ (Hocaoglu et al., 2011; Shen and Guiot, 1996; Wang et al., 2011). However, the short exposure of O_2 in the oxic-anoxic woodchip bioreactors treating agricultural drainage can enhance denitrification rates because O_2 can help break down organic matters in woodchip and increase the labile carbon availability for denitrification (Maxwell et al., 2019; McGuire et al., 2021). Although the impact of DO on denitrification and GHG emission in WWTPs and woodchip bioreactors treating agricultural drainage has been well studied, little information is available about this relationship in woodchip bioreactors treating onsite wastewater.

The evaluation of NO_3^- removal rate and the prediction of GHG emission from woodchip bioreactors in OWTSs are challenging because environmental conditions such as flow rate, temperature, influent NO_3^- concentration and pore water chemistry were fluctuating and difficult to control. In this study, to evaluate the impact of DO and woodchip age on denitrification kinetics and GHG production from woodchip bioreactors at controlled conditions during onsite wastewater treatment, batch reactors of aged (5 years) and fresh (6 months) woodchips were set up at two DO levels (0 and 1–3 mg L^{-1}). Digital PCR (dPCR) analysis was also performed to evaluate the change of functional gene abundances associated with denitrification (*nirS, nirK, norB* and *nosZ*) and methanogenesis (*mcrA*) at different environmental conditions (DO and woodchip age). The results of this study can provide guidance for the design, operation, and maintenance of woodchip bioreactors for long-term (>5 years) onsite wastewater treatment to achieve efficient NO_3^- removal with minimized climate change impact.

2. Materials and methods

2.1. Woodchip samples collection

Aged woodchips (5 years) were collected from a pilot-scale woodchip bioreactor, and fresh woodchips (6 months) were collected from a field-scale woodchip bioreactor. Both aged and fresh woodchips were from the same woodchip stockpile at the Water Research Innovation Facility of the New York State Center for Clean Water Technology. The configuration and operational conditions of the pilot-scale woodchip bioreactor were described in a previous study (Figure S8) (Chen et al., 2022b). The field-scale woodchip bioreactor has similar configuration with the pilot-scale system with upscaled size (1.2 m × 1.2 m × 0.5 m). Both woodchip bioreactors were fed with nitrified septic tank effluent (STE) from a nitrification reactor (i.e., sand filters).

2.2. Batch incubation experiment

Four groups of batch incubation experiments were conducted in triplicates: a) aged woodchips with high DO (1–3 mg L^{-1}), b) fresh woodchips with high DO (1–3 mg L^{-1}), c) aged woodchips with no DO (0 mg L^{-1}) and d) fresh woodchips with no DO (0 mg L^{-1}) (Figure S1). The high DO condition (1–3 mg L^{-1}) in the incubation experiment represented the DO concentrations in effluents from the pilot-scale up-flow woodchip bioreactor treating nitrified onsite wastewater where

woodchip samples for the incubation experiment were collected (Figure S8) (Chen et al., 2022b). The no DO level incubation groups simulated the woodchip bioreactors at fully anaerobic condition. Homogenized woodchips (350 g) were added to an 1150 mL GL 45 laboratory glass bottle (DWK Life Science, Germany) containing 400 mL nitrified STE collected from a nitrification reactor. The characteristics of the nitrified STE were summarized in Table S2. Helium gas was purged into the liquid phase of all bottles for 10 min and then the gas phase for 5 min to remove O_2. Then all bottles were immediately sealed with rubber stoppers (DWK Life Science, Germany). For high DO (1–3 mg L^{-1}) incubation experiment, headspace helium gas (30 mL) in the incubation bottle was replaced with 30 mL pure O_2 gas by a 60 mL syringe (Fisher Scientific, United States). Contactless DO sensors (Pyroscience, Germany) were attached to the side walls of all high DO incubation bottles for DO analyses. All bottles were incubated in shaking incubators (Corning, United States) at 120 rpm and 25 °C. Pure O_2 (10 mL) was injected to fresh woodchip bottles incubated at high DO every 10–15 h due to the high O_2 consumption rates (Figure S2). The injection of pure O_2 led to the alternating oxic-anoxic condition in fresh woodchip incubated at high DO (Figure S2).

2.3. Samples collection and analysis

Liquid sample (5 mL) was collected from each bottle every 4–10 h by a 60 mL gas-tight syringe (Fisher Scientific, United States) connected to a two-way stopcock (Cole Parmer, United States), then 30 mL air was introduced to the syringe for mixing. After vigorously shaking the syringe for 30 min, the headspace gas (30 mL) in the syringe was transferred to another syringe for dissolved N_2O and CH_4 analysis. The remaining liquid sample was then filtered through a 0.45 μm filter (Fisher Scientific, United States), acidified with concentrated sulfuric acid (18 M), and stored at 4 °C. Gas sample (3 mL) was also taken from the headspace of each bottle by a 5 mL gas-tight syringe (Fisher Scientific, United States) every 4–10 h for gaseous N_2O and CH_4 measurement.

NH_4^+-N, NO_2^--N, and NO_3^--N were analyzed by a Lachat QuikChem 8500 autoanalyzer (Hach, USA) according to the manufacturer's instruction. Both gaseous and dissolved N_2O and CH_4 were analyzed by a Shimadzu GC-2014 gas chromatography (Shimadzu, Japan) equipped with an electron capture detector (ECD) for N_2O analysis and a flame ionization detector (FID) for CH_4 analysis. The detection limit was 0.02 mg N L^{-1} for NH_4^+-N, NO_2^--N, and NO_3^--N, 0.03 ppm for N_2O and 2.0 ppm for CH_4.

Total organic carbon (TOC) and total nitrogen (TN) were also measured in aged and fresh woodchip samples utilized in the batch incubation experiment. Around 40 g of aged and fresh woodchip were dried at 70 °C overnight, and then ground and homogenized to fine powders for TN analysis (Shahraki et al., 2020). Additional 5 g of aged and fresh woodchip samples were treated with 10 % hydrochloric acid (HCl) (v:v) for 5 h to remove carbonate, then washed with deionized water and dried at 70 °C overnight for TOC analysis (Shahraki et al., 2020). Elemental analyzer Carbon/Nitrogen/Sulfur (CNS) (Carlo Erba, Italy) was used to measure TOC and TN in the woodchip samples.

