

NITROUS OXIDE AND METHANE EMISSIONS FROM DENITRIFYING BIOREACTORS
WITH AND WITHOUT A SOIL COVER

BY

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THESIS

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ABSTRACT

Denitrifying woodchip bioreactors reduce nitrate loads in subsurface agricultural drainage water, thus reducing agriculture's impact on water quality. However, bioreactors can emit the greenhouse gases nitrous oxide (N_2O) and methane (CH_4) as unwanted byproducts both through the bioreactor surface and as dissolved gas in the liquid outflow. The current United States Department of Agriculture design standard for denitrifying bioreactors allows flexibility as to whether a bioreactor is built with or without a soil cover, but it is unknown how greenhouse gases vary with or without a soil cover at the field-scale in real-world conditions. The objective of this study was to compare both surface and dissolved N_2O and CH_4 emissions at three full-scale bioreactors with a soil cover versus three without a soil cover in Illinois, USA to determine the impact of a soil cover on bioreactor greenhouse gas emissions. Each of the six bioreactors were monitored at least five times over March to August 2021 for: surface N_2O and CH_4 emissions; bioreactor flow rate; inflow and outflow dissolved N_2O and CH_4 and nitrate-nitrogen; and inflow and outflow temperature, dissolved oxygen (DO), and oxidation reduction potential. Hydraulic retention times varied widely between sites (means 1.8 to 21.6 h) but did not equally span the two treatments, preventing meaningful comparison of the covered vs. uncovered treatments and resolution of the stated objective; other trends outside of the two treatments were instead analyzed. Across all six sites, combined (surface and dissolved) mean nitrous oxide emissions (-1.57 to 14.5 mg $\text{N}_2\text{O}\text{-N}/\text{m}^3\text{-d}$) were 1-3 orders of magnitude lower than mean nitrate mass removal (0.291 to 2.97 g $\text{NO}_3^-\text{N}/\text{m}^3\text{-d}$). Methane emissions (mean -1.16 to 412 mg $\text{CH}_4\text{-C}/\text{m}^3\text{-d}$) were nearly negligible from bioreactor surfaces, and like N_2O , were mostly observed in the dissolved form. Methane and nitrous oxide approximately equally contributed to an impact on climate change, but methane was only released in noticeable quantities at half the bioreactors. Bioreactor #4 – No Cover consistently removed both N_2O and CH_4 due to low loading as well as possible young age impacts. Inducing low inflow DO levels or targeting a mid-range design HRT could mitigate climate change impacts from greenhouse gas emissions at subsurface drainage denitrifying bioreactors.

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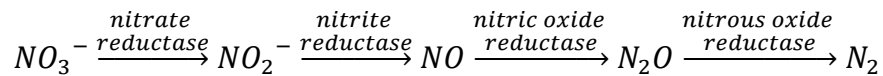
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CHAPTER 1: INTRODUCTION

Nutrient export to natural water bodies is an increasingly recognized global issue. Modern agricultural modifications have increased anthropogenic nutrient export to the environment, with agriculture accounting for about 80% of riverine nitrate-nitrogen export in the state of Illinois, USA (IEPA and IDOA, 2015). Such cropland modifications in the U.S. Midwest include the application of nitrogen-based fertilizers and the use of subsurface drainage to decrease over-saturation of wet fields. Although subsurface drainage increases crop yield, it also short-circuits natural processes that attenuate nutrients in the soil, thus increasing nutrient export to water bodies via dissolved nitrate and phosphorous in the drainage water (Randall and Goss, 2008). Elevated nitrate and phosphorous levels can lead to eutrophic and hypoxic conditions that can cause dead zones, fish kills, algae blooms, and threats to human health (US EPA, 2021). In the United States, nutrient-rich flow draining from the Mississippi River Basin creates an annual hypoxic “dead” zone in the Gulf of Mexico. The Illinois Nutrient Reduction Strategy, created in 2015 to direct the Illinois response to this issue, recognizes denitrifying bioreactors as a conservation practice for water quality improvement in the state.

Denitrifying bioreactors reduce nitrate-nitrogen export in watersheds via the process of denitrification, or the reduction of nitrate-nitrogen to dinitrogen gas (Schipper et al., 2010; Addy et al., 2016; Christianson et al., 2021). Bioreactors are trenches filled with carbonaceous material, typically woodchips, which receive nitrate-laden water. The woodchips provide habitat, a carbon source and electron donors for the microbes; the drainage water provides electron acceptors (in this case N-oxides such as NO_3^-), and stop logs regulate hydraulic retention to facilitate an anoxic environment (Christianson et al., 2012). Denitrification is mediated by microbes living in the pores of the woodchips in the following pathway:



where the labels indicate enzymes responsible for completing the chemical reduction. Common functional genes for nitrite (NO_2^-) reductase include *nirS* and *nirK* and *nosZ* for nitrous oxide (N_2O) reductase (Healy et al., 2015). Since different enzymes are responsible for each reduction step, different microbes may perform different steps of the denitrification process depending on genes expressed (Warneke et al., 2011c; Griebmeier and Gescher, 2018). Sometimes the process

of nitrate reduction to dinitrogen gas remains incomplete, resulting in the production of nitrous oxide (N₂O), a gas with 298 times the global warming potential of carbon dioxide (IPCC, 2007) or 265 times the potential accounting for a 100-y adjustment and ignoring carbon climate feedbacks (Myhre et al., 2013).

A handful of studies have explored the possibility of denitrifying woodchip bioreactors to produce nitrous oxide as a tradeoff of water treatment. Two main summary findings are that (1) the majority of nitrous oxide losses are in the dissolved form rather than via bioreactor surface fluxes, and (2) total nitrous oxide emissions (surface + dissolved) are consistently less than 6% of nitrate removed (Christianson et al., 2021). A pilot-scale study that measured emissions across 5.8 m long bioreactors found the greatest emissions in the first 1.4 m, with a correlation between decreasing N₂O emissions at decreasing dissolved oxygen (DO) concentrations below 2.0 mg/L (Davis et al., 2019). The same study tested hydraulic retention times (HRTs) of 2, 8, and 16 h and found the greatest nitrous oxide emissions at the lowest HRTs (Davis et al., 2019). A column study involving HRTs from 3.7-17.4 d also found that nitrous oxide emissions from woodchip bioreactors were greatest (7.1 mg N₂O-N/m²-d) at low HRTs (Healy et al., 2015). Higher temperature has been shown to increase bacteria containing *nirS* (nitrite reductase gene) and lacking *nosZ* (nitrous-oxide reductase gene) in bioreactor barrels, possibly explaining increased nitrous oxide emissions at higher temperatures (Warneke et al., 2011c).

Additionally, microbes in bioreactors may perform methanogenesis, using carbon dioxide as electron acceptors to produce methane, a gas 28 times more potent than carbon dioxide (Myhre et al., 2013). Methanogenesis, a strictly anaerobic, less energetically favorable process compared to denitrification, occurs at oxidation-reduction potentials of < -200mV (Le Mer and Roger, 2001), generally once other electron donors such as nitrate are depleted. However, methanogenesis and nitrate reduction have occurred simultaneously in bioreactors (Rivas et al., 2020). Methane emissions have been observed to increase at higher retention times (Davis et al., 2019) and low hydraulic loading (Elgood et al., 2010). There have been mixed results concerning the significance of methane emissions compared to nitrous oxide emissions, with Elgood et al. (2010) concluding that CH₄ emissions were ~20 times more potent than N₂O emissions while Bock et al. (2018) concluded that greenhouse gas emissions were driven by N₂O, not methane.

Current bioreactor practice standards allow flexibility in choosing to cover a field bioreactor with soil or to allow the presence of woodchips all the way to the surface (NRCS,

2020). The presence of such a soil cover could influence, and possibly mitigate, bioreactor nitrous oxide emissions. A pilot-scale study evaluating surface nitrous oxide emissions at different flow regimes showed significantly lower surface fluxes from bioreactors with a soil cover compared to those without (0.05 vs 0.40 mg $\text{N}_2\text{O-N/m}^2$ -h, respectively) (Christianson et al., 2013). More recently, Manca et al. (2021) showed the same result for bench-scale bioreactor surface emissions and reported dissolved emissions were unaffected by the presence of a soil cover. Researchers hypothesized the soil cover induced N_2O to reside longer in the liquid phase and complete full denitrification. Neither of these studies addressed methane emissions. Recently increased interest in “climate-smart” agriculture in tandem with continued pressure to achieve Mississippi River Basin water quality goals sets an important stage to better understand denitrifying bioreactor nitrous oxide and methane emissions (surface + dissolved), especially from the perspective of controllable design factors like the presence of a soil cover.

The objective of this study was to compare both surface and dissolved N_2O and CH_4 emissions at three full-scale bioreactors with a soil cover versus at three full-scale bioreactors without a soil cover in Illinois, USA. It was hypothesized that the treatment group with soil covers would have lower surface N_2O and CH_4 emissions than the non-cover treatment because of 1) N_2O transformation in the soil cover and/or 2) because the soil cover would reduce gas transfer and force more dissolved N_2O and CH_4 out of the bioreactor in the liquid outflow.

CHAPTER 2: METHODS

2.1 Bioreactor descriptions

Three full-size bioreactors in Illinois, USA with a soil cover and three without were monitored from March to August 2021 for nitrate removal and greenhouse gas production (Fig. 1, Table 1). Four of the six bioreactors were designed by the United States Department of Agriculture Natural Resources Conservation Service (USDA NRCS) according to the Conservation Practice Standard (CPS) 605 (bioreactors #3 through #6; Table 1). Wickramaratne et al. (2020) previously described tannic acid leaching from bioreactors #1, 5, and 6, and Dougherty et al. (2020) previously described nitrate removal from 2017-2020 at bioreactor #1.

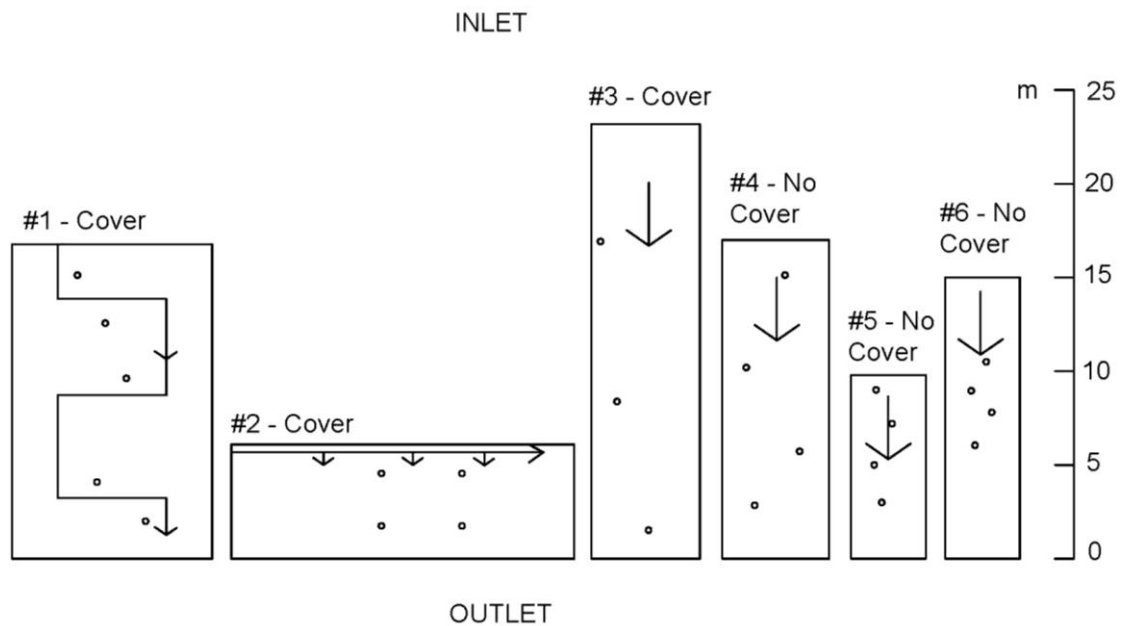


Figure 1. Scale drawings of the six bioreactors. The circles represent surface chamber locations, and the arrows indicate flow direction. Note that bioreactors 1 and 2 have non-traditional flow patterns.

Table 1. Summary of the six bioreactors. Note that bioreactors 1 and 2 have non-traditional flow patterns.

<i>Site</i>	<i>Soil cover</i>	<i>Bioreactor Installation date</i>	<i>Dimensions (LWD)</i>	<i>Drained area</i>	<i>NRCS standard design</i>	<i>Design notes</i>
	--		<i>m</i>	<i>ha</i>		
#1 - Cover: Dudley Smith Farm	Yes	Oct 2016	16.8 x 10.7 x 0.91	14.2	No	Included two flow-routing baffles
#2 - Cover: Northwest Research and Education Center	Yes	Aug 2017	6.1 x 18 x 1.8	29	No	Longer width than length
#3 - Cover: Private Farm	Yes	Aug 2017	23 x 5.8 x 1.1	20	Yes	
#4 - No Cover: Private Farm	No	Nov 2020	16.5 x 4.9 x 1.5	8.1	Yes	Treated drainage from an organic-transition farm
#5 - No Cover: Private Farm	No	Sep 2017	9.8 x 3.7 x 0.9	6.9	Yes	
#6 - No Cover: Private Farm	No	Aug 2018	15.24 x 3.66 x 0.91	6.9	Yes	

Woodchip median particle diameters (D_{50}) and drainable porosities ranged from 13.2 - 23.0 mm and 40.7 - 44.6%, respectively, for five of the six bioreactors (Table 2). Woodchips from bioreactor #3 No Cover were not evaluated because it was constructed prior to lab involvement; a lab-based average value of 42.9% was used for drainable porosity at that site. Drainable porosity was estimated in the lab in triplicate for each woodchip type using a 1-L container, which were later corrected based on compaction testing (Johnson et al., under review 2022). Drainable porosity was used to calculate bioreactor daily HRT where HRT was the product of saturated bioreactor volume and woodchip drainable porosity divided by the flow rate based on on-site water depth readings. Particle size analysis was performed in triplicate following standard methods (ANSI/ASABE, 1992).

Table 2. Woodchip properties for the six bioreactors. For the D_{50} , 50% of the particles were smaller than the size listed in the table.

<i>Site</i>	<i>Drainable porosity (%)</i>	<i>D_{50} (mm)</i>	<i>Woodchip material</i>	<i>Reference if previously reported</i>
#1 - Cover: Dudley Smith Farm	44.6	14.9	Playground chips	Wickramarathne et al., 2020; Sanchez Bustamante Bailon, 2020; Dougherty et al., 2020
#2 - Cover: Northwest Research and Education Center	40.7	23.0	Playground chips, small chips	
#3 - Cover: Private Farm	42.9*	-	Pallet recycling chips	
#4 - No Cover: Private Farm	42.5	13.9	80/20 maple/oak	Johnson, 2021
#5 - No Cover: Private Farm	43.1	16.3	Maple	Dougherty, 2018; Wickramarathne et al., 2020; Sanchez Bustamante Bailon, 2020
#6 - No Cover: Private Farm	42.3	13.2	Maple	Wickramarathne et al., 2020; Sanchez Bustamante Bailon, 2020

*Estimated based on compaction-corrected mean of NRCS-approved woodchips.

2.1.1. #1 - Cover (Dudley Smith Farm bioreactor)

The Dudley Smith bioreactor (LWD: 16.8 x 10.7 x 0.91 m, Table 1; Fig. 2) was a covered bioreactor built in fall 2016 in Christian Co., Illinois, USA. A geotextile fabric separated the woodchips from the soil cover, which was ~0.9-1.0 m thick and composed of Virden silty clay loam soil. The bioreactor received drainage from 14.2 ha of research plots planted in continuous corn (*Zea mays L.*). It was not designed to the NRCS standard, and its most novel feature was two flow-routing baffles intended to increase the flow path (estimated 26 m; Fig. 2). Annual nitrate removal ranged from 3.0 - 4.4 kg N/ha, or 22 - 24%, in past work (Dougherty et al., 2020). During the current study, inflow control structure stop logs were set at 16 cm until 08 June 2021 then moved to 32 cm until the end of the study period; outflow control structure stop logs were set at 16 cm throughout the entire period.

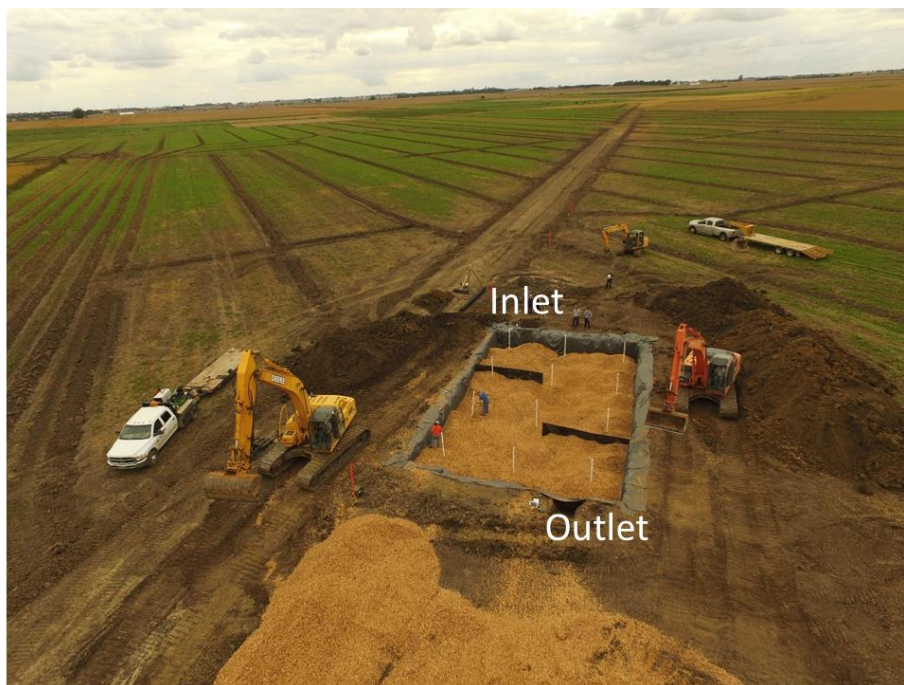


Figure 2. Bioreactor #1 – Cover: Dudley Smith Farm during construction in October 2016. The black plastic plates were the baffles inside the bioreactor. Photograph was taken before placement of the soil cover. Courtesy: N.D. Bowman, Illinois Extension.