2.4. Microbial analysis

After the incubation experiment, 300 g woodchips were collected from each treatment group and were stored at −80 °C for microbial analysis. Genomic DNA was extracted from woodchip samples using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Germany) according to the manufacturer's instruction. DNA extract yields and purities were quantified with a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, United States). The abundance of denitrifying microorganisms was estimated by *nirS* and *nirK* which encode for NO_2^- reductase, *qnorB* and *cnorB* which encode the enzyme for conversion of nitric oxide (NO) to N_2O, *nosZ* I and *nosZ* II which encode enzyme for N_2O reduction. The methanogen biomass was evaluated by *mcrA* which encodes methyl coenzyme for CH_4 production. The total biomass (16S rRNA) and selected functional genes (*nirS, nirK, qnorB, cnorB, nosZ* I, *nosZ* II, and *mcrA*) were measured by a dPCR (Qiagen, Germany). Detailed information of protocols, primers and denitrification pathways is provided in the supplemental material (Table S1 and Figure S3). The total *norB* abundance was calculated as the sum of *qnorB* and *cnorB* levels and the total *nosZ* abundance was calculated as the sum of *nosZ* I and *nosZ* II levels.

2.5. Data analysis

One-way analysis of variance (ANOVA) was used to evaluate the impact of DO and woodchip age on denitrification performance and GHG emission (CH_4 and N_2O) at a significance level of 0.05. All analyses were performed in R (version 3.5.3) and OriginLab 2018 (OriginLab, MA). The normalized N_2O and CH_4 mass was calculated as the ratio of accumulated N_2O/CH_4 mass to woodchip volume. The calculation details for the average NO_3^- removal rate was provided in the supplemental materials.

3. Results

3.1. NO_3^- Removal Kinetics

Throughout the entire incubation experiment, NO_3^- was the major nitrogen specie (0–22.3 mg NO_3^--N L^{-1}) detected in the liquid phase, while NH_4^+ and NO_2^- concentrations were below 1 mg N L^{-1} (Fig. 1 and S4). The reduction of NO_3^- in all treatment groups except for fresh woodchip incubated at no DO followed first-order kinetics (R^2=0.98–0.99), indicating that NO_3^- concentration was the limiting factor for denitrification (Fig. 1 and Table S3). For fresh woodchip incubated at no DO, since NO_3^- concentration rapidly reduced to nearly 0 mg NO_3^--N L^{-1} after 4 h, the active data points were too limited to support kinetic model development. Without DO, fresh woodchips achieved the NO_3^- removal rate of 119.0 ± 1.2 g NO_3^--N $m^{-3}d^{-1}$, 3 times higher than that observed at 1–3 mg L^{-1} DO (37.5 ± 0.8 g NO_3^--N $m^{-3}d^{-1}$) (Fig. 1 and Table 1). Aged woodchips also showed over 80 % higher NO_3^- removal rate without DO (16.6 ± 0.1 g NO_3^--N $m^{-3}d^{-1}$) compared with the level observed at 1–3 mg L^{-1} DO (8.8 ± 0.7 g NO_3^--N m^3d^{-1}) (Fig. 1 and Table 1). In this study, the NO_3^- removal rates of aged woodchip were comparable with those reported in other long-term (>5 years) operated woodchip bioreactors treating agricultural and mineral drainage (0.1–12.0 g NO_3^--N $m^{-3}d^{-1}$) with similar influent NO_3^- levels (3–35 mg NO_3^--N L^{-1}) and HRTs (4–58 h) (Table 2) (Elgood et al., 2010; Ghane et al., 2015; Warneke et al., 2011; White et al., 2022). However, the NO_3^- removal rates of fresh woodchip observed in this incubation experiment were higher than the rates reported in our field woodchip bioreactor (0.9–7.4 g NO_3^--N $m^{-3}d^{-1}$, 0–4 years) treating onsite wastewater and other relatively new woodchip bioreactors (5.1–76.2 g NO_3^--N m^{-3} d^{-1}, <5 years) treating agricultural drainage (Audet et al., 2021; Chen et al., 2022b; Davis et al., 2019; Nordström and Herbert, 2018).

3.2. Greenhouse gas emission

3.2.1. N_2O production

Significant N_2O production was observed only in woodchips at high DO (1–3 mg L^{-1}) (Fig. 2). Specifically, N_2O accumulated to 179.5 mg N_2O—N m^{-3} in fresh woodchips at high DO during the first 4 h of incubation. Then the level decreased to below detection limit after 25-hour incubation. On the contrary, N_2O was slowly released from aged woodchips at high DO to 243.6 mg N_2O—N m^{-3} after 35-hour incubation, 36 % higher than the peak level observed in fresh woodchips. Then the level decreased to below detection limit after 83 h of incubation (Fig. 2). When DO was not presented, aged and fresh woodchips produced 5.6 and 6.9 mg N_2O—N m^{-3} during the initial 15 and 23 h,

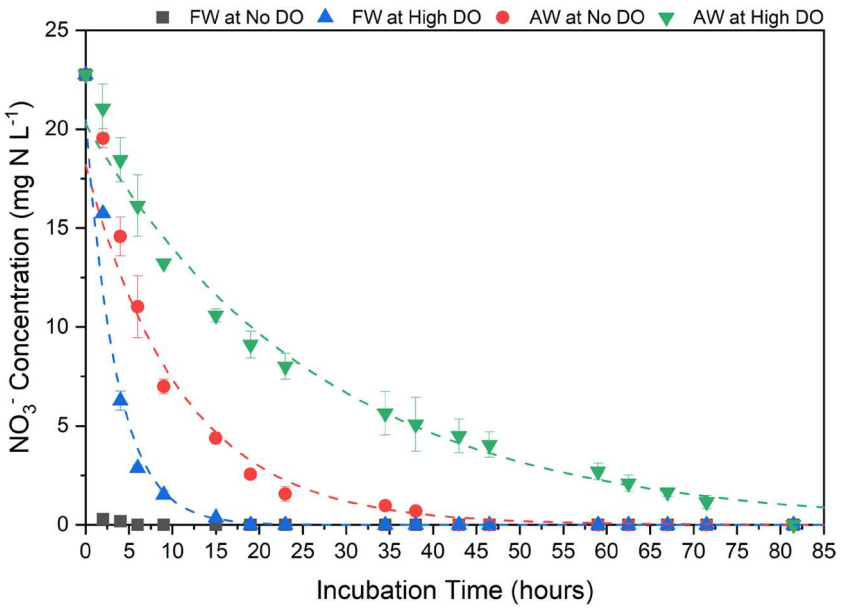

Fig. 1. The variation of NO_3^- concentration in aged woodchips and fresh woodchips at different DO levels during the incubation experiment (High DO: 1–3 mg L^{-1}, No DO: 0 mg L^{-1}). Error bars represent the standard errors for experimental triplicates. FW: fresh woodchips, AW: aged woodchips. The dashed lines show the first-order kinetic model fittings.