2.1.2. #2 - Cover (Northwest Research and Education Center bioreactor)

The Northwest Research and Education Center bioreactor (LWD: 6.1 x 18 x 1.8 m, Table 1; Fig. 3) was a covered bioreactor in Warren Co., Illinois, USA that was a part of a paired-bioreactor design constructed in 2017. A geotextile fabric protected the woodchips from the ~1.5 - 2 m thick Muscatine silt loam and Cisco silt loam soil cover. The studied bioreactor was the “main” or “baseflow” bioreactor of the pair, which received the majority of flow; a booster bioreactor, which was not studied here, treated bypass flow. This research bioreactor was designed with a length-to-width ratio (L: W) of 1.6:1, which was lower than the 4:1 ratio recommended by the NRCS CPS 605. This paired bioreactor system received drainage from 28.6 ha of research plots, 21.4 of which were in corn and 7.1 of which were in soybeans (*Glycine max* (L.) Merr.) during the study period. Control structure stop logs were set at 49 cm and 14 cm for the inflow and outflow, respectively. Rainfall data was collected onsite by a National Weather Service cylinder rain gauge and Nimbus digital thermometer.

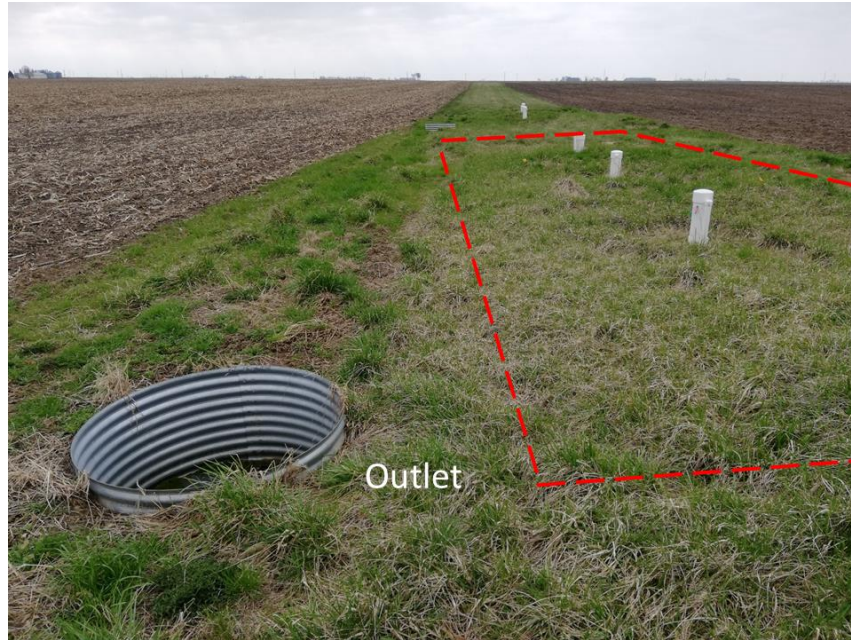


Figure 3. Bioreactor #2 – Cover: Northwest Research and Education Center bioreactor (Monmouth, Illinois) with dashed lines indicating approximate bioreactor location. The PVC pipes were woodchip bag ports and monitoring wells that were not used in this study. The inflow control structure was near the top right of the grassed area.

2.1.3. #3 - Cover (Private bioreactor)

Bioreactor #3 – Cover (LWD: 23 x 5.8 x 1.1 m, Table 1; Fig. 4) was a covered bioreactor located in Peoria Co., Illinois, USA installed in summer 2017 to the NRCS CPS 605. A geotextile fabric separated the woodchips from the ~1 m thick Sylvan silty clay loam soil cover. This was the longest bioreactor included in this study, although its L: W was consistent with the NRCS CPS. The bioreactor received drainage from a private field in a corn-soybean rotation planted in corn in 2021. Control structure stop logs were set at 93 cm and 14 cm for the inflow and outflow, respectively. Rainfall data was collected from a neighboring farm rain gauge. This was the only site to have a surface gas chamber measuring nearby grassed soil, which was installed and had vegetation removed in the same way as the soil cover chambers (section 2.2.1).

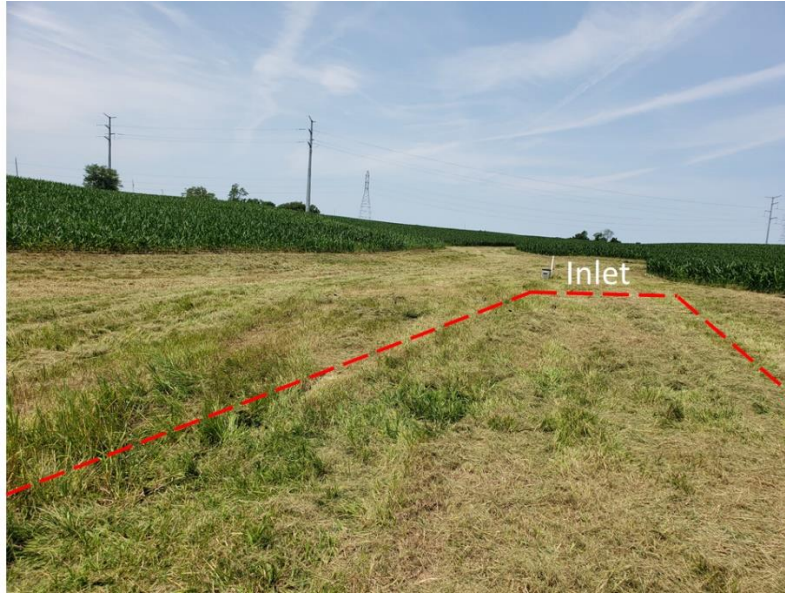


Figure 4. Bioreactor #3 - Cover pictured with dashed lines indicating approximate bioreactor location. The outflow structure was behind and to the left of the camera.

2.1.4. #4 - No Cover (Private bioreactor)

Bioreactor #4 – No Cover (LWD: 16.5 x 4.9 x 1.5 m, Table 1; Fig. 5) in Peoria Co., Illinois, USA was uncovered. It was installed to the NRCS CPS 605 in fall 2020 and was in its first year of operation during the study period. The bioreactor received drainage from a private transitioning-organic plot planted in clover (*Trifolium pretense*). Control structure stop logs were set at 82 cm and 49 cm for the inflow and outflow, respectively. Rainfall data was gathered on site via rain gauge by the landowner.



Figure 5. Bioreactor #4 - No Cover shown with the inflow control structure near the two people pictured. The outflow was to the right of the camera.

2.1.5. #5 - No Cover (Private bioreactor)

Bioreactor #5 – No Cover (LWD: 9.8 x 3.7 x 0.9 m, Table 1; Fig. 6) installed in fall 2017 in Bureau Co., Illinois, USA was uncovered and designed to the NRCS CPS 605. It received drainage from a 6.9 ha private farm planted in a corn-soybean rotation with soybeans in the 2021 season. Control structure stop logs were set at 66 and 59 cm for the inflow and outflow, respectively.



Figure 6. Bioreactor #5 - No Cover with the outflow control structure identified at the top left. The inflow control structure was to the left of the camera.

2.1.6. #6 - No Cover (Private bioreactor)

Bioreactor #6 – No Cover (LWD: 15.24 x 3.66 x 0.91 m, Table 1; Fig. 7) installed in summer 2018 in Bureau Co., Illinois, USA, was uncovered and designed to the NRCS CPS 605. It received drainage from a 6.9 ha private farm planted in a corn-corn-soybean rotation with soybeans planted during the study period. Inflow and outflow control structure stop logs were both set at 44 cm during the study period. There was a drawdown plate with orifice flow as described by Maxwell et al. (2021) at the outflow. Rainfall data was gathered from the NOAA weather station in Geneseo, IL (GHCND: USC00113384).



Figure 7. Bioreactor #6 - No Cover pictured from the side. The inflow control structure was to the left of the camera and the outflow was forward and to the right.

2.2 Gas measurement

Nitrous oxide, methane, and carbon dioxide were captured from both the surface of the bioreactors and as dissolved gas from the inflow and outflow control structures of the bioreactors. Samples were collected at each bioreactor at least seven times from mid-March 2021 through August 2021. The sampling plan was designed to capture each bioreactor over a range of flow conditions, from relatively high flow in the late spring to low flow in the summer. Because each bioreactor had a different design and volume, the overall aim was to capture observations from the two treatments (cover versus no cover) under a relatively similar range of hydraulic retention times, albeit this was ultimately beyond experimental control.

2.2.1 Surface gas measurement

Each bioreactor was fitted with three to five 31 cm diameter PVC static chambers across the length of the bioreactor (Table 3; Fig. 1). Each chamber consisted of a base that was installed into the ground and left in place throughout the sampling period and a cap that was placed on top during a sampling event. The chambers were constructed per recommendations in the 2010 USDA-ARS GRACEnet Project Protocols (Parkin and Venterea, 2010). Temperature and pressure perturbations were minimized with reflective tape on the chamber top and a chamber

vent, respectively. Four of the bioreactors had surface chambers installed two years prior (2019) for previous research (unpublished; Bioreactors #1, 2, 5, and 6), which were reused here after resecuring. Chamber rings were newly installed at Bioreactors #3 and #4 more than 24 hours in advance of the first sampling event (Parkin and Venterea, 2010). Bioreactor #2 had chambers in a square pattern with duplicates at the same longitudinal distance from the bioreactor edge inflow (Table 3; Figure 1).

Table 3. Estimated longitudinal distances of surface gas flux chambers (C) from upstream bioreactor edge inflow at six bioreactors in Illinois, USA.

<i>Site</i>	Longitudinal distance of chambers from upstream bioreactor edge (m)					<i>Chamber installation date</i>
	<i>C1</i>	<i>C2</i>	<i>C3</i>	<i>C4</i>	<i>C5</i>	
#1 - Cover: Dudley Smith Farm	1.6	4.2	7.1	14	16	Spring 2019
#2 - Cover: Northwest Research and Education Center	3.9	1.1	1.1	3.9	-	Spring 2019
#3 - Cover: Private Farm	6.3	15	22	-	-	26 Apr. 2021
#4 - No Cover: Private Farm	1.9	6.8	11	14	-	26 Apr. 2021
#5 - No Cover: Private Farm	0.8	2.6	4.8	6.8	-	Spring 2019, adjusted 28 Apr. 2021
#6 - No Cover: Private Farm	4.5	6.1	7.2	9.0	-	Spring 2019, adjusted 28 Apr. 2021

Chambers at Bioreactors #3 (covered) and 4 (uncovered) were installed by first removing debris and vegetation from the chamber location. For the uncovered site, a ~10 cm deep hole was dug, in which the chamber was placed and sealed with water-soaked bentonite (Fig. 8). Woodchips then covered the bentonite and were filled inside to make the woodchip height even inside and outside. For the covered site, a ring for the chamber was dug instead of a hole to minimize soil disturbance. Once the ring was deep enough (~10 cm depth) and the chamber placed inside, soil was packed into the edges.



Figure 8. Chamber base installation at an uncovered site. Bentonite surrounded the edges, was soaked, and then was covered with woodchips.

Surface gas vials consisted of a 10 mL glass vial (Fisherbrand™ Headspace Vials, 20mm, 10mL), a gray butyl stopper (20 mm round bottom vial stopper), and a crimp cap (20 mm Hole punch seal). Gas vials were prepared within a week prior to sampling by evacuating using a 25G needle either to 250 mbar or for a time of 140 seconds, depending on the vacuum pump used (Welch 8905 or 2546B-01; Mt. Prospect, IL, USA).

The headspace height was measured at four locations (approximately 0°, 90°, 180°, and 270° around the base) in each chamber prior to each sampling (Fig. 9). These heights averaged 5.03 ± 0.22 cm across all chambers during the study (range: 4.4 - 6.1 cm). At the start of sampling, the chamber top was installed on the first chamber, sealing it tight with the tire tube connector. The syringe and needle (BD 20 mL Luer-Lok syringe, 20G1 needle) were flushed with atmospheric air twice before drawing each sample. The first 15 mL gas surface sample was drawn from the air immediately outside the chamber since each sample withdrawn from the chamber dilutes the analyte in the chamber headspace (Parkin and Venterea, 2010). After an interval ranging from 1-2 minutes, the next chamber top was installed, and a sample drawn, which was repeated for all chambers. Thirty minutes after the first chamber was closed, a second 15 mL sample was drawn from within each chamber through a sampling port. This process was repeated at 60 minutes to form a 0-, 30- and 60-minute time series.



Figure 9. Headspace measurement of a chamber before sampling. Chamber heights were measured at approximately 0°, 90°, 180°, and 270° around the chamber base before each sampling event.

Other bioreactor surface data included bioreactor surface temperature, relative humidity, woodchip temperature, and soil temperature (Traceable Hygrometer humidity and temperature meter, Fisher Scientific; Waltham, MA, USA; #1- Cover used HOBOLink weather data).

Sampling was intended to be completed between 9 a.m. – 2 p.m. to limit diurnal flux variations (Parkin and Venterea, 2010), but the distance to some sites resulted in actual sampling times between 8:30 a.m. – 4:00 p.m.

2.2.2 Dissolved gas measurement

The dissolved gas vials were constructed and evacuated similarly to the surface gas vials but used a 20 mL glass vial (Fisherbrand™ Headspace Vials, 20mm, 20mL; Waltham, MA, USA) instead of a 10 mL vial. After the 20 mL vials were evacuated, 0.3 mL of 80% ZnCl was added to each, making sure the seal held by observing if the syringe moved upon insertion into the vial.

Dissolved gas samples were collected as described in Davis et al. (2019). A peristaltic pump fitted with tubing (Solinist Model 410; Georgetown, ON, CA) or a groundwater pump (Proactive Mini Typhoon 12V, Proactive Environmental Products; Hamilton, NJ, USA) pulled water from the bioreactor inflow and outflow control structures, purging leftover water in the tubing for

several seconds before water was collected. A 20 mL Leur Lock syringe was inserted into the tubing and filled with water to limit atmospheric exposure. The syringe was purged three times; the fourth, fifth, and sixth samples were kept as triplicate samples. For each sample, the syringe was held upwards and shaken to let air bubbles escape as excess water was squirted from the syringe. A 20G1 needle was fitted to the Leur Lock syringe, and water was squirted out of the needle and syringe until 10 mL remained, which was injected into the prepared 20 mL vials. Dissolved samples were transported on ice and were stored at 4°C for up to 17 d.

Dissolved samples were adjusted to atmospheric pressure using a syringe filled with 20mL He; the volume of He that freely entered the vial was recorded. Sample vials were shaken for 13 minutes at room temperature with the vials upright on a reciprocal shaker at 400 rpm (Jahangir et al., 2012). After standing for 63 minutes, 5 mL of the headspace of the sample vial were injected into a 10 mL GC vial filled with 10 mL He. The 10 mL GC vial was evacuated ahead of time and filled with He during the 63-minute wait period. If samples were to be stored for more than a week before this processing occurred, flowable sealant was added to the caps (Dow Corning RTV Sealant 734; Midland, MI, USA).

2.2.3 Gas Sample Analysis

Gas concentrations were measured using a gas chromatograph (GC-2014 Shimadzu GC, Shimadzu AOC-5000 Plus Autosampler; Kyoto, Japan) using helium as the carrier gas (Airgas; Radnor, PA, USA) through a packed column at 80°C (80/100 HAYESEPT N 1.5m x 1/8 in x 2.1 mm SS, preconditioned). Detection for N₂O was done by an electron capture detector (ECD) while detection for CH₄ was performed by a flame ionization detector (FID) with detection limits of 0.1 ppb and 0.1 ppm for the two detectors, respectively. Standards for N₂O, CO₂, and CH₄ (ppm/L air) were made from 100 ppm canisters of N₂O, CO₂, and CH₄ (Airgas; Radnor, PA, USA) on the morning of the gas chromatograph operation (Table 4). While CO₂ data was collected, it was not analyzed for this thesis.

Table 4. Standards used for the gas chromatograph in ppmv.

Standard	CO ₂ (ppmv)	CH ₄ (ppmv)	N ₂ O (ppmv)
He	0	0	0
1	965.69	1.307	0.65
2	812.71	3.300	0.99
3	645.90	6.56	1.64
4	459.67	13.33	3.33
5	328.33	26.67	6.67
6	0	40	13.33

Standard curves were constructed for N₂O and CH₄ concentrations for each run of the gas chromatograph. In some cases, the standard curves appeared to plateau at higher concentrations, so a standard curve composed of a subset of the lower concentration standards was used for low concentrations.

A linear slope of concentration versus time was used to calculate surface flux ($\mu\text{L min}^{-1} \text{m}^{-2}$) as follows:

$$\text{Surface flux} = \frac{mV}{A} \quad (1)$$

where m is slope (ppm/min), V is headspace volume (L), and A is chamber top surface area (m^2). Unit conversion for surface flux was used to achieve units in terms of $\text{g NO}_3\text{-N/ m}^2\text{-h}$ or $\text{g CH}_4\text{-C/m}^2\text{-h}$. Slope was calculated using the SLOPE function in Microsoft Excel.

Chamber flux detection limits for the gas chromatograph were determined using methods from Parkin et al. (2012). Thirty-five 10 mL vials were filled with air samples and analyzed on the gas chromatograph. Based on these thirty-five samples, the following equation was used to find the detection limits of surface sampling for N₂O and CH₄.

$$DL = f/60 * C_{gas} * Cov \frac{V}{A} \quad (2)$$

where DL is the detection limit ($\mu\text{L/min-m}^2$), f is the scaled slope factor for three time points over an hour of sampling (1/h) from Parkin et al. (2012), C_{gas} is the average analyte gas concentration with outliers removed (ppm), Cov is the coefficient of variation of analyte gas with outliers removed, V is surface chamber volume (L), and A is surface chamber surface area (m^2). Detection limit N₂O and CH₄ surface fluxes were -0.393 and 0.391 $\mu\text{L/min-m}^2$ and 3.528 and -3.544 $\mu\text{L/min-m}^2$, respectively.