Table 1
Denitrification and CH_4 production from batch incubations at various DO levels.

Woodchip type	DO Level [a]	CH_4 Production Rate (g CH_4-C $m^{-3}d^{-1}$)		NO_3^- Removal Rate (g NO_3^--N $m^{-3}d^{-1}$)
		Before Complete Removal of NO_3^-	After Complete Removal of NO_3^-	
Fresh	High	1.0 ± 0.1	3.9 ± 0.1	37.5 ± 0.8
	No	6.1 ± 0.1	8.8 ± 0.1	119.0 ± 1.2
Aged	High	0.1 ± 0.0	1.1 ± 0.0	8.8 ± 0.7
	No	0.1 ± 0.1	1.4 ± 0.0	16.6 ± 0.1

[a] High DO level: 1–3 mg L^{-1}, No DO level: 0 mg L^{-1}.

respectively. Then the N_2O level reduced to below detection limit after 30 and 47 h of incubation, respectively (Fig. 2).

The N_2O—N produced from all woodchips accounts for 0–0.8 % of NO_3^--N removed by denitrification during the incubation (Figure S5). This result fell in the lower range of the mass ratio of N_2O production to NO_3^- removal (0.1–4.7 %) reported in other woodchip bioreactors treating agricultural wastewater (Christianson et al., 2013; David et al., 2016; Davis et al., 2019; McGuire et al., 2023; Warneke et al., 2011), suggesting nitrogen gas (N_2) rather than N_2O was the main product of denitrification in woodchips during the incubation period.

3.2.2. *CH_4 production*

Before NO_3^- was fully removed in each incubation group, aged woodchips released 81 mg CH_4–C m^{-3} at no DO (by 30 h) and 218 mg CH_4–C m^{-3} at high DO (by 66 h), however, fresh woodchips produced

Table 2
Comparison of GHG emission and NO_3^- removal in different woodchip bioreactors.

Wastewater source	HRT (hours)	Influent NO_3^- Concentration (mg NO_3^--N L^{-1})	Woodchip Age (years)	N_2O Production Rate (mg N_2O—N $m^{-3}d^{-1}$)	CH_4 Production Rate (g CH_4-C $m^{-3}d^{-1}$)	NO_3^- Removal Rate (g NO_3^--N $m^{-3}d^{-1}$)	NO_3^- Removal Efficiency (%)	Effluent DO (mg L^{-1})	Reference
Agricultural	4–6	3–24	0.1–2	20.3–56.4	n/a [a]	5.4–76.2	5.1–85.2	<2	(White et al., 2022)
Agricultural	2–16	14	0.1–5	36.6–478.4	0.2–0.9	0.1–12.5	9.0–53.8	1–2	(Davis et al., 2019)
Agricultural	23–153	5–15	2–7	0–80.0	n/a	n/a	17.0–82.0	n/a	(Audet et al., 2021)
Agricultural	5	10–35	4	0.1–0.5	<0.1	2.1–5.8	16.7–90.7	1–5	(Ghane et al., 2015)
Agricultural	9	14–17	1–2	55.7–110.4	<0.1	4.0–12.0	27.1–93.5	0–3	(Warneke et al., 2011)
Agricultural	24–48	1–6	2–3	0–14.6	0.1–1.2	0.2–1.2	29.1–100.0	<1	(Elgood et al., 2010)
Mine Drainage	46–58	22	2	0–3.4	<0.1	n/a	22.3–90.1	n/a	(Nordström and Herbert, 2018)
Synthetic Wastewater	3–12	n/a	1	0–89.4	<0.1	n/a	n/a	n/a	(Bock et al., 2018)
Synthetic Wastewater	204–436	1.2–1.8	n/a	62.5–268.8	0.1–6.7	1.5–3.0	>99.6	<2	(Healy et al., 2012)
Nitrified STE	4–83	22	0.5–5	0.1–13.5	0.1–8.8	8.8–119.0	100.0	0–3	This study

[a] n/a indicates the data is not provided in the reference.

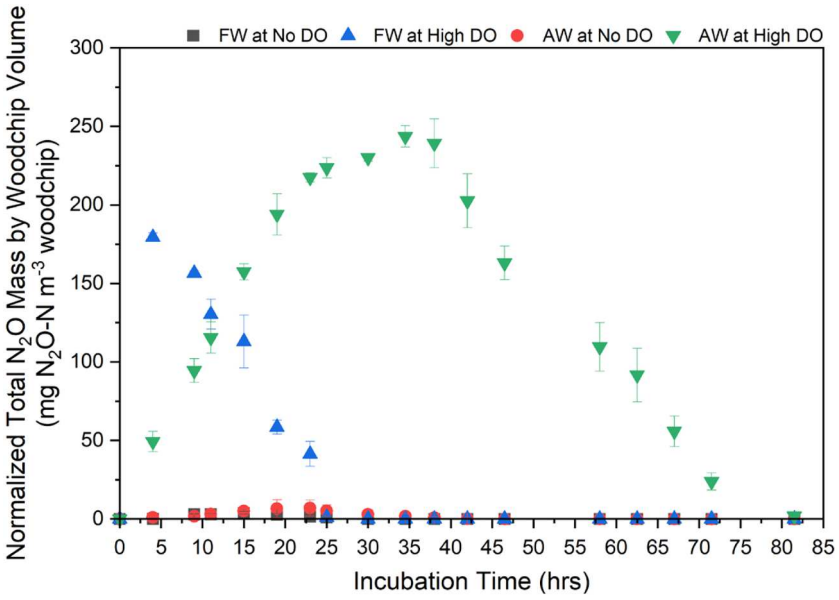

Fig. 2. The N_2O accumulation in aged and fresh woodchips at different DO levels during the incubation experiment (High DO: 1–3 mg L^{-1}, No DO: 0 mg L^{-1}). Error bars represent the standard errors for experimental triplicates. FW: fresh woodchips, AW: aged woodchips.

higher level of CH_4 at both no DO (1017 mg CH_4—C m^{-3} by 4 h) and high DO (659 mg CH_4—C m^{-3} by 15 h) conditions (Fig. 1 and 3). The CH_4 production kinetics could be fitted with a zero-order kinetic model (R^2=0.93–1.00), suggesting the CH_4 production rate was constant before NO_3^- was completely removed (Fig. 3 and Table S4). The CH_4 production rates were 1.0 ± 0.1 g CH_4—C m^{-3} d^{-1} in fresh woodchips at high DO, 83 % lower than that (6.1 ± 0.1 g CH_4—C m^{-3} d^{-1}) without DO (Table 1). On the contrary, the CH_4 production rates in aged woodchips were significantly lower at both high DO (0.1 ± 0.0 g CH_4-C $m^{-3}d^{-1}$) and no DO (0.1 ± 0.1 g CH_4-C $m^{-3}d^{-1}$) conditions (Table 1).

After NO_3^- was completely removed, a significant increase in CH_4 production was observed in all incubations and could also be fitted with a zero-order kinetic model (R^2=0.97–0.99) (Fig. 1, 3 and Table S4). CH_4 production from fresh woodchips was inhibited when high DO was present, which was indicated by a 55 % lower CH_4 production rate observed at high DO (3.9 ± 0.1 g CH_4—C m^{-3} d^{-1}) than that at no DO (8.8 ± 0.1 g CH_4—C m^{-3} d^{-1}) (Table 1). On the other hand, in aged woodchips, the presence of DO did not affect CH_4 production rates (Table 1). The CH_4 production rates in this study were comparable with values (0–6.7 g CH_4—C m^{-3} d^{-1}) observed in other woodchip bioreactors treating agricultural and mineral drainage with effluent DO ranged from 0 to 3 mg L^{-1} (Table 2) (Bock et al., 2018; Davis et al., 2019; Elgood et al., 2010; Ghane et al., 2015; Warneke et al., 2011; White et al., 2022).

3.3. Microbial abundance of denitrifiers and methanogens

In fresh woodchip incubations, the abundance of most functional genes related to nitrogen transformations was significantly higher in the no DO group except for *norB* (Fig. 4). Specifically, 16S rRNA abundance was 81 % higher at no DO condition (3.8 ± 0.1 × 10^{11} 16S rRNA copies g^{-1}) (Fig. 4a, Table S5 and S7). *NirS* and *nirK* levels were 6.5 ± 0.5 × 10^{10} *nirS* copies g^{-1} and 9.4 ± 0.4 × 10^9 *nirK* copies g^{-1} in no DO group and decreased by 35–44 % to 4.2 ± 0.6 × 10^{10} *nirS* copies g^{-1} and 5.3 ± 1.0 × 10^9 *nirK* copies g^{-1} in high DO group (Fig. 4b, c, Table S5). The *nirS* abundance was one order of magnitude higher than the *nirK* level, consistent with the observation in the woodchip bioreactor of a continuous flow nitrogen removing biofilter (Chen et al., 2022b). Over 50 % higher *nosZ* abundance was observed in the no DO group (4.6 ± 0.8 × 10^{10} *nosZ* copies g^{-1}) than that in the high DO group (3.0 ± 0.2 × 10^{10} *nosZ* copies g^{-1}) (Fig. 4e, Table S5 and S7). The abundance of *mcrA* in the no DO group was 6.8 ± 0.6 × 10^{10} *mcrA* copies g^{-1}, over 100 % higher than the level observed in the high DO group (3.3 ± 0.1 × 10^{10} *mcrA* copies g^{-1}) (Fig. 4f, Table S5 and S7). On the contrary, *norB* level increased by over 100 % at high DO (2.8 ± 0.8 × 10^{10} *norB* copies g^{-1}), compared to fully anaerobic condition (1.3 ± 0.2 × 10^{10} *norB* copies g^{-1}) (Fig. 4d, Table S5 and S7).

At the end of the incubation, the abundance of most selected functional genes in aged woodchips was significantly lower (26–98 %) than those observed in the fresh woodchips, while comparable *norB* levels were detected in both aged and fresh woodchips in the high DO group (Fig. 4). Similar to the observations in fresh woodchip incubations, significantly lower abundance of 16S rRNA, *nirS, nosZ,* and a higher level of *norB* were observed in the high DO groups. The 16S rRNA abundance in no DO group (2.7 ± 0.3 × 10^{11} 16S rRNA copies g^{-1}) was 125 % higher than that observed in the high DO group (1.2 ± 0.4 × 10^{11} 16S rRNA copies g^{-1}), *nirS* level in the no DO group (2.8 ± 0.1 × 10^{10} *nirS* copies g^{-1}) was 47 % higher than that in the high DO group (1.9 ± 0.7 × 10^{10} *nirS* copies g^{-1}) and *nosZ* level in the no DO group (2.1 ± 0.2 × 10^{10} *nosZ* copies g^{-1}) was 75 % higher than that in the high DO group (1.2 ± 0.2 × 10^{10} *nosZ* copies g^{-1}) (Fig. 4a, b, e, Table S5 and S7). On the contrary, an 87 % decrease of *norB* level was observed in the no DO group (3.6 ± 0.5 × 10^9 *norB* copies g^{-1}) compared with that in the high DO group (2.8 ± 0.8 × 10^{10} *norB* copies g^{-1} at high DO) (Fig. 4d, Table S5 and S7). However, comparable abundances of *nirK* (3.2 ± 0.8 × 10^9 *nirK* copies g^{-1} with no DO and 3.9 ± 0.6 × 10^9 *nirK* copies g^{-1} with high DO) and *mcrA* (1.2 ± 0.2 × 10^9 *mcrA* copies g^{-1} with no DO and 1.4 ± 0.1 × 10^9 *mcrA* copies g^{-1} with high DO) were observed at both DO conditions, suggesting these two functional genes abundance were insensitive to DO level changes (Fig. 4c, f, Table S5 and S7).