Dissolved concentration detection limits were the relative standard deviation (in this case the coefficient of variation, Cov) as proscribed for precision measurement by the American

Public Health Association (2017, p. 223-225). When calculated dissolved concentrations fell below the detection limit, the concentration value was set to the detection limit, which was 0.251 and 0.347 ppmv for N₂O and CH₄, respectively.

Dissolved concentrations were converted from ppmv from the standard curves to $\mu\text{mol/L}$ using the ideal gas law:

$$C_{\mu\text{mol/L}} = \frac{C_{\text{ppmv}} * 1}{RT} \quad (3)$$

where $C_{\mu\text{mol/L}}$ is the gas concentration in $\mu\text{mol/L}$, C_{ppmv} is the concentration in ppmv from the standard curve, R is the ideal gas constant in $\text{L atm K}^{-1} \text{mol}^{-1}$, and T is incubation temperature in K. At times when incubation temperature (i.e., room temperature while processing dissolved samples as described in section 2.2.2) was not measured, it was estimated as 23°C based on room temperature during other incubation sessions.

Mass of gas was calculated using the Bunsen coefficients (Table 5) defined as the volume of gas (standard temperature and pressure) absorbed per unit volume of liquid solution, at the temperature of measurement when total pressure and fugacity are 1 atm (Weiss, 1974). The mass calculation is as follows:

$$M = C_{\mu\text{mol/L}} * (V_h + V_l * \beta) \quad (4)$$

where M is the number of moles in μmol in the gas and liquid of a dissolved sample vial, $C_{\mu\text{mol/L}}$ is the gas concentration in $\mu\text{mol/L}$, V_h is the vial headspace volume, V_l is the total liquid volume, and β is the Bunsen coefficient.

Table 5. Bunsen coefficients with T as incubation temperature.

	Bunsen coefficient, β	Reference
Nitrous oxide (N ₂ O)	$\beta = 1.3668 - 0.0487T + 0.00068145T^2$	(Young, 1981)
	with T as temperature in Celsius	
Carbon dioxide (CO ₂)	$\beta = e^{-58.0931 + 90.5069 * (\frac{100}{T}) + 22.294 * \ln(\frac{T}{100})}$	(Weiss, 1974)
	with T as temperature in Kelvin	
Methane (CH ₄)	$\beta = e^{-68.8862 + 101.4956 * (\frac{100}{T}) + 28.7314 * \ln(\frac{T}{100})}$	(Wiesenburg and Guinasso, 1979)
	with T as temperature in Kelvin	

2.3 Water quality and flow

Inflow and outflow grab samples were taken at each control structure concurrently with the gas sampling events. Water samples were transported on ice, filtered (0.45 μm membrane filter), and stored at 4°C until nitrate analysis which was within 24 d (measured as $\text{NO}_3^- \text{N} + \text{NO}_2^- \text{N}$; Lachat Quickchem, 10-107-04-1-A, Loveland, CO, USA). Occasionally the #6 – No Cover bioreactor outflow water level was too low in the control structure for the grab sampling stick and so a peristaltic pump (Solinist Model 410; Georgetown, ON, CA) was used.

There were nine instances of low water level or personnel miscommunication which precluded grab sample collection at bioreactors #1, #2 and #6, so nitrate concentrations from the nearest date from a companion long-term monitoring dataset were used (long term dataset method: samples either hand-collected twice weekly or every 4-d with Teledyne ISCO automated sampler models 6712 or 3700, Lincoln, NE, USA). The time difference between substitute nitrate concentrations and the sampling date was no longer than 1 d. Nitrate removal rate was calculated by dividing mass removal by the volume of the bioreactor. Dissolved oxygen (DO), oxidation reduction potential (ORP), and water temperature were recorded in both the inflow and outflow structures during each sampling event (YSI Professional Plus; Xylem Inc.; Yellow Springs, OH, USA).

Manually measured water depths in the outflow control structure at each sampling event were used with a corresponding weir equation to calculate bioreactor flow rate. These bioreactor flow rates were used to calculate mass NO_3^- and N_2O fluxes through the bioreactor and hydraulic retention times. Water was manually measured by coating a PVC pipe with a color-changing paste (Kolor Kut Products; Houston, TX, USA), extending that pipe to the bottom of the control structure, ensuring the pipe was aligned straight vertically, then measuring the height of the color change with a tape measure. Depth and flow rate were cross-referenced with 15-min depths and calculated daily flow rates logged from pressure transducer data (Onset HOBO U2OL-04; Bourne, MA, USA) from the companion long-term monitoring dataset. In cases when hand-collected temperature was not recorded due to equipment availability or water-level measurements were not recorded ($\sim 1/6^{\text{th}}$ of sampling events), temperature or flow rate was substituted by the closest value from the long-term monitoring. Water level measurements were occasionally adjusted based on best judgement to match observations with long-term monitoring.

Bioreactor control structure v-notch weir equations differed by site depending on the structure dimensions and type of top stop log (Table 6).

Table 6. Control structure details and weir equations for the six bioreactors.

Site	Outflow control structure pipe size		Outflow control structure pipe width, W inches	Flow equation	Weir type
	<i>nominal size, inches</i>	<i>nominal size, cm</i>			
#1 - Cover	8	20.3	10.31	5a, 5b, 8	Rounded V-notch
#2 - Cover	8	20.3	10.31	5a	V-notch
#3 - Cover	6	15.2	6.31	5a	V-notch
#4 - No Cover	6	15.2	6.31	6	Steel-edged v-notch
#5 - No Cover	6	15.2	6.31	5a	V-notch
#6 - No Cover	6	15.2	6.31	7	Steel-edged v-notch; inverted-v outflow drawdown plate

Weir equations were as follows:

$$\text{If } H \leq 6.56, Q = 2.5866H^{2.0464} \quad (5a)$$

(Christianson et al., 2019)

$$\text{If } H > 6.56 \text{ and } H > 0.27W, Q = 2.5866(6.56)^{2.0464} + 3.0318WH^{1.37} \quad (5b)$$

(Chun and Cooke, 2008)

where Q is flow rate (gpm), W is control structure width (in.), and H is height of water above the weir crest (in.).

$$\text{If } H_1 \leq 13.84, Q_1 = 0.011H_1^{2.28} \quad (6)$$

(Christianson et al., 2019)

$$\text{If } H_1 \leq 16.66, Q_1 = 0.011H_1^{2.28} + 1.1736(H_1 - H_{downstream})h^{0.5} \quad (7)$$

(Christianson et al., 2019; Maxwell et al., 2021)

where Q_1 is flow rate (L/s), H_1 is height of water above the outflow weir crest (cm), $H_{\text{downstream}}$ is the water level on the downstream side of the outflow control structure (cm), and h is the head water differential (m).

At bioreactor #1 – Cover, where drainage was especially flashy with frequent flooding, Darcy's equation (Eqn. 8) for flow through a porous medium was used in place of the weir equation when the value from the weir equation exceeded the value from Darcy's equation (9 times).

$$Q = K[(H_A - H_B + r)/L] * (wH_B) \quad (8)$$

where K is the hydraulic conductivity (0.1476 ft/s), H_A and H_B are the heights above the bottom of the v-notch (in.) for the inflow and outflow, respectively, r is the vertical gradient across the bioreactor (0.285 ft), L is the length of the bioreactor (ft), and w is the width of the bioreactor (35 ft). Appropriate unit conversion put Q in units of gpm which was later converted to L/s.

2.4 Impact on climate change

Total gas emission rates were found by summing area normalized surface flux mass per day (g N_2O -N/d or g CH_4 -C/d) and dissolved mass per day (g N_2O -N/d or g CH_4 -C/d). Emission factors (EF, %) were calculated as the ratio of the mass of N_2O produced (g) to the mass of NO_3^- removed (g) multiplied by 100 as calculated by Manca et al. (2021) and Nordström and Herbert (2018). To compare the climate change impact from the different bioreactor greenhouse gases, carbon dioxide equivalents were calculated by multiplying the 100-yr global warming potential (265 and 28 for N_2O and CH_4 , respectively) by the production rates (Davis et al., 2019) in terms of mass of N_2O and CH_4 .

2.5 Statistical analysis

The Shapiro Wilk test in the stats package in R v4.1.1 showed that NO_3^- mass removal and removal efficiency, N_2O and CH_4 production, and inflow N_2O concentration were non-normal ($p < 0.001$), implying the use of non-parametric tests. Conditions or response variables between covered and uncovered treatments were compared using the non-parametric Mann Whitney U Test in R at a significance of 0.05. The non-parametric Kruskal Wallis test was used in R to compare all six bioreactors with each other. Correlation of variables excluding outliers

was done using Spearman's rank correlation coefficient in R. The magnitude of a Spearman's Rho indicates the correlation strength with +1 for perfect positive correlation, -1 for perfect negative correlation, and 0 indicating no linear relationship. The following ranges of values of Rho were used to define the strength of relationships: weak from ± 0.2 to ± 0.39 , moderate from ± 0.4 to ± 0.59 , strong from ± 0.6 to ± 0.79 and very strong from ± 0.80 to ± 1 .

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Results per site

Across the six bioreactors, regardless of presence/absence of a cover, mass nitrate removal exceeded mass N_2O produced by 1-3 orders of magnitude on any given day (Fig. 10), and emission factors (EF) were less than 3% (Table 7). Dissolved greenhouse gas emissions contributed more significantly to total mass emissions compared to surface emissions (Table 7), which commonly fell within the detection limit. In terms of the study period as a whole, the bioreactors emitted net greenhouse gases, except for #4 – No Cover, which removed nitrous oxide. However, at the daily scale, nitrous oxide removal, in dissolved or gaseous form was not uncommon. A comparison between the cover/no cover treatments was not possible due to hydraulic differences among treatments (see Section 3.2.1) despite an experimental design intended to capture all sites across a variety of flow trends. With the inability to compare cover/no cover treatments, this study recommends leaving the cover decision to the landowner until further research emerges since there are few other aspects of bioreactor design that allow for landowner preference.

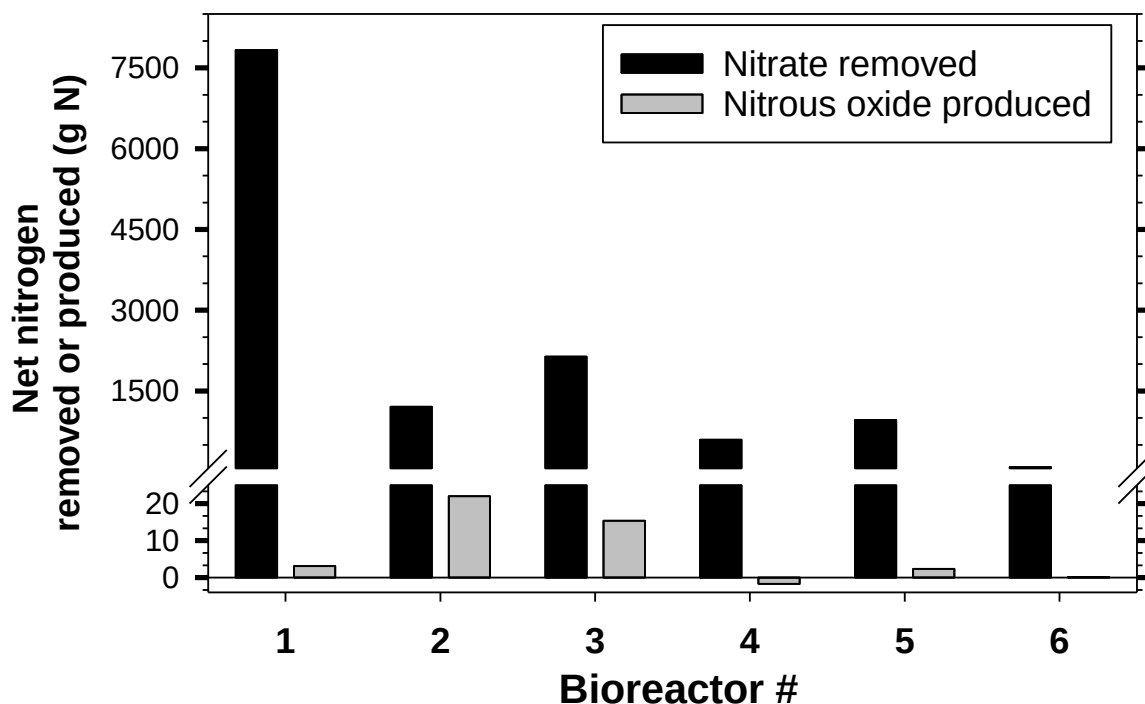


Figure 10. Nitrate removal and nitrous oxide production across the six bioreactors on the x axis. There is a break on the y axis to accommodate different scales between the nitrate removal and nitrous oxide production. The negative bar for bioreactor #4 shows there was nitrous oxide removal instead of production. Bars reflect between 7 and 21 sample events per site.

Table 7. Summary of nitrate removal, nitrous oxide production, and emission factors (EFs) for all sites. Means exclude dates in which only surface or only dissolved samples were collected.

Site	Number of sample events, n	Mean (stdev) of nitrate removal efficiency %	Mean (stdev) nitrate removal rate $g\ NO_3^- / m^3 \cdot d$	Mean (stdev) dN_2O produced $g\ N/d$	Mean (stdev) surface flux $mg\ N_2O-N / m^2 \cdot h$ $g\ N/d$		Net NO_3^- removed $g\ NO_3^- N$	Net (surf. + diss.) N_2O produced $g\ N_2O-N$	Mean (stdev) emission factor %
#1 - Cover	21 ^[a]	82.1 (13.2)	2.28 (2.84)	0.099 (1.82)	2.21e-2 (3.66e-2)	0.10 (0.16)	7831.9	3.13	0.27 (0.65)
#2 - Cover	8 ^[b]	6.42 (8.32)	0.75 (0.74)	2.90 (3.24)	6.34e-3 (1.28e-2)	0.02 (0.03)	1207.7	20.47	2.95 (2.57) ^[c]
#3 - Cover	7	90.6 (13.5)	2.01 (0.25)	2.01 (1.78)	5.69e-2 (5.79e-2)	0.18 (0.19)	2138.7	15.38	0.69 (0.60)
#4 – No Cover	9 ^[d]	87.5 (4.93)	1.24 (1.17)	-0.421 (0.408)	-5.51e-4 (1.66e-4)	-0.001 (0.03)	593.9	-1.69	-0.35 (0.26)
#5 – No Cover	10	16.5 (11.4)	2.97 (1.84)	0.212 (0.696)	2.98e-2 (5.43e-2)	0.03 (0.05)	962.8	2.37	1.56 (3.89)
#6 – No Cover	7 ^[d]	7.31 (7.44)	0.291 (0.403)	0.0437 (0.0737)	1.55e-3 (1.02e-2)	0.002 (0.01)	84.4	0.19	2.11 (4.47)

[a] #1 – Cover had 21 sampling events overall, including 8 dissolved-only samples and 2 surface-only samples.

[b] #2 – Cover had 7 sampling events with dN_2O data.

[c] #2 – Cover avg EF excludes a sampling event that resulted in the production of both nitrous oxide and nitrate nitrogen.

[d] #4 – No Cover and #6 – No Cover had only 4 sampling events with flow. Calculated means exclude events without flow.

3.1.1. #1 - Cover (Dudley Smith Farm bioreactor)

Consistently anaerobic and reduced conditions (Fig. 11) created across the bioreactor supported high nitrate removal efficiencies and removal rates at bioreactor #1 – Cover ($82.1 \pm 13.2\%$ removal efficiency; $2.28 \pm 2.84\ g\ NO_3^- N / m^3 \cdot d$ nitrate removal rate; Table 7) as well as a common hydrogen sulfide smell. It is possible these favorable conditions for denitrification resulted in the low average N_2O production ($0.15 \pm 1.64\ g\ N_2O-N/d$; Table 7) and low emission factor ($0.27 \pm 0.65\%$; Table 7). Dissolved N_2O was both produced and removed across the bioreactor (Fig. 12c). Bruun et al. (2017) reported similar fluctuations at a woodchip-based subsurface flow constructed wetland in Denmark and noted that increases in emissions may be

attributed to incomplete denitrification, causing N_2O production through an excess of NO_3^- and/or the presence of oxygen.

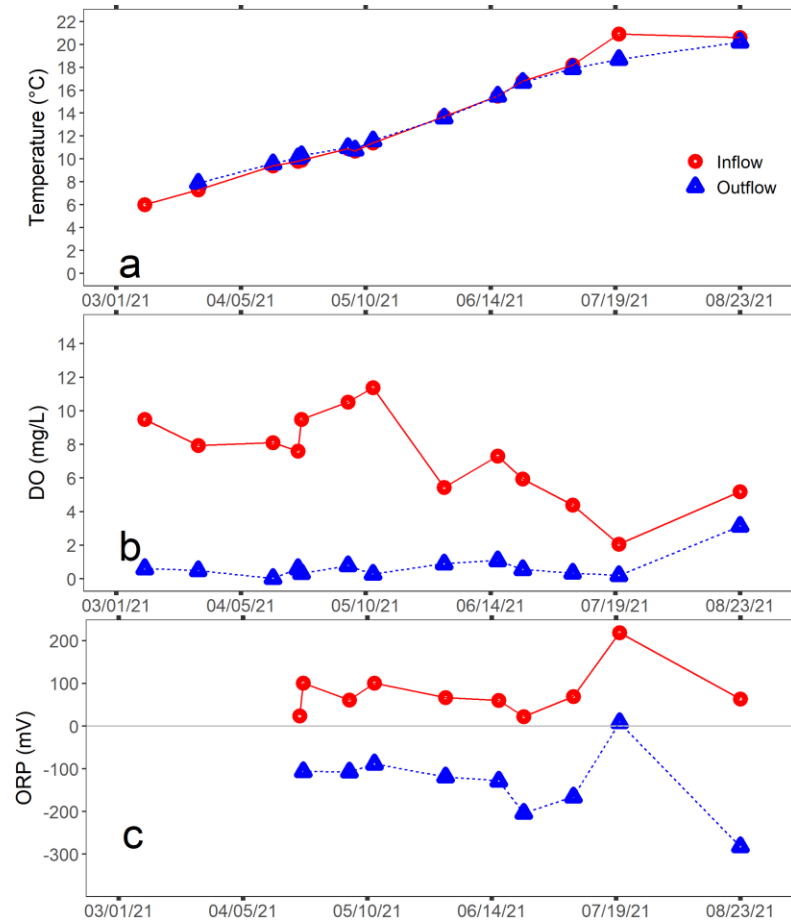


Figure 11. Time series plot of covered bioreactor #1 water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

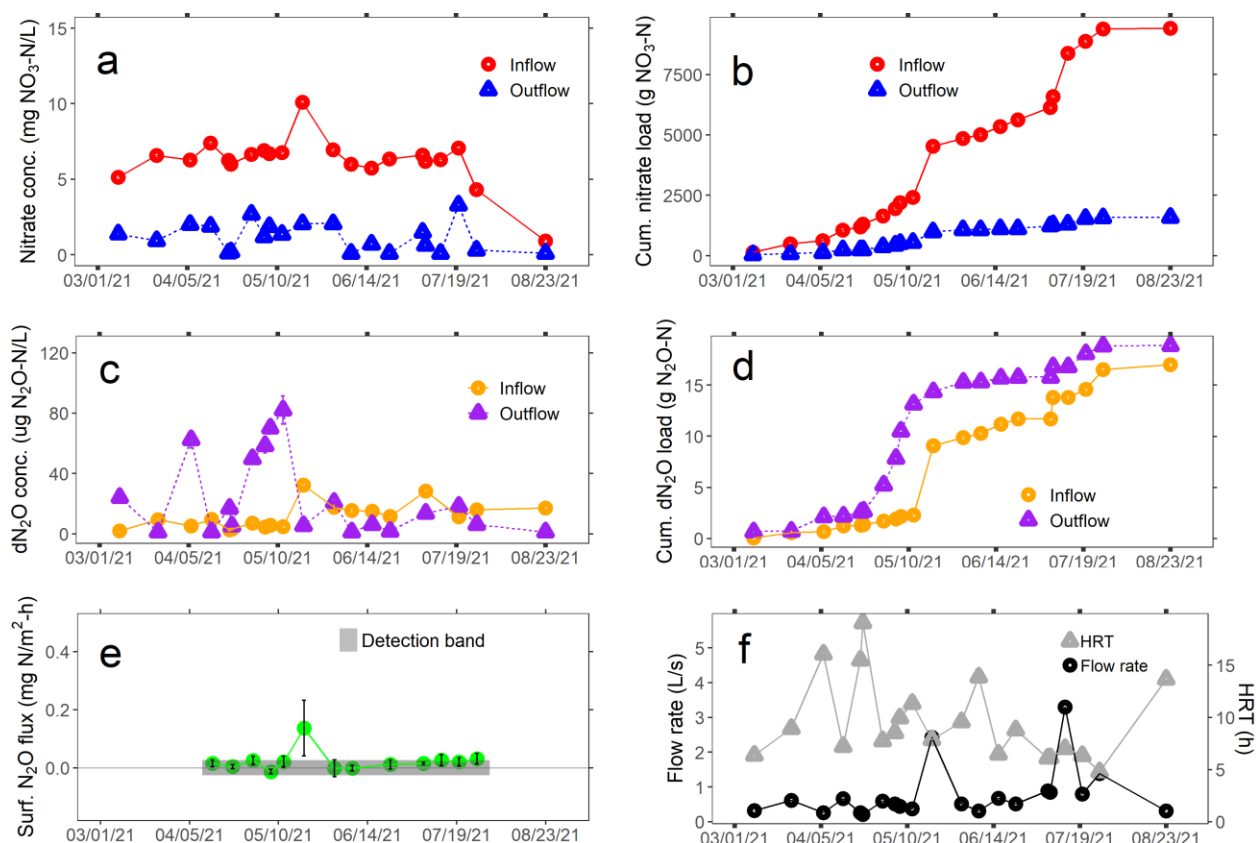


Figure 12 Nitrogen dynamics at covered bioreactor #1 including nitrate concentrations in mg/L(a) and loads (b); dN₂O concentrations in µg/L (c) and cumulative dN₂O loads (d); and surface N₂O flux (e); with flow and hydraulic retention time (HRT, f).

Data showed that dissolved nitrous oxide (dN₂O) removal coincided with higher inflow dN₂O concentrations, higher nitrate removal efficiencies, and lower inflow DO concentrations. Inflow dN₂O concentrations and nitrate removal efficiencies were significantly higher on dates with dN₂O removal compared to dates with high dN₂O production ($p < 0.001$ and $p = 0.006$, respectively). This supported the hypothesis that less N₂O in bioreactors is produced during periods of complete denitrification (Elgood et al., 2010; Nordström and Herbert, 2018). From Mar. 9 to May 12, inflow DO concentrations were significantly higher than later inflow concentrations ($p < 0.001$; Fig. 12b) whereas the inflow dN₂O concentrations increased into the warmer months. Four sampling events from Apr. 30 to May 12 contributed to over half of the dN₂O production (Fig. 11d). These results coincided with observations by Elgood et al. (2010) who found greater d2NO emissions in colder months that had greater influent DO concentrations.

A large precipitation event from May 16 - 18 (68 mm) was a driver of both one of the study's highest surface N₂O fluxes on May 20 (Fig. 12e: 0.136 mg N₂O-N/m²-h) and the bioreactor operating at one of the highest flow rates of this period (Fig. 12f: 2.4 L/s). The high surface flux was the only instance of emissions above detection for all five chambers across all sites. Despite having the greatest surface emissions, May 20 also had the greatest dN₂O reduction, resulting in a net N₂O sink and removal of 4.66 g N₂O-N/d. Thus, while some of the high surface emissions may have been due to conversion of N₂O from aqueous to gaseous form, a greater portion was reduced in the bioreactor. This interesting point also illustrated that surface fluxes and dissolved fluxes may not always be directionally linked at a bioreactor. Temperature, DO, and ORP were not recorded on this day.

3.1.2. #2 - Cover (Northwest Research and Education Center bioreactor)

Bioreactor #2 – Cover had the lowest nitrate removal efficiency ($6.4 \pm 8.3\%$, $n = 8$ sampling events; Table 7) and one of the highest average daily combined nitrous oxide emissions (2.92 ± 3.25 g N₂O-N/d). Low nitrate removal was likely related to the low HRT (< 4 h; Fig. 13f), which was a function of the very low L:W ratio design (that is, a wide bioreactor with a short flow path). While DO concentrations nevertheless decreased across the bioreactor under these HRTs (Fig. 14b), the outflow was not as strongly reduced as at most other sites (Fig 14c). The low HRTs especially in mid-May resulted in low nitrate removal efficiencies but also resulted in relatively high daily nitrate removal rates (e.g., 1.51 - 2.13 g NO₃⁻N/m³-d) due, in part, to the high loading. Despite the relatively high overall N₂O production (Table 7), one sampling date, which had the highest inflow dN₂O concentrations, resulted in dN₂O removal across the bioreactor (May 24: inflow and outflow: 9.04 ± 0.40 and 5.71 ± 0.88 µg N₂O-N/LN₂O; Fig. 13c).

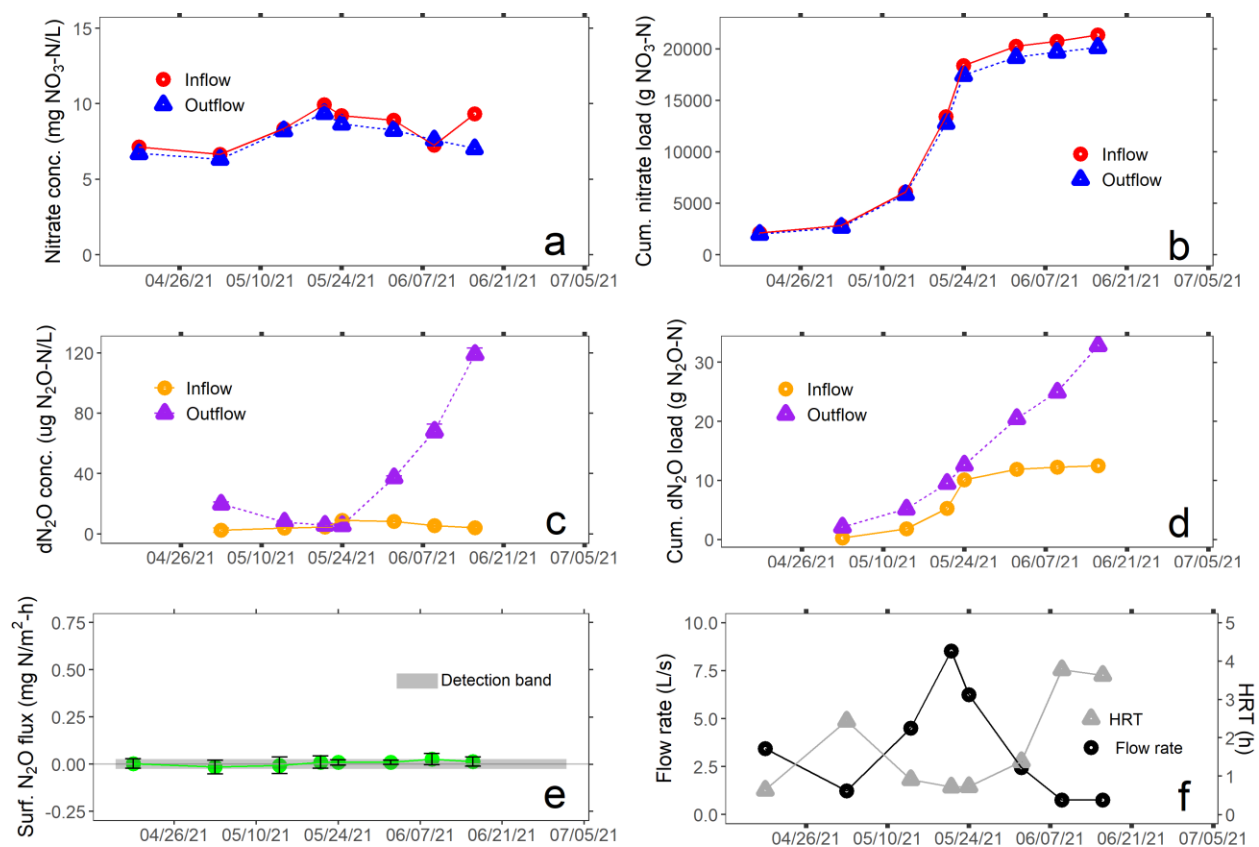


Figure 13. Nitrogen dynamics at covered bioreactor #2 including nitrate concentrations in mg/L (a) and cumulative loads (b); d N₂O concentrations in µg/L (c) and cumulative dN₂O loads (d); and surface N₂O flux (e); with flow and hydraulic retention time (HRT, f).

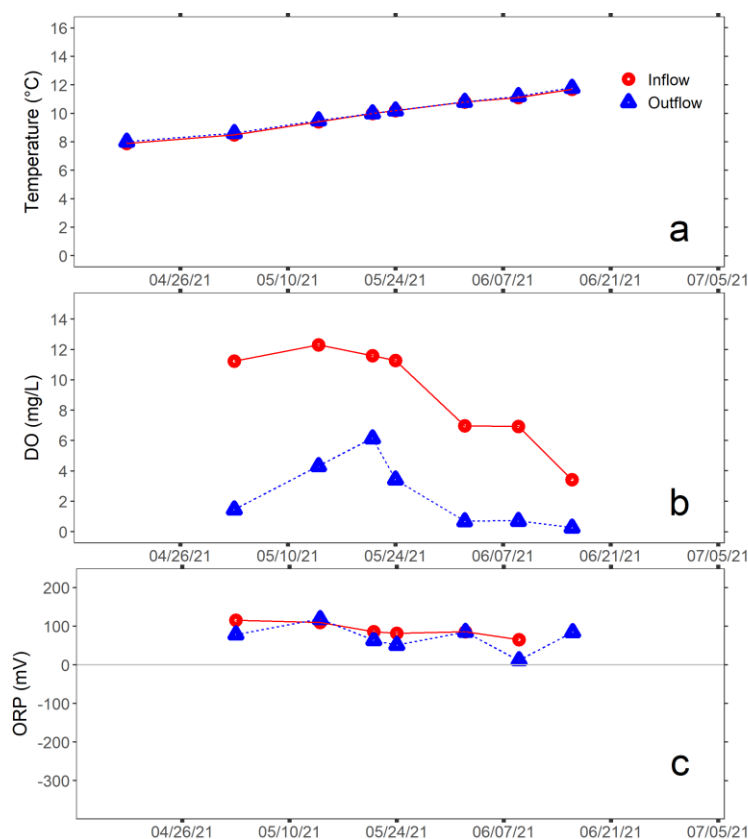


Figure 14. Time series of covered bioreactor #2 water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

Outflow nitrate concentrations consistently decreased from the end of May through June, which was partially driven by decreases in inflow nitrate concentrations, but during that same time, outflow dN₂O concentrations increased (Fig. 13a and c). The very late May and June samples showed more consistently anaerobic conditions (outflow DO < 0.72 mg/L) coincident with lower inflow DO concentrations and a trend of increasing HRT. The higher dN₂O production during a period of lower inflow DO concentrations was opposite to that observed at bioreactor #1 – Cover, illustrating that many factors interplay in bioreactor dN₂O dynamics. One additional factor here may have been that the saturated depth in the bioreactor consistently fell from its peak on May 20. McGuire and Reid (2019) observed the possibility of episodic N₂O fluxes in lab-scale bioreactors under falling water levels due to the release of entrapped air.

3.1.3. #3 – Cover (Private bioreactor)

The consistent and nearly complete nitrate removal for most sampling dates ($91 \pm 14\%$ removal efficiency; 2.01 ± 0.25 g NO₃⁻N m³/d nitrate removal rate; $n = 7$ sampling events; Table

7) at covered Bioreactor #3 was a trend distinct from the variation of dN_2O , production of which peaked in mid-May (Fig 15a and c). This mid-May dN_2O peak was one of the highest observed across all sites and occurred in a similar timeframe as one of the highest surface N_2O fluxes across all sites (Fig. 15e; surface peaks of $\sim 0.2 \text{ mg N}_2\text{O-N/m}^2\text{-h}$ at bioreactor #1, 3, and 5). A 9.9 cm storm event in the 2 days preceding sampling may have been a causal agent in the surface N_2O dynamics because soil moisture is known to be a key driver of soil N_2O emissions with high nitrous oxide emissions expected at high soil moisture (Friedl et al., 2016).

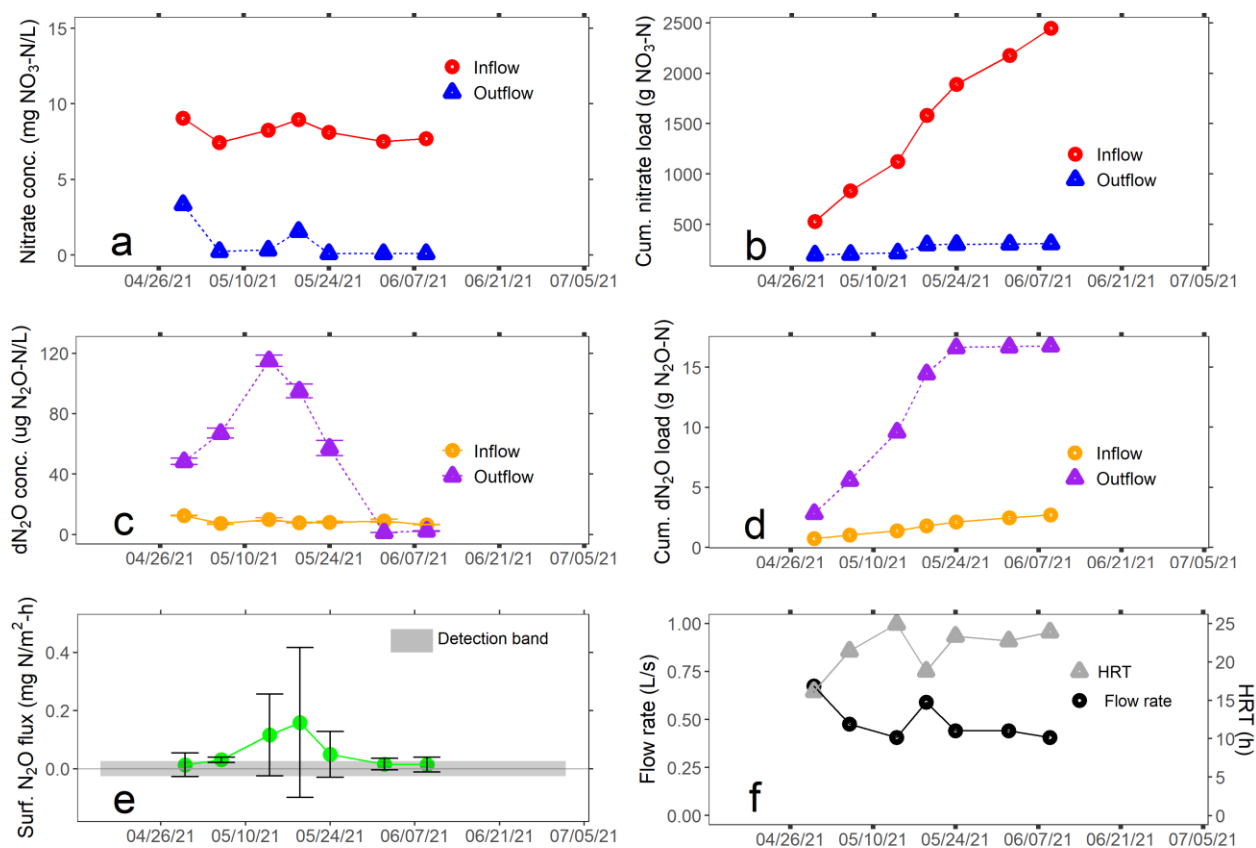


Figure 15. Nitrogen dynamics at covered bioreactor #3 including nitrate concentrations in mg/L (a) and loads (b); dN_2O concentrations in $\mu\text{g/L}$ (c) and cumulative dN_2O loads (d); and surface N_2O flux (e); with flow and hydraulic retention time (HRT, f).