4. Discussions

4.1. Impact of environmental conditions on GHG emission and denitrification kinetics

4.1.1. Hydraulic retention time (HRT)

Throughout the entire experiment, the incubation time played an important role in controlling NO_3^- removal efficiency in woodchip bioreactors treating onsite wastewater. Longer incubation time ensured sufficient contact time between water flow and the biofilm attached to

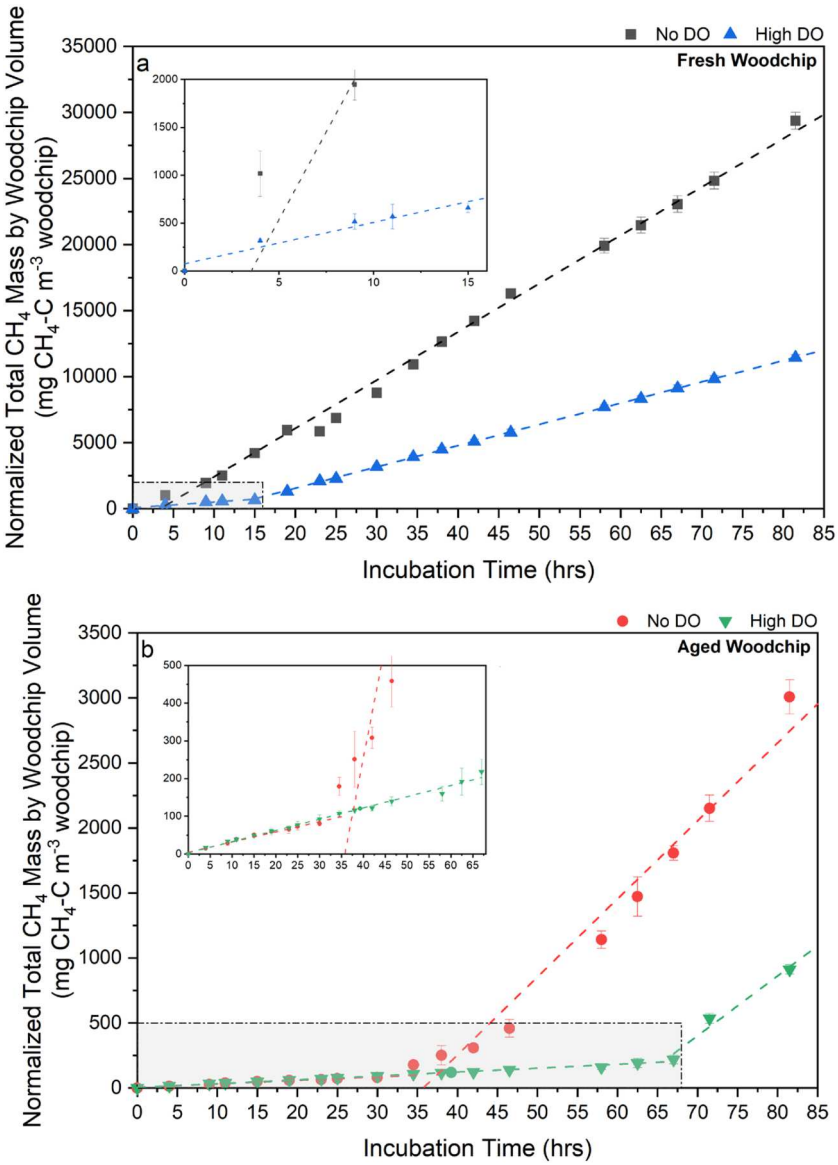

Fig. 3. The CH_4 accumulation in a) fresh and b) aged woodchips (High DO: 1–3 mg L^{-1}, No DO: 0 mg L^{-1}). Error bars represent the standard errors for experimental triplicates. The dashed lines show the zero-order kinetic model fittings. The small graphs represented CH_4 production in different treatment groups before NO_3^- removal was completed (highlighted in gray).

the surface of woodchips and promoted the NO_3^- reduction. That result may indicate that prolonged HRTs in woodchip bioreactors treating onsite wastewater may facilitate the denitrification and contribute to lower effluent NO_3^- concentration. Based on the calculation with NO_3^- removal rate observed in the batch incubations (Table 1), HRTs of 43–101 h with no DO, and 81–190 h with high DO (1–3 mg L^{-1}) were required for the studied 5-year-old woodchip bioreactor to fully remove 30–70 mg N L^{-1} from STE at the practical HLR (0.04 m^3 m^{-2} d^{-1}) for woodchip treatment units of OWTSs (calculation in SI) (Gobler et al., 2021).

With DO presence (1–3 mg L^{-1}), the incomplete denitrification promoted N_2O production during the first 4–35 h of incubation, and then N_2O level gradually reduced to below the detection limit after denitrification was completed (Fig. 2). These results suggested that N_2O may only be significantly produced at initial 4–35 h of detention in the woodchip bioreactor when high O_2 was present and longer HRT can promote full denitrification, thus reducing the N_2O emission potential. In other woodchip bioreactors treating agricultural wastewater, N_2O level was reported to increase during the first a couple of hours of detention, then the level reduced when wastewater was retained in systems for longer time (>4 h) (Davis et al., 2019; Hassanpour et al., 2020).

In this study, longer incubation time can only significantly promote the CH_4 production in all incubation groups after the completion of NO_3^- removal. This result suggested that labile carbon released from woodchips was primarily used for denitrification rather than methanogenesis (Fig. 3). Similar observations were reported in agricultural wastewater treatment studies that significant CH_4 emission (0.2–2.1 g CH_4–C m^{-3} d^{-1}) was observed in woodchip bioreactors when NO_3^- concentration decreased to <1 mg NO_3^--N L^{-1} (Elgood et al., 2010; Healy et al., 2012). Assuming cellulose with the empirical formula of $C_6H_{10}O_5$ was the sole carbon source for denitrification and methanogenesis, 2–13 % carbon (0.3–18.3 g C m^{-3} d^{-1}) was utilized by methanogens for CH_4 production before denitrification was completed, while most carbon (87–98 %, 22.5–127.5 g C $m^{-3}d^{-1}$) served as the electron donor for denitrification (calculation in SI) (Gray, 1926; Tugtas et al., 2010). Considering incomplete NO_3^- removal was detected in most field woodchip bioreactors treating agricultural and mineral wastewater, lower CH_4