Bioreactor solution was anaerobic and reduced which supported that nitrate removal was via denitrification (Fig. 16b and c). The outflow nitrate concentration often indicated the bioreactor was operating under nitrate limitation (i.e., concentrations $< 0.50 \text{ mg NO}_3\text{-N/L}$; Shih et al., 2011; note analytical detection limit: $0.10 \text{ mg NO}_3\text{-N/L}$). Conditions were so highly reduced that sulfate reduction was likely, especially given observed hydrogen sulfide odor on May 6, 14, 19, June 2, and June 9. Like bioreactors #1 – Cover and #6 – No Cover, this

bioreactor had dN₂O removal during the warmest period of sampling. As on many dates for this bioreactor, this period of dN₂O removal across the bioreactor corresponded with nitrate limitation, a hydrogen sulfide smell, and relatively high HRTs (>20 h), consistent with observations by Rivas et al. (2020) that increasing HRT coincides with increased sulfate and N₂O reduction.

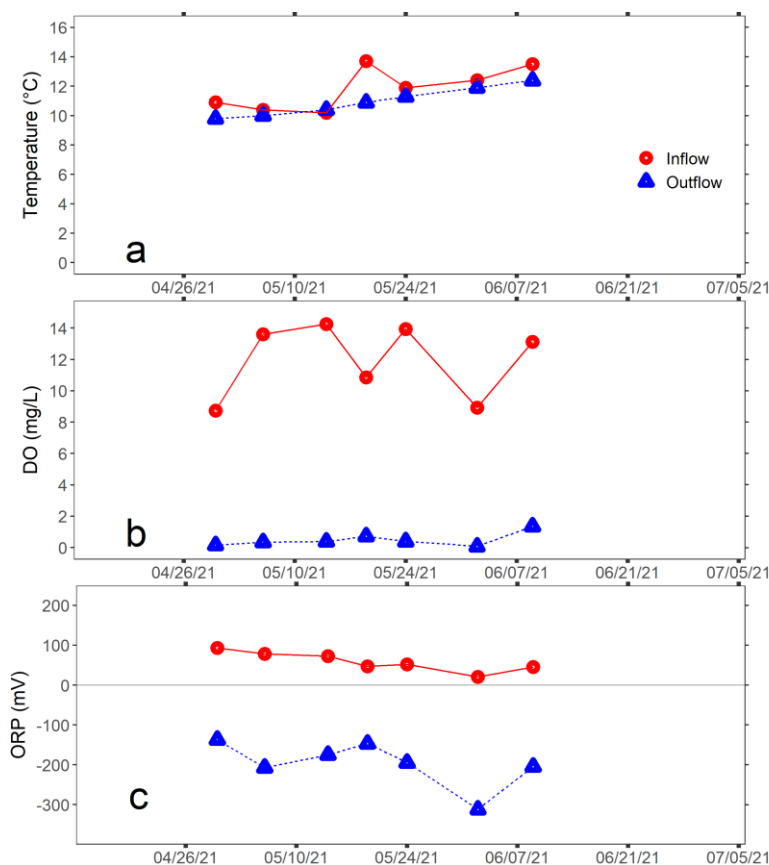


Figure 16. Time series plot of covered bioreactor #3 water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

Hydraulic retention times ranging from 16.2 to 24.9 h were higher than the other bioreactors studied here but were not out of range of a global bioreactor review which showed a median HRT of approximately 26 h (Christianson et al., 2021). This bioreactor was unique in that there was always bypass flow even though outflow flow was low. There may have been internal flow restrictions in this relatively long, 4-y old bioreactor.

Bioreactor #3 – Cover site was the only one with a soil chamber near the bioreactor as a control. The soil control never showed fluxes above the detection limit, suggesting that a bioreactor can produce more surface nitrous oxide emissions than grassed soil alone. Even so,

bioreactor surface N₂O fluxes vary spatially (Warneke et al., 2011a; Warneke et al., 2011b; Davis et al., 2019), and here, all the highest mean surface fluxes were driven by a chamber farthest from the bioreactor inflow (Fig. 17). Other studies have shown no significant increase in N₂O emissions from a denitrification wall compared to the soil control (Moorman et al., 2010) and no more significant emissions from bioreactors compared to agricultural soils (Goeller et al., 2019).

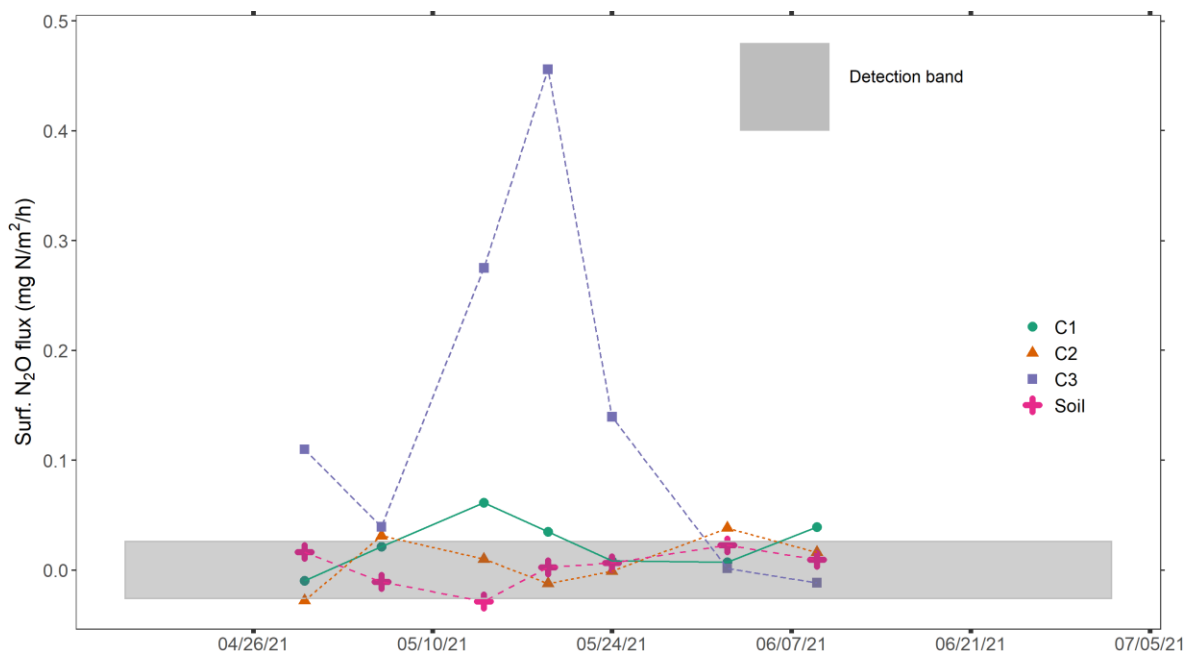


Figure 17. Soil and bioreactor #3 – Cover surface nitrous oxide emissions separated by chamber. Chamber 1 (C1) was closest to the inflow while chamber 3 (C3) was farthest from the inflow.

3.1.4. #4 – No Cover (Private bioreactor)

Low nitrate loading, sporadic flow, and consistent dN₂O removal distinguished bioreactor #4 – No Cover. This bioreactor was the only one of the six studied where dN₂O was consistently removed, often to the detection limit (0.251 ppmv N₂O). The low inflow nitrate concentrations at Site #4 – No Cover (< 3.03 mg NO₃⁻N/L) were a factor of cropping system where this drainage field was planted to clover, a perennial (Hertzberger et al., 2019). Low inflow concentrations along with very low flow during this study period resulted in low overall nitrate loading (Fig. 18b), as well as a low mean removal rate of 1.2 ± 1.2 g NO₃⁻N/m³-d but a high nitrate removal efficiency of $87 \pm 5\%$ (Table 7). This low loading led to highly reduced conditions (Fig. 19c) and a frequent hydrogen sulfide smell (observed 30 Apr, 6 May, 9 June, and 2 July). All these factors

which encouraged complete denitrification may have been casual factors in the observed dN_2O removal.

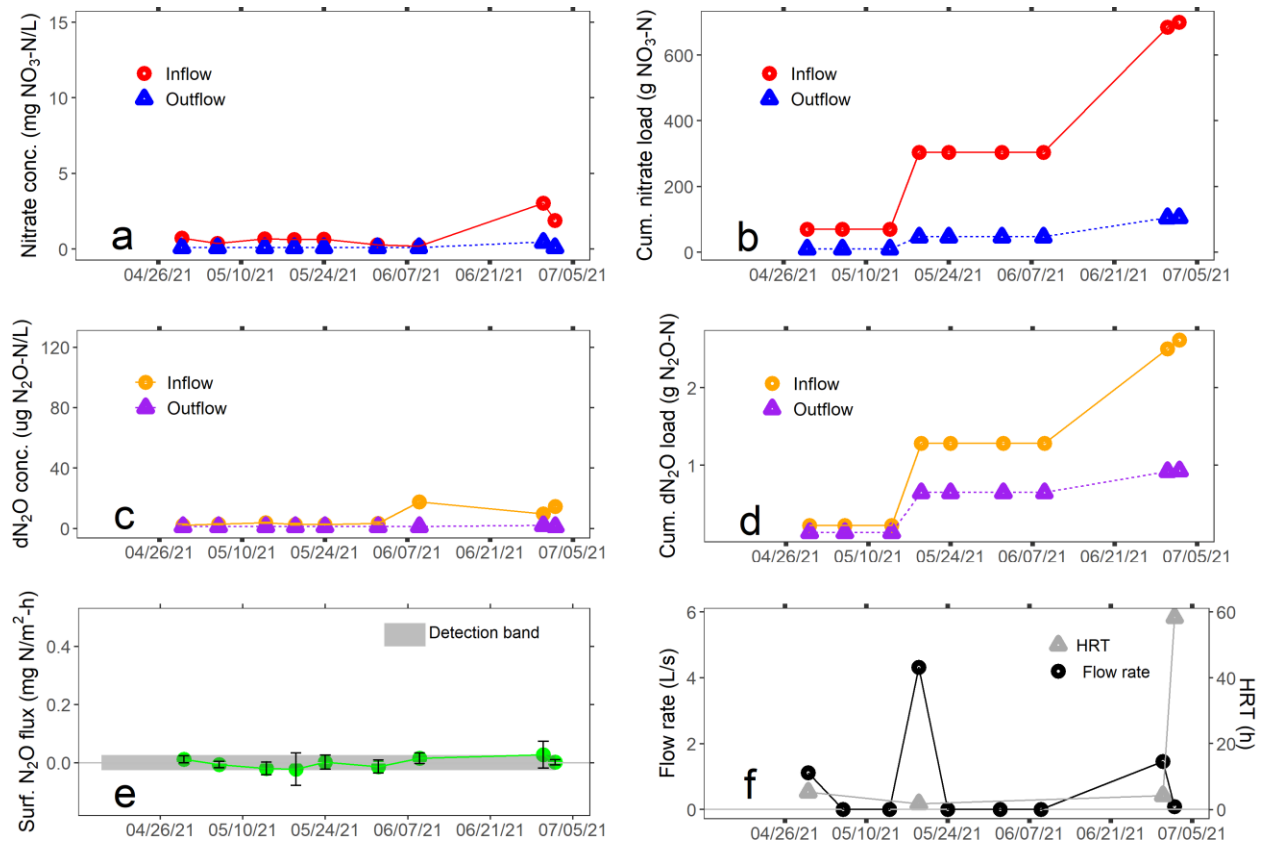


Figure 18. Nitrogen dynamics at uncovered bioreactor #4 including nitrate concentrations in mg/L (a) and cumulative loads (b); dN_2O concentrations in $\mu\text{g/L}$ (c) and cumulative dN_2O loads (d); and surface N_2O flux (e); with flow and hydraulic retention time (HRT, f).

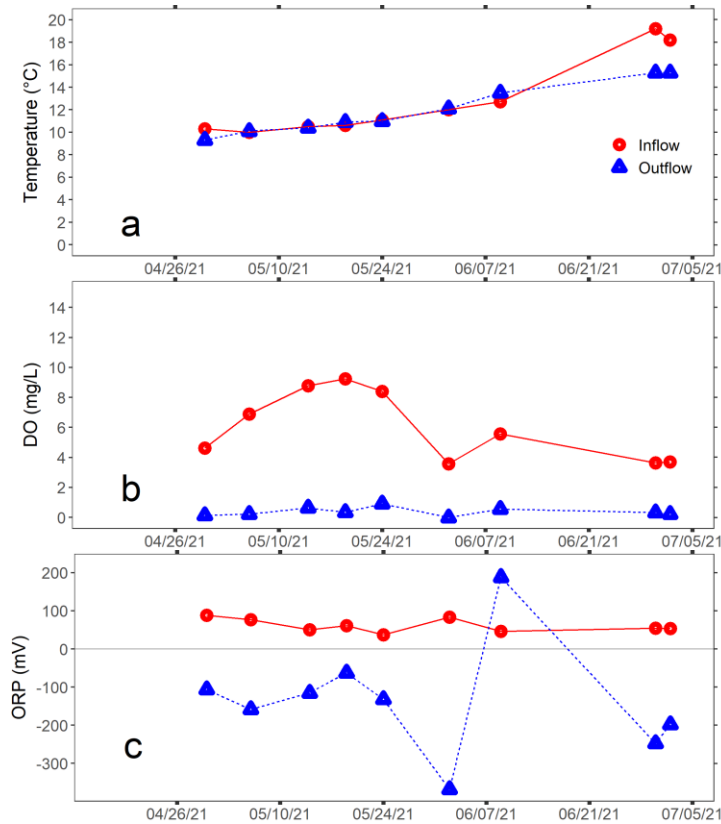


Figure 19. Scatterplot at uncovered bioreactor #3 of water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

Young age also could have been a factor in the dN₂O removal at bioreactor #4 – Cover (0.33 y from construction) especially given relatively more labile carbon availability in the first year of operation (Schipper et al., 2010; Healy et al., 2012; Rivas et al., 2020). Some bioreactor studies in New Zealand and Denmark reported dN₂O removal only in the first year of operation (Rivas et al., 2019; Audet et al., 2021) although this removal can happen at older bioreactors, too (Nordström and Herbert, 2018; Audet et al., 2021).

3.1.5. #5 – No Cover (Private bioreactor)

Bioreactor #5 – No Cover had relatively consistent performance (HRT most commonly 3.8 - 6.0 h; Fig. 20f) with $16.5 \pm 11.4\%$ nitrate removal efficiency (Table 7), but one of the highest nitrate removal rates ($2.97 \pm 1.84 \text{ g NO}_3\text{-N/m}^3\text{-d}$; Table 7) across sites due to the relatively high loading. Loading was a function of the high inflow nitrate concentrations ($12.57 \pm 1.85 \text{ mg NO}_3\text{-N/L}$, $n = 10$ sampling events) and relatively high flow rates ($0.57 \pm 0.14 \text{ L/s}$) for this small bioreactor. Even so, anaerobic and reduced conditions at the outflow were observed, confirming the likelihood of nitrate removal via denitrification (Fig. 21b and c).

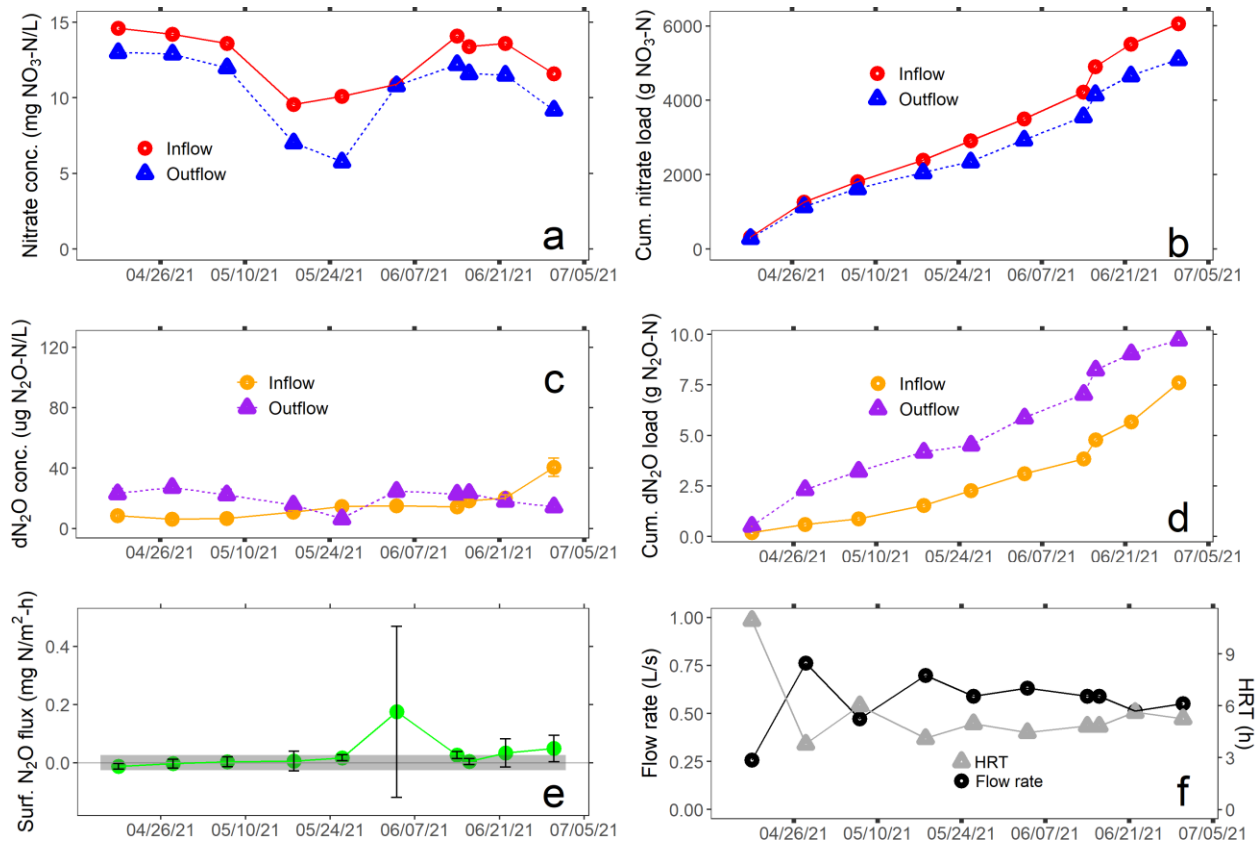


Figure 20. Nitrogen dynamics at uncovered bioreactor #5 including nitrate concentrations in mg/L (a) and loads (b); d N₂O concentrations in µg/L (c) and cumulative dN₂O loads (d); and surface N₂O flux (e); with flow and hydraulic retention time (HRT, f).

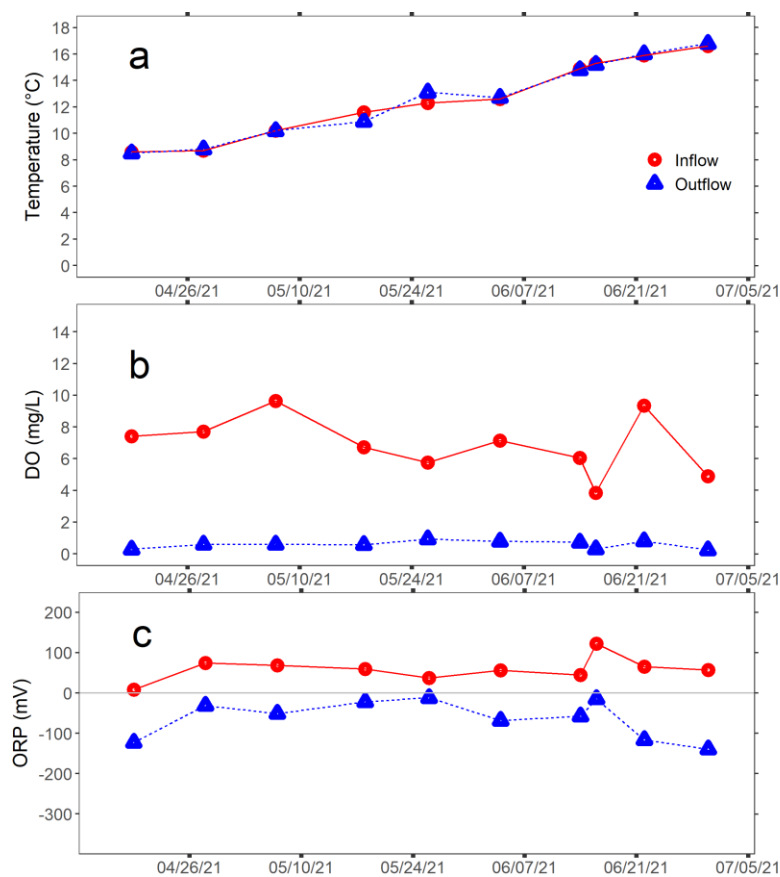


Figure 21. Time series of uncovered bioreactor #5 water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

Dissolved nitrous oxide was both produced and removed in the bioreactor, resulting in an average daily dissolved production of 0.21 ± 0.70 g $\text{N}_2\text{O-N/d}$ (Table 7). On May 26, the greatest nitrate mass removal (220.5 g N/d) and removal efficiency (43%) occurred alongside dissolved nitrous oxide removal. Negligible surface emissions on May 26 indicated that nitrous oxide was not simply exiting the bioreactor through the surface in the gaseous rather than aqueous phase through the water. The same conclusion was not necessarily applicable during dN_2O removal in late June when there were detectable surface emissions (Fig. 20e; Fig. 22).

On June 4, the highest N_2O surface emissions and above average dN_2O production coincided with minimal nitrate removal, which could not be explained by HRT, DO, nor ORP values since they did not differ significantly from other dates. The high average surface flux on June 4 (Fig. 20e) came from high surface emissions from chamber 4 (0.61 mg $\text{N}_2\text{O-N/m}^2\text{-h}$) with only minimally detectable emissions from chamber 2 and undetectable emissions from the other

two chambers (Fig. 22). Spatial inconsistency of the June 4 emissions may suggest the presence of microsites or hotspots with differing conditions (Davis et al., 2019; Rivas et al., 2020).

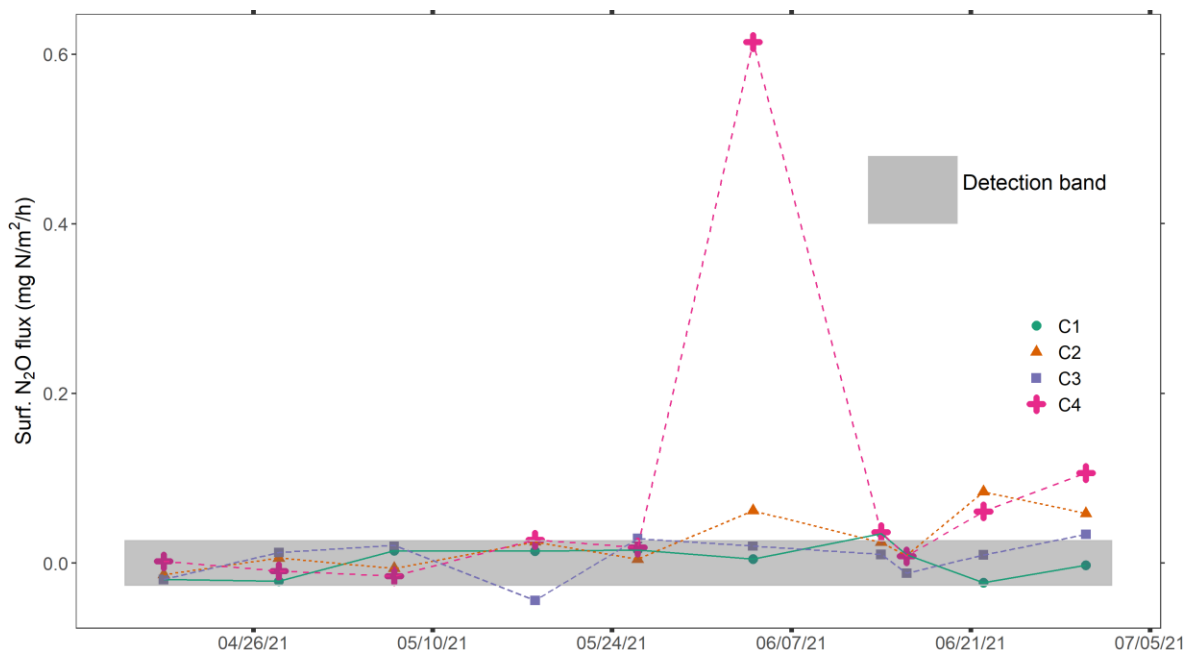


Figure 22. Soil and bioreactor #5 – No Cover surface nitrous oxide emissions separated by chamber. Chamber 1 (C1) was closest to the inflow while chamber 4 (C4) was farthest from the inflow.

3.1.6. #6 – No Cover (Private bioreactor)

The infrequent and low flow conditions at Bioreactor #6 – No Cover contributed to low nitrate removal ($7.3 \pm 7.4\%$ $\text{NO}_3^- \text{N}$; 0.29 ± 0.40 g $\text{NO}_3^- \text{N}/\text{m}^3\text{-d}$ removal rate; Table 7) and low dissolved nitrous oxide emissions (average 0.04 ± 0.07 g $\text{N}_2\text{O-N}/\text{d}$; Table 7). While water level measurement in the outflow control structure did not indicate flow on three of seven dates, there may have been very small flows seeping along the bottom of the bioreactor that could not be measured. This bioreactor was the only one in this study that had an outflow drawdown plate intended to limit sulfate reducing conditions (i.e., bottom outflow stop log with an orifice in it; USDA NRCS, 2020; Maxwell et al., 2021). Despite low flow rates, the HRTs during times of measurable flow were within range of other bioreactors (HRTs 3.4-18.1 h; Fig. 23f). Conditions were rarely ideal for denitrification. For example, the mean outflow DO concentrations was 3.82 mg DO/L and ORP was 66.00 mV (Fig. 24b and c).

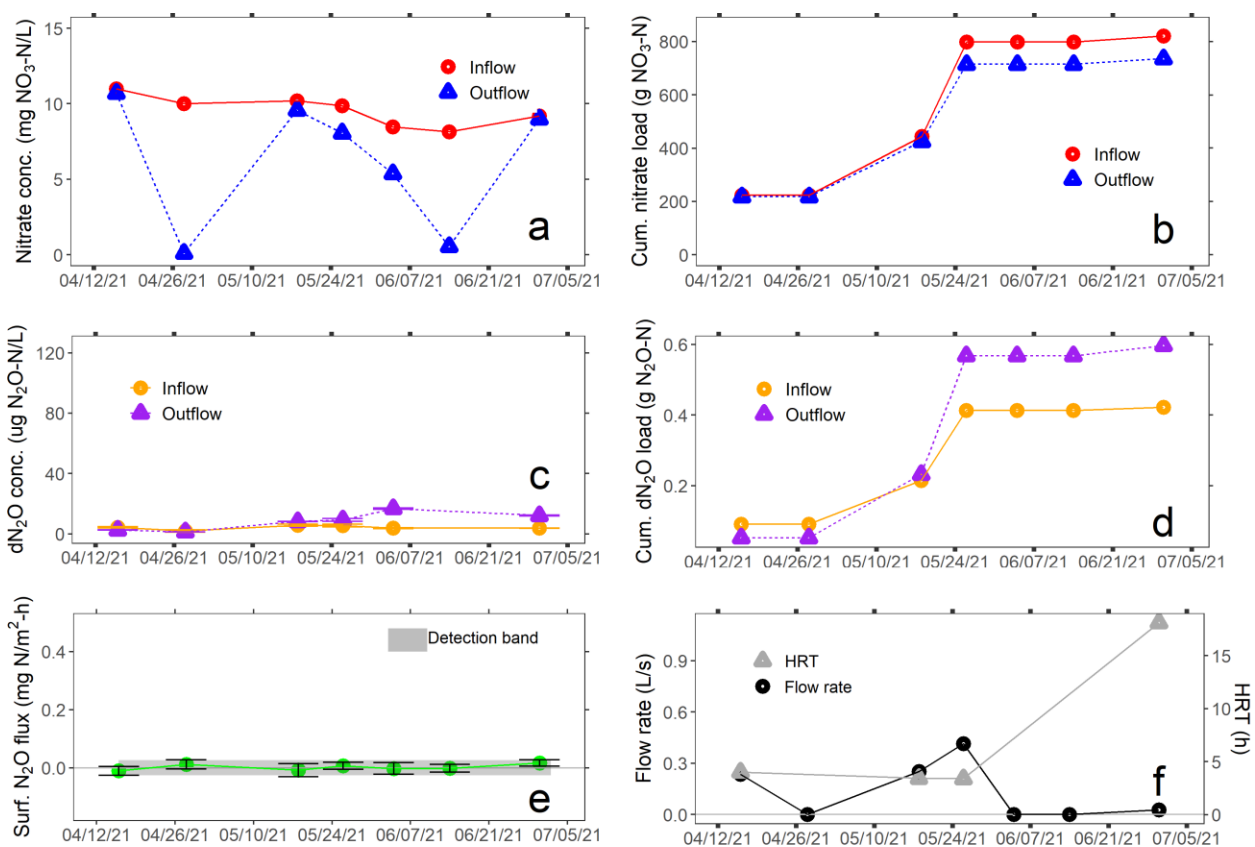


Figure 23. Nitrogen dynamics at uncovered bioreactor #6 including nitrate concentrations in mg/L (a) and cumulative loads (b); d N₂O concentrations in µg/L (c) and cumulative dN₂O loads (d); and surface N₂O flux (e); with flow and hydraulic retention time (HRT, f).

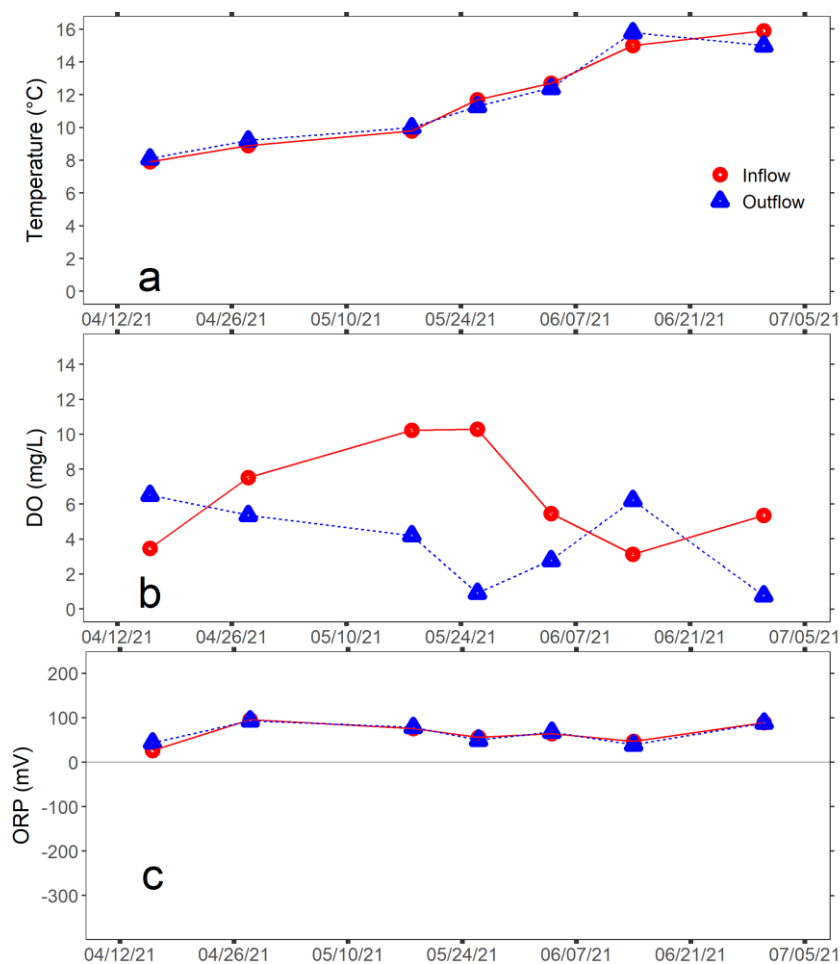


Figure 24. Scatterplot at uncovered bioreactor #6 of water temperature (a), dissolved oxygen (b), and oxidation reduction potential (c).

3.2 Nitrogen trends across sites

Data from all the sites together were examined for flow, water chemistry, and bioreactor characteristics as predictive parameters for nitrogen removal and nitrous oxide production.

3.2.1 Hydraulics and loading

The site-specific negative correlation between HRT and daily nitrate removal rates was strongest at #1 – and #3 – Cover (Spearman's $Rho = -0.71$ and -0.82 , $p < 0.001$ and $p = 0.03$; respectively; Fig. 25a). Higher nitrate removal rates at lower retention times have been documented before (Lepine et al., 2015; Martin et al., 2019). Nitrous oxide production showed no significant correlation with HRT over the dataset (Spearman's $Rho = 0.08$, $p = 0.55$; Fig. 25b) because this relationship differed in strength and direction at each bioreactor with a significant correlation at only bioreactor #2 (Spearman's $Rho = 0.81$, $p = 0.02$). Bioreactor #2 – Cover had

some of the highest nitrous oxide production, but this site had a relatively limited range of very short HRTs (0.6 - 3.8 h; Fig. 25b; Fig. 13f). These HRTs at #2 Cover were the most similar to the 2 h HRT tested by Davis et al. (2019), which resulted in the highest nitrous oxide mass loads. Those previous findings appeared to be consistent here, but there was not a full range of HRTs equally distributed across all sites (a realistic limitation of this field study) which limited this evaluation.

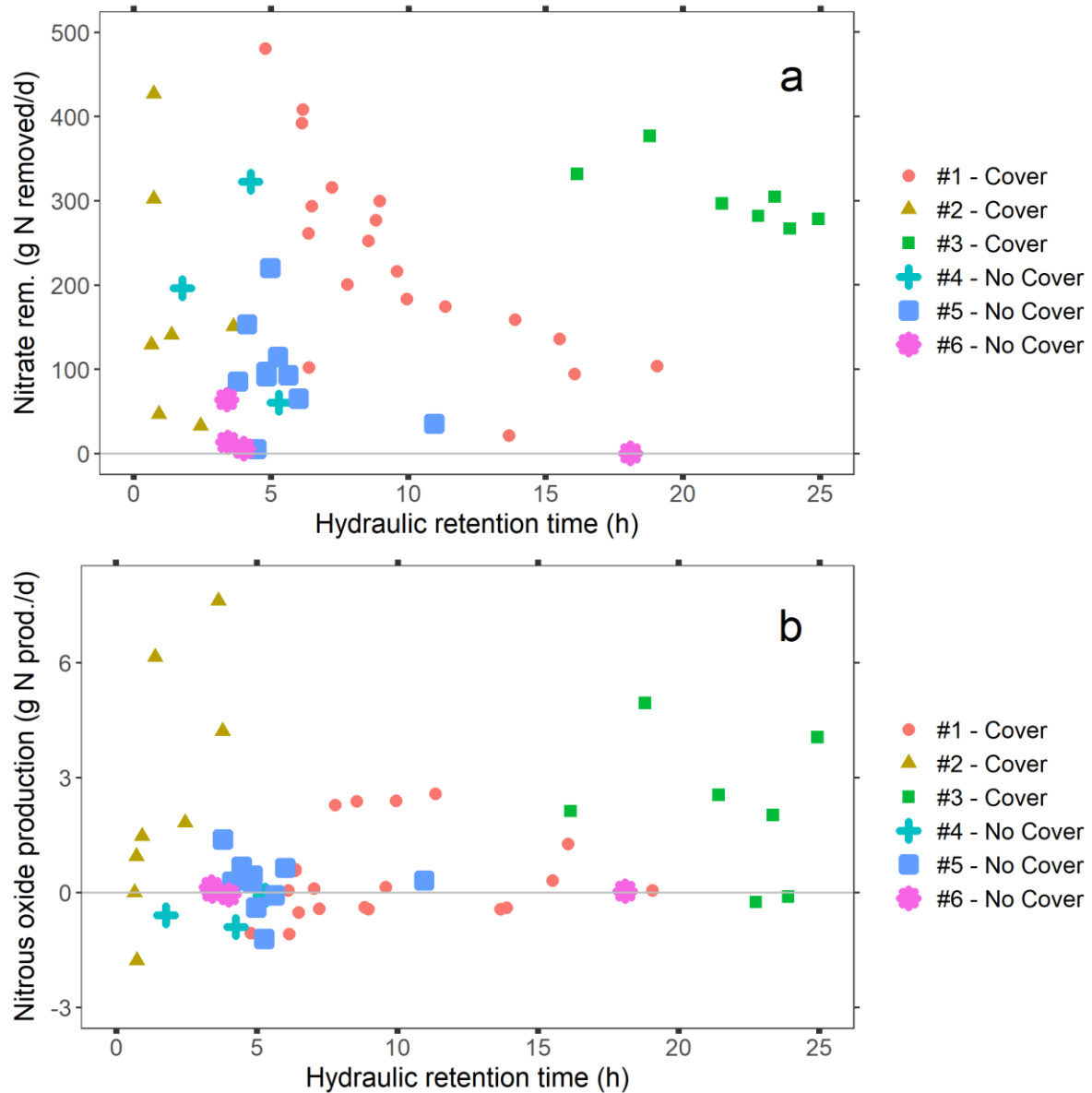


Figure 25. Mass nitrate removal (a) and nitrous oxide production (b) across hydraulic retention time (HRT) for six bioreactors in Illinois, USA.