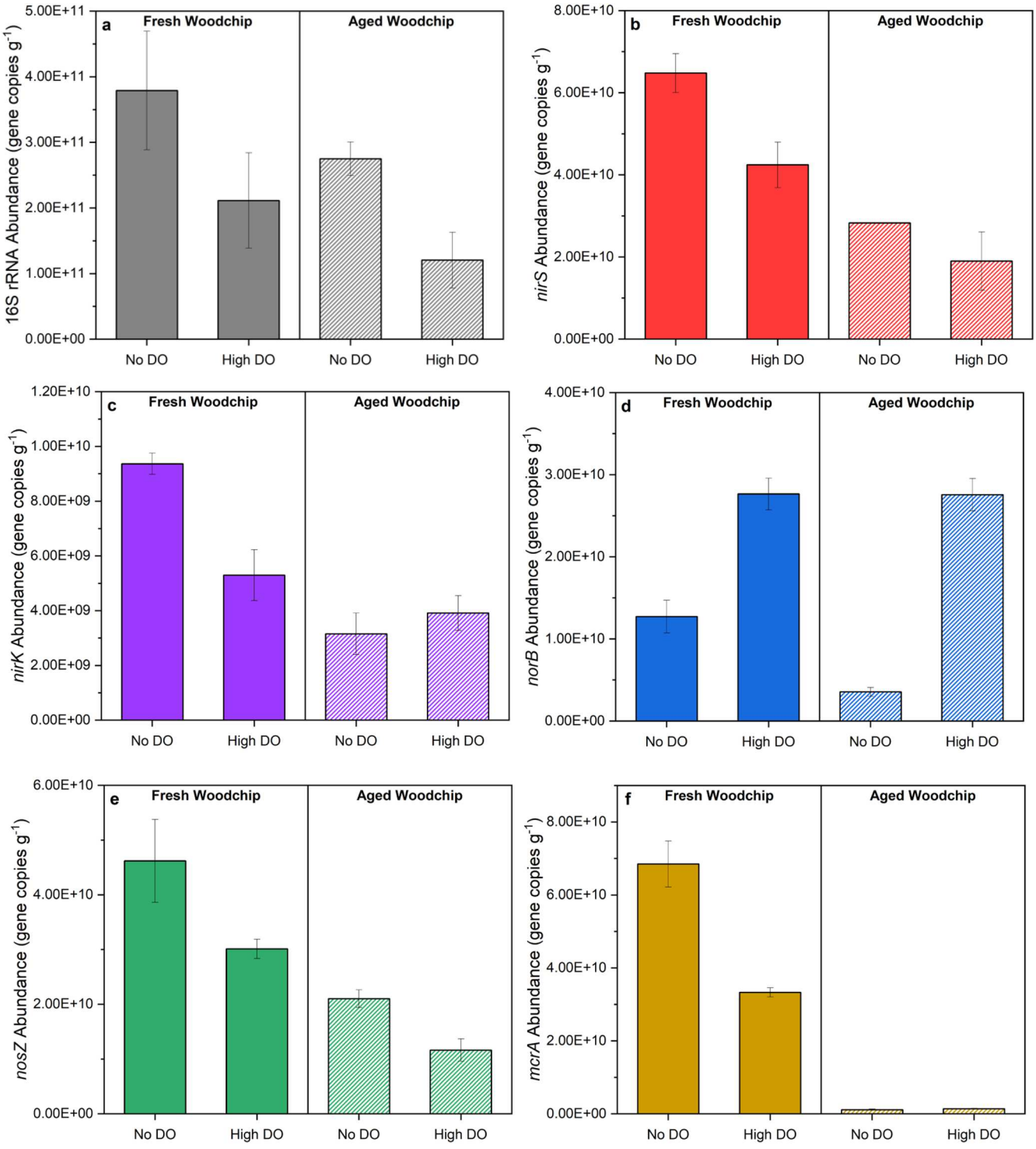

Fig. 4. Absolute abundance of a) 16S rRNA; b) *nirS*; c) *nirK*; d) *norB*; e) *nosZ* and f) *mcrA* in aged and fresh woodchips during incubation experiment (High DO: 1–3 mg L^{-1}, No DO: 0 mg L^{-1}). Error bars represent the standard errors for experimental triplicates.

production (0–0.9 g CH_4-C $m^{-3}d^{-1}$) was observed from these systems (Bock et al., 2018; Davis et al., 2019; Ghane et al., 2015; Warneke et al., 2011; White et al., 2022). However, when sufficient HRT was provided to achieve complete NO_3^- removal, quick accumulation of CH_4 (1.2–6.7 g CH_4-C $m^{-3}d^{-1}$) was expected as evidenced in this study and previous case studies (Elgood et al., 2010; Healy et al., 2012). Considering woodchip bioreactors are generally designed with higher HRTs for onsite wastewater treatment (>80 h) than agricultural drainage treatment (4–60 h), lower N_2O emission and higher CH_4 production may be observed in woodchip bioreactors treating onsite wastewater, which was rarely investigated in the previous studies (Davis et al., 2019; Ghane et al., 2015; Gobler et al., 2021; Nordström and Herbert, 2018; White et al., 2022).

4.1.2. DO

In municipal WWTPs, denitrification was fully inhibited when DO increased to >1–4 mg L^{-1} (Tallec et al., 2008; Wang and Chu, 2016). On the contrary, in this study, significant NO_3^- reduction was still observed

at elevated DO levels (1–3 mg L^{-1}) and this was probably attributed to the quick consumption of O_2 at woodchip surface, which led to a thin layer of anoxic micro-environment to facilitate denitrification. However, this was hardly detected by the contactless DO sensors, which were attached to the side wall of incubation bottles. Efficient denitrification (10–12 g N m^{-3} d^{-1}) with the presence of O_2 (>2 mg L^{-1}) was also reported in an oxic-anoxic cycling woodchip bioreactor treating synthetic wastewater (McGuire et al., 2021). On the other hand, reduced NO_3^- removal rate (47–69 %) and decreased NO_2^- reductases (*nirS* + *nirK*) abundance (37–55 %) were observed at 1–3 mg DO L^{-1}, compared with the no DO groups, suggesting the presence of O_2 inhibited the denitrification process by suppressing NO_2^- reduction process (Fig. 4b, c, Table 1, S5 and S7). When woodchip bioreactors were used to treat wastewater containing high DO (3 - 8.5 mg L^{-1}), such as tile drain water or nitrified wastewater effluent, a reduction of NO_3^- removal rate was expected (Chen et al., 2022b; Christianson, 2011).