Differences in the HRT range both between and within the covered and uncovered treatment types limited the ability to fully evaluate differences in N dynamics between treatments. For example, the covered bioreactors as a treatment group spanned HRTs from 0.6 - 25 h with relatively distinct trends by site (e.g., #2 - Cover) and limited overlap between sites. The uncovered treatment group was most generally studied under a very narrow HRT range of about 2 - 6 h which was much smaller than the range spanned by the covered bioreactors. The covered treatments showed more variation in nitrous oxide emissions (Fig. 25b), but whether this is attributable to the range of HRTs, the covered treatment itself, or another factor, remains unclear.

Beyond the important design and performance metric of bioreactor HRT, inflow nitrate loading also provided a comparison basis for these treatment groups. Inflow nitrate mass loading at bioreactors #1, #3, #4, and #6 very strongly positively correlated with mass of nitrate removal within each individual bioreactor (Spearman's $Rho > 0.92$). The correlation was still strong but not statistically significant at an α of 0.05 at #2 – Cover (Spearman's $Rho = 0.67$, $p = 0.08$). Bioreactor#5 – No Cover, which had similar inflow loading across all dates, had no significant relationship (Spearman's $Rho = -0.10$, $p = 0.79$) (Fig. 26a). Nitrate mass loading overall had no significant effect on nitrous oxide production (Spearman's $Rho = 0.17$, $p = 0.18$) and a mix of positive and negative effects within each site. There was a significant difference in inflow nitrate mass loading among sites ($p < 0.001$; Kruskal Wallis test) and between treatments ($p = 0.04$; Mann Whitney U test).

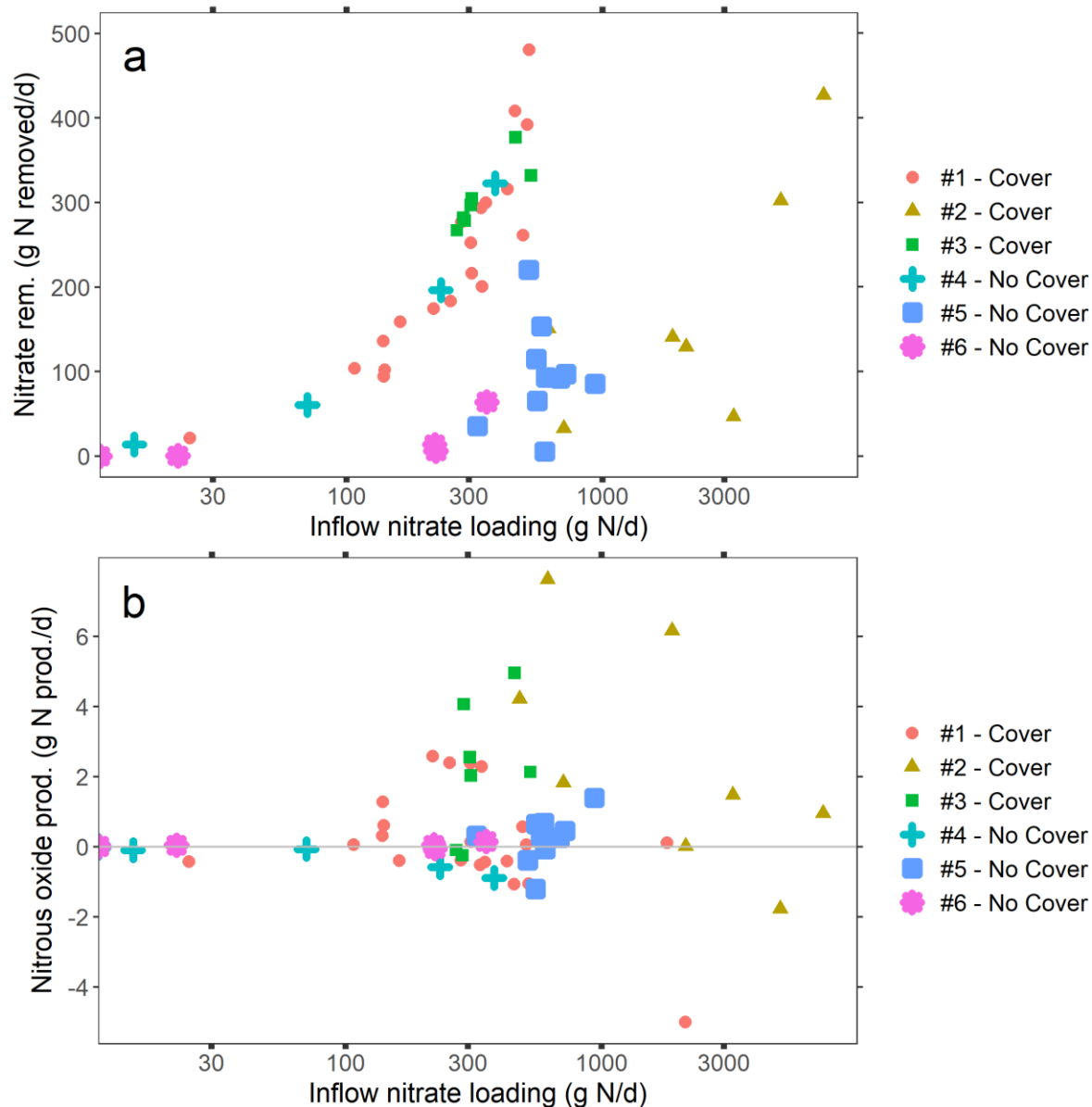


Figure 26. Inflow nitrate mass loading vs. mass nitrate removal (a) and nitrous oxide production (b). X-axis on log scale.

3.2.2 Inflow water temperature and DO

Temperature has been well related to bioreactor NO_3^- removal performance (Schipper et al., 2010; Addy et al., 2016) but was not correlated as strongly as expected here across all sites (Spearman's $\text{Rho} = 0.09$, $p = 0.50$; Fig. 27a). Nitrous oxide production appeared to peak at moderate, relatively average groundwater temperatures of 10 - 14 °C (Fig. 27c). The higher N_2O production below 15 °C may have been due to temperature-limited denitrification (i.e., limited nitrate removal, Fig 27a) with more complete denitrification (and thus less N_2O production) at

higher temperatures (Elgood et al., 2010). These conclusions were nonetheless somewhat limited across the full dataset because bioreactors #2 and #3 were never sampled at temperatures exceeding $\sim 13^{\circ}\text{C}$ (Fig. 14a and 16a).

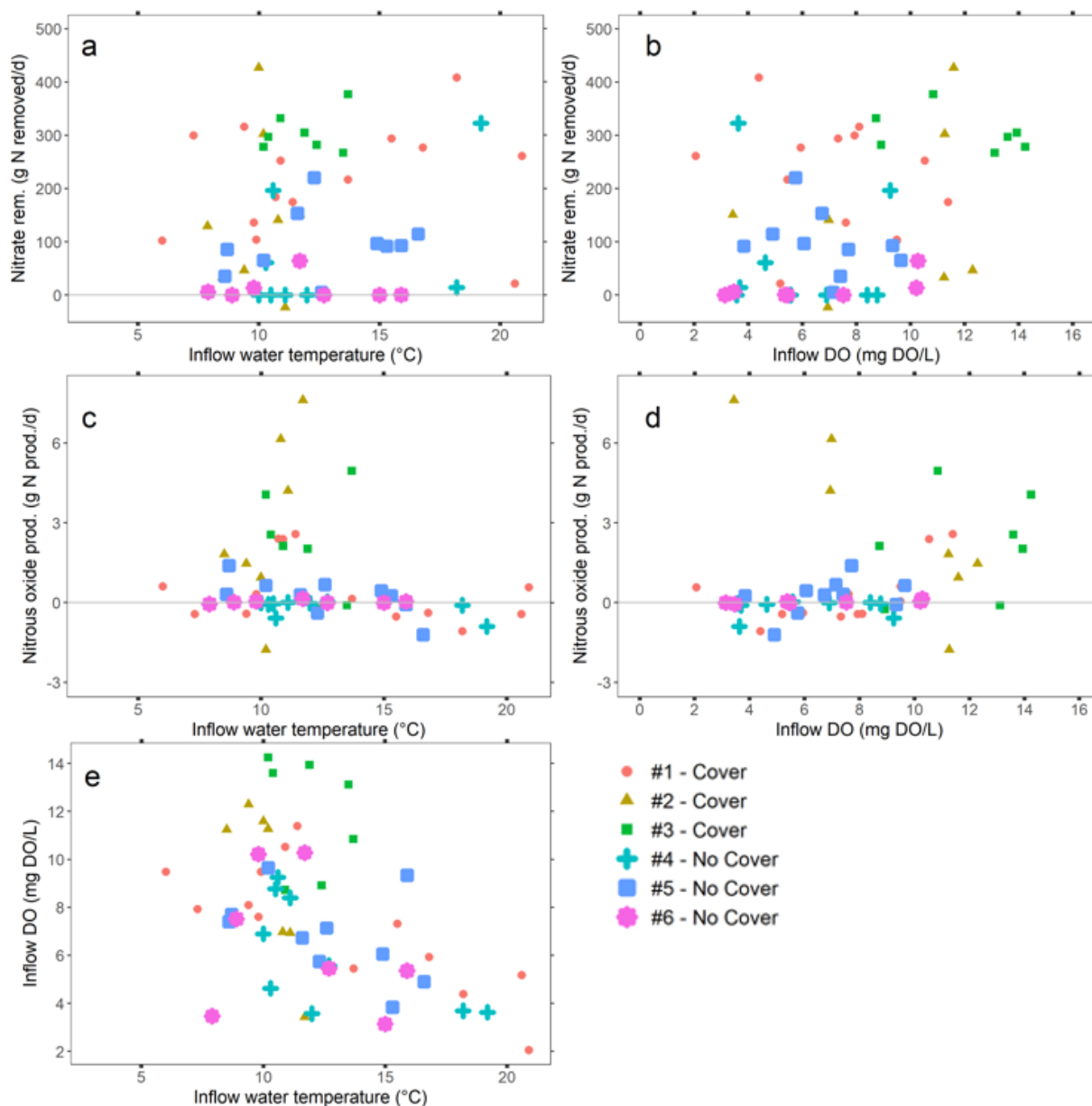


Figure 27. Mass nitrate removal (a and b) and nitrous oxide production (c and d) across all inflow water temperatures and inflow dissolved oxygen concentrations and inflow dissolved oxygen concentrations across inflow water temperatures (e).

Inflow DO concentration was weakly positively correlated with nitrate removal (Spearman's $Rho = 0.31$, $p = 0.02$) and N_2O production (Spearman's $Rho = 0.34$, $p = 0.01$) across all sites (Fig. 27b and d). Bioreactor #2 – Cover was the only site with a significant trend

with inflow DO and N₂O production ($p = 0.03$) within its own samples and showed a distinct contradictory trend (strong negative correlation: Spearman's $Rho = -0.82$) possibly due to the falling water level during N₂O production (McGuire and Reid, 2019) or the short hydraulic retention time that limited complete denitrification. When this site was excluded from the analysis, the across-site correlation between DO and N₂O production was moderate (Spearman's $Rho = 0.50$, $p=0.001$; Fig. 27d). Higher levels of DO have been found to favor the production of N₂O over N₂ (Knowles et al. 1982; Kampschreur et al., 2009).

Inflow temperature and inflow DO concentration were negatively correlated (Spearman's $Rho = -0.48$, $p < 0.001$; Fig. 27e). This may be explained by greater oxygen-depleting soil microbial activity plus the lower solubility of DO at high temperatures. Since lower temperatures (e.g., 10 - 15 °C) and higher DO concentrations coincided with greater N₂O production (Fig. 27c and d), these two factors may interrelate to influence nitrous oxide production in a bioreactor via less complete denitrification. From a practical perspective, early season drainage water would have the coolest water and highest DO concentrations, and correspondingly, some of the highest N₂O productions at #1 and #3 occurred prior to mid-May. Strategies to increase temperature and/or lower dissolved oxygen could help mitigate N₂O production. Previous attempts to heat denitrifying bioreactors have had limited success (Cameron and Schipper, 2011; Rendall, 2015). Management such as controlled drainage to increase drainage water contact with the soil microbiome prior to entering the bioreactor could decrease dissolved oxygen heading into the bioreactor and possibly mitigate N₂O production.

3.2.3 Bioreactor age and design geometry

The NRCS Conservation Practice Standard recommends a 10-y design life for denitrifying bioreactors (USDA NRCS, 2020), and bioreactors in this study ranged in age from 0.3 to 4.4 y old. The strong positive correlation between age and nitrate removal (Spearman's $Rho = 0.60$, $p < 0.001$; Fig. 28) conflicted with previous research which has shown that nitrate removal decreases after the first year of bioreactor operation but may not significantly change after that (Addy et al., 2016). However, only bioreactor #4 was in its first year and was operating under nitrate limited conditions which resulted in low nitrate removal rates that might have skewed this age analysis.

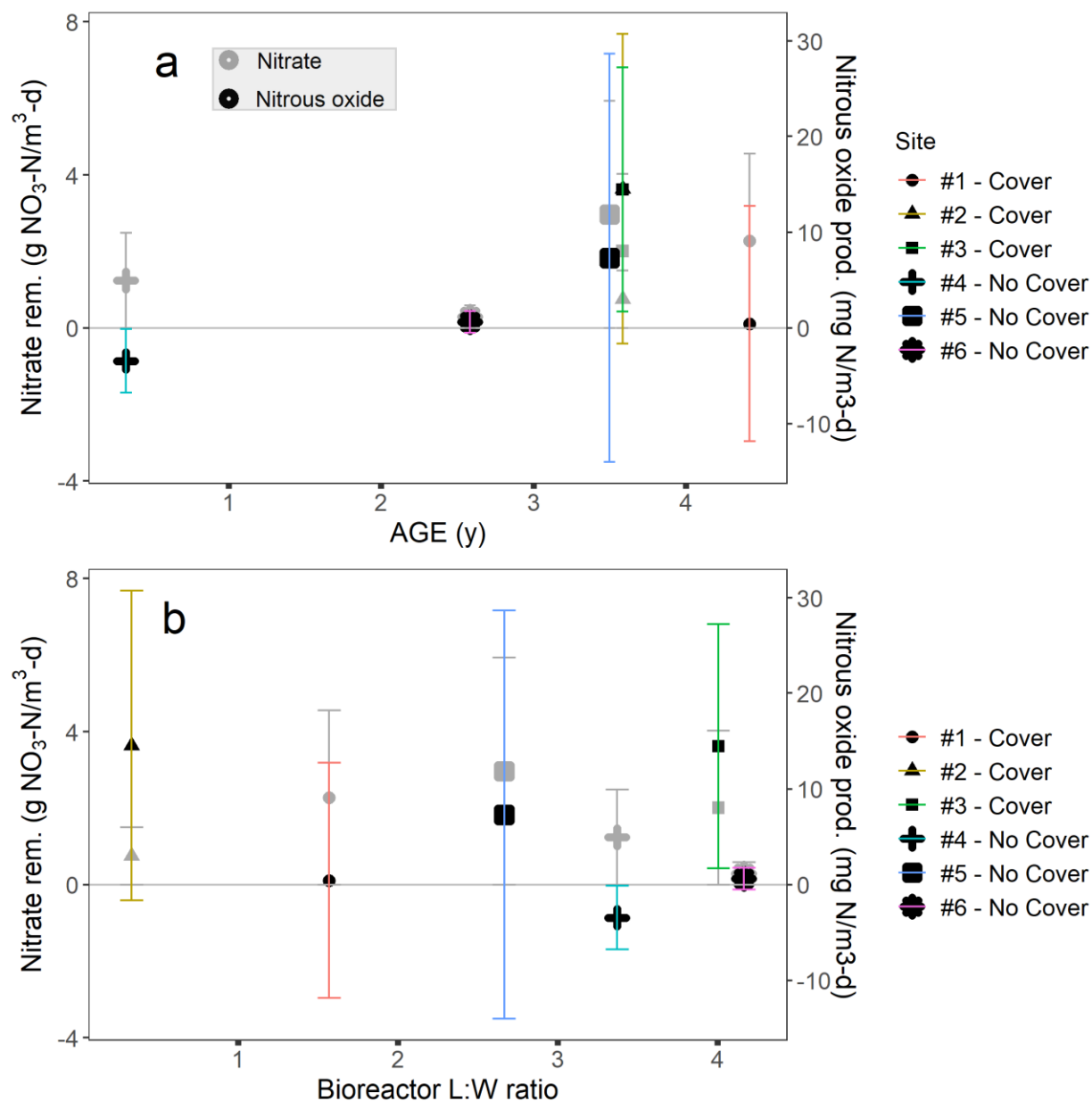


Figure 28. The effect of bioreactor age (a) and length: width ratio (b) on mean nitrate removal rate (left axis; gray points) and mean nitrous oxide production (right axis; black points). Error bars are standard deviation.