The presence of high DO also induced N_2O production (179.5–243.6 mg N_2O—N m^{-3}) in both aged and fresh woodchips during the incubation (Fig. 2). This could be explained by the different impacts of DO on N_2O reductase (*nosZ*) and NO reductase (*norB*). Compared with the no DO group, 115–680 % higher *norB* abundance and 35–43 % lower *nosZ* abundance were observed at high DO (Fig. 4d, e, Table S5 and S7). These results indicated the presence of DO may promote the growth of NO reducing microorganisms but inhibit the growth of N_2O reducing microorganisms. Higher *norB/nosZ* ratios were observed in the high DO group (0.9–2.4) than that in the no DO group (0.2–0.3), suggesting a higher fraction of N_2O generated by *norB*-containing microorganisms may be converted to N_2 by *nosZ*-containing microorganisms at anaerobic condition (Table S5). The impact of O_2 on NO reducing microorganisms was rarely reported in WWTPs or OWTSs. Previous work only demonstrated that the increased N_2O emission at high DO was majorly attributed to the higher sensitivity of N_2O reducing microorganism to O_2 change than NO_2^- reducing bacteria (Otte et al., 1996). That was indicated by quicker reduction of *nosZ* abundance than *nirS*+*nirK* level during the transition of anaerobic to aerobic conditions in a pure acetate-limiting bacterial culture (Otte et al., 1996). However, in this study we observed similar (*nirS*+*nirK*)/*nosZ* ratios in both high DO group (2.9–3.2) and no DO group (2.8–3.0) (Table S5). These results indicated the *norB/nosZ* ratio was a good biomarker to indicate N_2O emission from woodchip bioreactors, which agreed with results in a previous study that *norB/nosZ* was positively correlated with N_2O production (Warneke et al., 2011), because these two functional genes were directly related to the generation and consumption of N_2O during denitrification. Although limited studies reported the impact of DO on N_2O emission from woodchip bioreactors, a similarly positive relationship between DO (0–1 mg L^{-1}) and N_2O production (0–60 μg N_2O—N g^{-1} suspended solid hr^{-1}) was observed in the denitrification zone of a municipal WWTP (Tallec et al., 2008). Therefore, to reduce the potential of N_2O production from the OWTS, it is recommended to keep anaerobic/anoxic condition in woodchip bioreactors.

In addition, the presence of DO has different impacts on woodchips at various ages. A significantly lower CH_4 production rate was observed in fresh woodchips at high DO (1.0–3.9 g CH_4—C m^{-3} d^{-1}) compared with that at no DO (6.1–8.8 CH_4-C $m^{-3}d^{-1}$) (Table 1), supported by the over 100 % higher abundance of *mcrA* in the no DO group (Fig. 4f, Table S5 and S7). However, in aged woodchips, similar CH_4 production rate was observed at different DO levels (Table 1), supported by the microbial data that approximately the same level of *mcrA* was observed with or without DO presence. The collective results indicated that DO was the limiting factor for methanogenesis when sufficient labile carbon was available in fresh woodchips. However, after long-term (>5 years) operation, CH_4 production was primarily controlled by carbon availability rather than DO.

4.1.3. Woodchip age

A significant decrease (75–85 %) of NO_3^- removal rate was observed in aged woodchips compared with fresh woodchips during the incubation (Table 1). Similar observations (40–60 % loss of NO_3^- removal rate) were reported in a 7-year woodchip bioreactor treating agricultural wastewater (Robertson, 2010). The reduced NO_3^- removal rate in aged woodchips may be attributed to the consumption of labile carbon by various reduction/oxidation processes which limited the carbon availability for denitrification (Robertson, 2010). In this study, around 8.7 % carbon in aged woodchip was consumed during the 5-year operation of a continuous flow woodchip reactor treating nitrified onsite wastewater (Table S6) (Chen et al., 2022b). This result was comparable with the carbon loss (5–11 %) in woodchips after 4–7 years of treatment of onsite wastewater (Ghane et al., 2018; Moorman et al., 2010). The loss of carbon in aged woodchip was majorly contributed by the consumption of labile carbon and may lower the woodchip reactivity. Previous literature reported 30–80 % greater Lignocellulos Index (LCI), which was calculated as the fraction of bio-refractory lignin in woodchip and was negatively correlated with the woodchip bioavailability, in woodchip bioreactors treating agricultural drainage after 4–7 years of operation (Feyereisen et al., 2016; Ghane et al., 2018; Moorman et al., 2010). The lower reactivity of aged woodchip may reduce the carbon release rate from woodchip to the liquid phase, decreasing the C:N ratio for denitrification and may explain the lower NO_3^- removal rate in the aged woodchip during the incubation experiment. However, in this study, the mass and characteristics of carbon released from woodchip were difficult to measure during the incubation experiment. The carbon concentration during the incubation can only represent the net change of organic matters caused by the carbon release from woodchip and carbon consumption by a series of biological reduction/oxidation processes. The release of carbon from woodchip with different ages should be solely characterized in the future study to get a deeper understanding of the impact of woodchip age on denitrification.

However, the reduced NO_3^- removal rates observed in aged woodchips may not necessarily indicate lower NO_3^- removal efficiencies over long-term (>5 years) operation. Consistent high NO_3^- removal efficiency (50–90 %) has been reported in woodchip bioreactors treating onsite and agricultural wastewater over 5–9 years of operation (Gobler et al., 2021; Moorman et al., 2010; Robertson et al., 2000; Schipper and Vojvodić-Vuković, 2001). Those woodchip bioreactors were designed with long HRTs (7–13 days) which provided sufficient reaction time for denitrification and offset the negative effect of reduced NO_3^- removal rate. The impact of woodchip age on N_2O production was observed only in the high DO group. Fresh woodchips generated 26 % less N_2O compared with aged woodchips at high DO, possibly because the consumption of labile carbon by aerobic respiration reduced the carbon availability for denitrification in aged woodchips, led to incomplete reduction of NO to N_2O (Fig. 2). However, comparably low level of N_2O was observed in both aged and fresh woodchips without DO, which may be attributed to the less competition of aerobic respiration and denitrification for labile carbon. The impact of woodchip age on N_2O emission observed in this study was also supported by the microbial data that same level of *norB/nosZ* ratio (0.3) was observed in both aged and fresh woodchips without DO, while fresh woodchips had a lower *norB/nosZ* ratio (0.9) than aged woodchips (2.4) at high DO condition (Table S5). These results emphasized that *norB/nosZ* ratio could serve as a biomarker to indicate N_2O emission from woodchip bioreactors. The results also indicated woodchip bioreactors could release a higher level of N_2O treating wastewater with high DO levels (>1 mg L^{-1}) wastewater after long-term (>5 years) operation, which have not been well investigated by previous studies.

The higher CH_4 production rate and higher *mcrA* abundance observed in fresh woodchips (Figs. 3, 4f, Tables 1 and S7) suggested that higher CH_4 production may be observed initially when woodchip bioreactors were used to treat onsite wastewater, then the level would decrease over time due to reduced carbon availability. Similar results were reported in subsurface wastewater infiltration systems treating onsite wastewater and anaerobic sludge digestion process that increased

organic loading (3.3 g VS L^{-1} d^{-1}) and higher C:N ratio (16:1) resulted in 1–5 times higher CH_4 generation and 1–2 magnitude higher *mcrA* abundance (Berninghaus and Radniecki, 2022; Zhang et al., 2023). However, the production of CH_4 from field woodchip bioreactors treating onsite wastewater over long-term (>5 years) operation was rarely reported by previous literatures and shall be evaluated in future research.

4.2. Environmental implications

4.2.1. Design optimization

In this study, the DO and woodchip age showed different importance in controlling the GHG emission and NO_3^- removal. Fresh woodchip showed even higher CH_4 production rate and NO_3^- removal rate at high DO condition than aged woodchip incubated at no DO, indicating that woodchip age played a more important role in controlling the NO_3^- removal and CH_4 production in woodchip bioreactors treating onsite wastewater. However, both aged and fresh woodchips showed significantly lower N_2O emission at no DO compared with high DO. This result suggested that the N_2O emissions from woodchip bioreactors were mainly controlled by DO rather than woodchip age. Based on these results, the operational strategy for woodchip bioreactors can be optimized for efficient NO_3^- removal. The woodchip replacement frequency should be increased to achieve more efficient NO_3^- removal. In order to reduce the DO levels in woodchip bioreactors for lower N_2O emission, low hydraulic loading could be applied to limit the transport of atmospheric O_2 to the system and saturated condition (i.e., up-flow pattern) was preferably utilized for the design of woodchip bioreactors because it can inhibit natural reaeration within the internal pore space of woodchip (Gobler et al., 2021; Greenan et al., 2009; Schaefer et al., 2021).

In addition, the results of this study indicated that extended HRT played an important role in controlling N_2O emission and maintaining effective NO_3^- removal from onsite wastewater. Conservative reactor size, internal recycling, and lower hydraulic loading could ensure sufficient HRT for complete denitrification and minimize the production of N_2O. Although longer HRT, increased woodchip replacement frequency and lower DO may promote CH_4 production, CH_4 was easily to be removed via natural processes. Natural soil showed high potential to remove CH_4 and was reported to be responsible for 94 % of global CH_4 sink capacity (Wuebbles and Hayhoe, 2002). Since woodchip bioreactors were usually installed underground for onsite wastewater treatment, the released CH_4 could be removed by the top soil.

4.2.2. Greenhouse gas analysis optimization

In this study 17–64 % N_2O and 3–26 % CH_4 were detected in dissolved form during the incubation experiment, indicating a significant portion of N_2O and CH_4 was released in gaseous form (Figure S6 and S7). This result also agreed with the observation in another gas tracer test that significant N_2O (16 %) and CH_4 (58 %) were partitioned in the gas-filled void of woodchip bioreactor and may be finally released to the atmosphere (McGuire and Reid, 2019). However, most woodchip bioreactors studies analyzed only the dissolved portion of CH_4 and N_2O in aqueous samples and ignored the gaseous portion (Christianson et al., 2013; David et al., 2016; Davis et al., 2019), leading to an underestimate of the overall GHG emission potential. A close floating chamber, which was extensively utilized for gas capturing from activated sludge tanks, could be applied for gas collection from woodchip bioreactors to provide a more precise evaluation of GHG emission (Czepiel et al., 1995).

Although the incubation experiment was designed to simulate the operation condition of field woodchip bioreactors treating nitrified wastewater containing different DO levels, limitations of the batch incubations may impact the accuracy of GHG evaluation. For example, in this study, the incubation bottles were continuously homogenized on a shaker table which simplified the flow pattern. However, in most field woodchip bioreactors, the flow pattern was a combination of plug flow which was attributed to the advection of water flow and continuous mixed flow due to the dispersion of the turbulence (Halaburka et al., 2017), which may result in decreased NO_3^- and DO concentrations and various GHG production rates alongside the reactor (Halaburka et al., 2017; Waugh et al., 2020). In addition, seasonal changes (e.g., temperature) have a strong impact on denitrification and methanogenesis activity which was not captured in the current study (David et al., 2016). Hence, continuous monitoring of GHG at multiple depths of field woodchip bioreactors could be employed for more accurate quantification of N_2O and CH_4 from woodchip bioreactors at wider spatial and temporal scales.

4.2.3. Microbial analysis optimization

This study correlated the GHG emission and denitrification with microbial organisms by quantifying the functional genes in DNA samples. However, the quantification of functional genes abundances may not represent the active function changes of microorganism because the DO and woodchip age can impact the activity of methanogens and denitrifying microorganisms without necessarily changing the abundance of functional genes (Ferrera and Sánchez, 2016). Both DNA and RNA analysis to determine changes in gene activity resulting from environmental and physical alterations should be conducted in future studies.

5. Conclusion

The batch incubation experiment conducted in this study unraveled the impact of DO and woodchip age on NO_3^- removal, N_2O and CH_4 emission from woodchip bioreactors treating onsite wastewater. Woodchip age negatively impacted NO_3^- removal and CH_4 production, which was suggested by decreased NO_3^- removal rate (8.8–16.6 g NO_3^--N $m^{-3}d^{-1}$) and CH_4 production rate (0.1–1.4 g CH_4-C $m^{-3}d^{-1}$) in aged woodchips. These results may be attributed to the inhibited growth of NO_2^- reducing microorganisms and methanogens in aged woodchips due to the limitation of available carbon. High DO (1–3 mg L^{-1}) inhibited NO_3^- removal (8.8–16.6 g NO_3^--N $m^{-3}d^{-1}$) by suppressing the growth of NO_2^- reducing microorganism (37–55 % reduction of *nirS*+*nirK* abundance), while promoted N_2O emission (179.5–243.6 mg N_2O—N m^{-3}) by increasing the abundance of NO reducing microorganisms (1–7 times higher *norB* level) and inhibiting the growth of N_2O reducing bacteria (53–75 % lower *nosZ* abundance). However, high DO had a negative impact on CH_4 production in fresh woodchips, while showed insignificant impact on CH_4 production in aged woodchips. Collectively, the results suggested that for onsite wastewater treatment, woodchip bioreactors should be designed with long HRT (>2–5 days) and be operated at anaerobic conditions to achieve efficient NO_3^- removal and minimize N_2O emission for long-term (>5 years) treatment.

CRediT authorship contribution statement

Siwei Chen: Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mian Wang:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Maggie Wu:** Methodology, Investigation, Formal analysis. **Yuhang Lu:** Formal analysis, Data curation. **Ao Fu:** Methodology, Data curation. **Christopher J. Gobler:** Project administration, Funding acquisition. **Caitlin Asato:** Data curation, Formal analysis, Methodology. **Xinwei Mao:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Xinwei Mao reports financial support was provided by New York State Department of Environmental Conservation. Xinwei Mao reports

financial support was provided by National Science Foundation. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

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