The highest mean nitrous oxide production occurred at the three bioreactors with very similar ages (#2, #3, and #5; 3.6, 3.6, and 3.5 y old, respectively), but overall, age and nitrous oxide production were not significantly correlated (Spearman's $Rho = 0.13$, $p = 0.30$; Fig. 28a). While studies have analyzed effects of age on nitrate removal, there is a paucity of studies that provide insight into the effect on nitrous oxide emissions. For instance, field-scale bioreactor nitrous oxide studies have been limited in their ability to analyze treatment of N₂O across age

since they tend to have nitrous oxide data for at most two years (David et al., 2016; Rivas et al., 2020; Audet et al., 2021). McGuire and Reid (2019) suggested that as woodchips age, the pore spaces inside wood media would increase, storing more bio-unavailable greenhouse gases and releasing more N₂O at falling water levels. Thus, falling water levels may have contributed to N₂O flux at bioreactor #2 – Cover but was not strongly suggested by data at other sites.

Bioreactor length: width (L: W) ratio itself appeared to weakly influence nitrate removal (Spearman's Rho = -0.28, p = 0.03; Fig. 28b) but did not influence nitrous oxide production (Spearman's Rho = -0.12, p = 0.35; Fig. 28b). Yet bioreactor #2, which was purposely designed to have an unusually low L:W ratio, had several peculiarities that set it apart from the other bioreactors. The low L:W ratio induced shorter retention times than the other bioreactors and likely led to a relatively low nitrate removal rate (0.75 ± 0.74 g NO₃⁻N/m³-d; Table 7). A higher flow rate because of less flow bypassing contributed to higher nitrate and nitrous oxide loading than the other bioreactors. These factors may have contributed to incomplete denitrification and higher nitrous oxide emissions.

3.3 Methane

Surface methane emissions were consistently low, and at times, orders of magnitude lower than dissolved methane emissions (Table 8). There was a sharp difference in methane production among the bioreactors, with noticeable methane production from half of the bioreactors (#1, #3, and #5) and near zero production from the other half (#2, #4, and #6). Differences in methane production did not depend on the presence/absence of a soil cover and were more dependent on nitrate removal efficiency, flow, and HRT. Methanogenesis is supported by strictly anaerobic conditions. Bioreactors #1, #3 and #5 had the highest methane production and were more anaerobic than #2 and #6, but not all instances of high methane emissions occurred under fully anaerobic conditions, such as occurred during the last sampling at #1 – and #3 – Cover. The occurrence of methane production during non-anaerobic conditions suggested the presence of anaerobic microsites within or among the woodchips. At #3 – Cover, the grassed soil control showed surface methane fluxes in a similar range to the nearby bioreactor.

Table 8. Dissolved and surface methane emissions across all bioreactors. Means exclude dates in which only surface or only dissolved samples were collected.

Site	Number of sample events, n	Mean (stdev) dCH ₄ produced g C/d	Mean (stdev) surface flux mg CH ₄ -C/m ² -h g CH ₄ -C/d		Net combined CH ₄ produced g CH ₄ -C
#1 - Cover	21 ^[a]	22.41 (46.12)	-2.68e-2 (3.55e-2)	-0.07 (0.13)	357.08
#2 - Cover	8 ^[b]	0.095 (0.117)	-2.46e-2 (3.47e-2)	-0.07 (0.09)	0.14 ^[c]
#3 - Cover	7	62.53 (25.95)	1.85e-2 (3.77e-2)	0.06 (0.12)	438.1
#4 – No Cover	9 ^[c]	-0.092 (0.220)	-5.08e-2 (0.617)	-0.10 (1.2)	-1.25
#5 – No Cover	10	11.92 (6.36)	0.821 (1.42)	0.70 (1.2)	126.3
#6 – No Cover	7 ^[c]	-0.00638 (0.00682)	1.54e-2 (3.87e-2)	0.02 (0.05)	0.12

[a] #1 – Cover had 21 sampling events overall, including 8 dissolved-only samples and 2 surface-only samples.

[b] #2 – Cover had 7 sampling events with dCH₄ data.

[c] #4 – No Cover and #6 – No Cover had only 4 sampling events with flow. Calculated means exclude events without flow.

Bioreactors #1, #3, and #5 produced substantial methane, with outflow methane concentrations within the range of levels reported by Elgood et al. (2010), up to 7.8 mg CH₄-C/L (Fig. 29). There was a general increase in outflow methane concentrations as the season progressed for bioreactors #1, #3, and #5, consistent with temperature trends observed by Elgood et al. (2010) and Bruun et al. (2017) (Fig. 29). High concentrations at the end of July and Aug. for #1 – Cover occurred during near nitrate limited conditions (Shih et al., 2011), though there were several earlier instances of nitrate limited conditions at #1 – Cover without high methane emissions. Bioreactor #3 – Cover, with the highest nitrate removal efficiency and frequent sulfur smell, had the highest average emission (Table 8), especially due to the high outlet concentrations during highly reduced and nitrate limited conditions on the last three sampling dates (Fig. 29e). Bioreactor #5 – No Cover had a comparatively low nitrate removal efficiency, consistently emitted methane at a lower concentration than #1 and #3 (Fig. 29), and had some of the highest, though still low, surface emissions (Fig. 30).

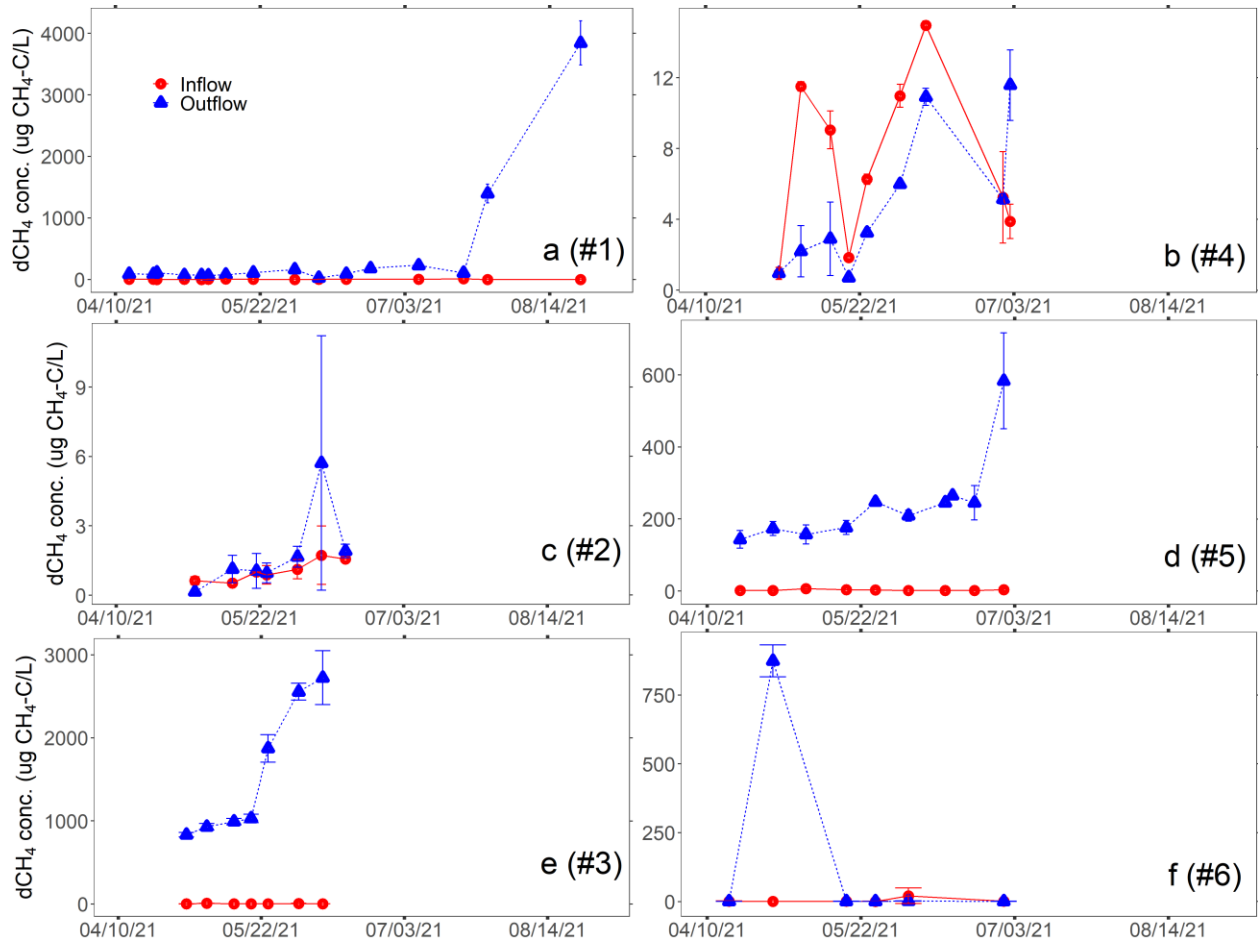


Figure 29. Time series plot of dissolved methane concentrations at six bioreactors in Illinois, USA, with covered bioreactors on the left and uncovered bioreactors on the right. Y-axes scales by panel differ across orders of magnitude.

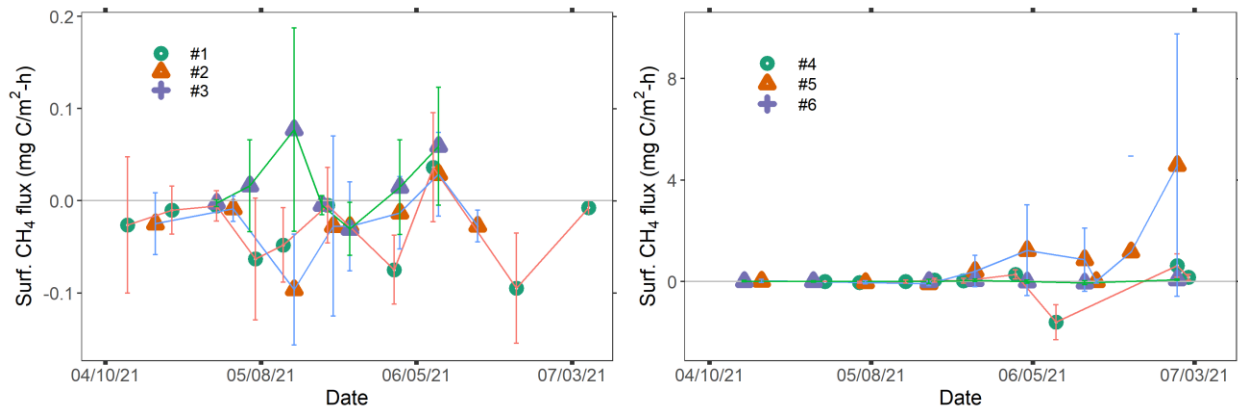


Figure 30. Time series plot of surface methane emissions from the covered bioreactors (a) and uncovered bioreactors (b). Y-axes scales by panel differ across orders of magnitude.

Bioreactors #2, #4, and #6 produced little dissolved methane, with outflow concentrations within the range, 5.28 - 34.24 $\mu\text{g CH}_4\text{-C/L}$, reported by Warneke et al. (2011b), or lower, with

the exception of a high concentration from #6 – No Cover in late April (Fig. 29). Bioreactor #2 – Cover’s shorter length to width ratio resulted in shorter retention times that minimized methane production as observed by Davis et al. (2019), suggesting that a short L: W may be effective in limiting methane and overall greenhouse gas impact. Bioreactor #2 most often had a negative surface methane flux, suggesting methane oxidation in the surface (Fig. 30a). Although complete nitrate removal and reduced conditions can lead to methanogenesis (Davis et al., 2019; Christianson et al., 2021), that was not the case at Bioreactor #4 – No Cover, which commonly removed methane despite frequent near-complete nitrate removal and a hydrogen sulfide smell. Unlike the bioreactor in Rivas et al. (2020) that removed nitrous oxide yet produced high levels of methane in the first year of operation, #4 – No Cover removed both nitrous oxide and methane in its first season of operation. Additionally, surface methane emissions comprised a larger portion of total emissions at bioreactor #4 – No Cover. Bioreactor #6 – No Cover’s minimal methane emissions are evidence that its drawdown plate can limit methane emissions as intended. Bioreactor #6 – No Cover had one instance of high outflow methane concentration ($874 \pm 58 \mu\text{g/L}$) during a dry period with no flow but complete nitrate removal.

The highest methane emissions generally occurred at the longest retention times of greater than 15 h (Spearman’s $Rho = 0.59$, $p < 0.001$), but this was heavily influenced by bioreactor #3 – Cover (HRTs: 16.2 - 24.9 h). Davis et al. (2019) found the highest methane emissions at higher HRTs when comparing 2, 8, and 16 h HRTs. This trend may be caused by the use of carbon dioxide as an electron acceptor after nitrate is depleted.

3.4 Climate change effects and bioreactor design implications

Methane contributed approximately equally to the net global warming potential compared to nitrous oxide when accounting for the entire period of study across all bioreactors (51%; Fig. 32; Table 9). Some studies have observed that CH_4 , rather than N_2O , can be a greater contributor to global warming potential in bioreactors under certain conditions (Elgood et al., 2010; Davis et al., 2019). Healy et al. (2012) and Healy et al. (2015) reached the same conclusion, but they analyzed solely surface emissions, which were found here to be much less significant than dissolved emissions. Notably, Bock et al. (2018) concluded that greenhouse gas contributions in columns were driven by nitrous oxide flux, but again, this study analyzed solely surface

emissions, for which methane flux was found to be quite low in the results stated here. This highlights the need to monitor aqueous greenhouse gas concentrations from bioreactors.

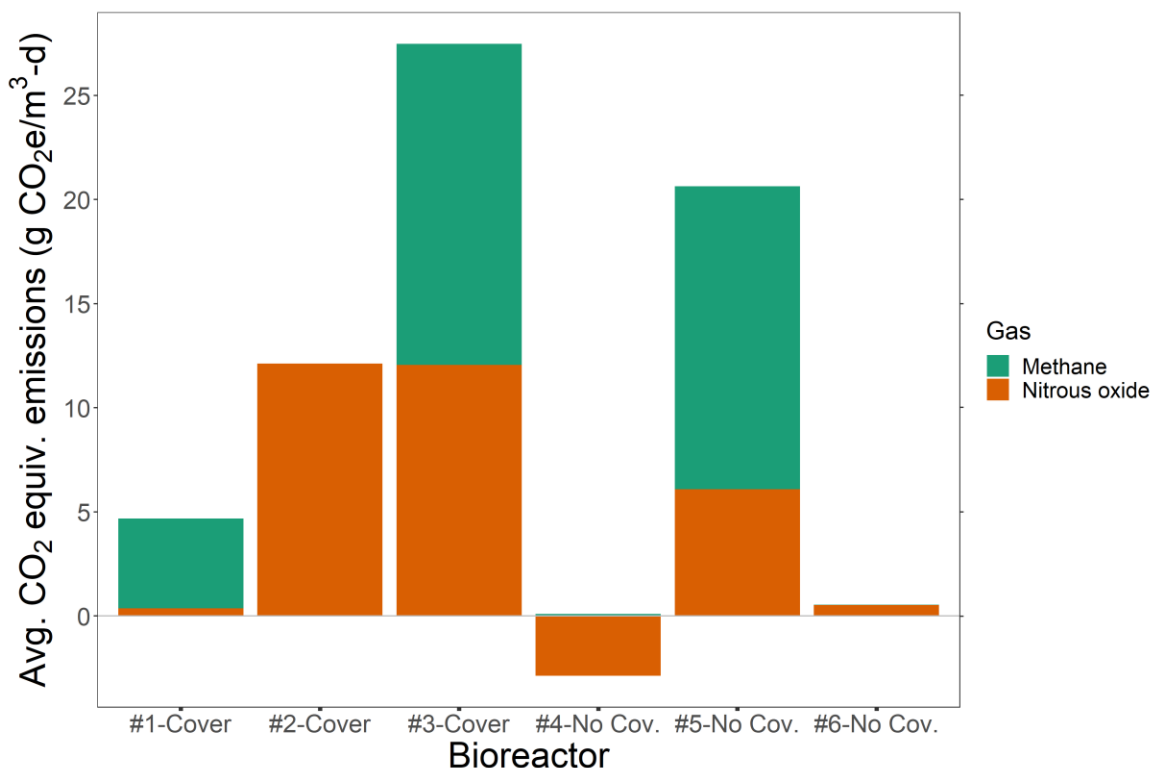


Figure 31. Average carbon dioxide emission equivalents for nitrous oxide and methane, excluding dates in which only surface or only dissolved samples were collected.

Table 9. Mean carbon dioxide emission equivalents for nitrous oxide and methane. Means exclude dates in which only surface or only dissolved samples were collected.

Site	Number of sample events, n	Mean (stdev) N ₂ O produced <i>g CO₂ e/m³-d</i>	Mean (stdev) CH ₄ produced <i>g CO₂ e/m³-d</i>	Mean combined CO ₂ equivalent <i>g CO₂ e/m³-d</i>
#1 – Cover	21 ^[a]	0.369 (10.2)	4.31 ()	4.68
#2 – Cover	8 ^[b]	12.1 (13.5)	5.44e-3 (2.71e-2)	12.1
#3 – Cover	7	12.1 (10.6)	15.4 (6.39)	27.5
#4 – No Cover	9 ^[c]	-2.87 (2.78)	0.100 (0.204)	-2.77
#5 – No Cover	10	6.09(17.8)	14.6 (8.57)	20.6
#6 – No Cover	7 ^[c]	0.517 (0.937)	0.0221 (0.0247)	0.539

[a] #1 – Cover had 21 sampling events overall, including 8 dissolved-only samples and 2 surface-only samples.

[b] #2 – Cover had 7 sampling events with dissolved data.

[c] #4 – No Cover and #6 – No Cover had only 4 sampling events with flow. Calculated means exclude events without flow.

Methane's site-specific warming impact highlighted the potential to design and manage bioreactors against greenhouse gas production. Though bioreactors #4 and #6 had the least greenhouse emissions, they rarely treated flow and thus had the lowest nitrate removals. Additionally, while a shorter HRT (#2 – Cover) and a drawdown plate (#6 – No Cover) minimized methane emissions and a sulfide smell as intended, the shorter HRT at #2 – Cover resulted in a low nitrate removal rate and one of the highest productions of nitrous oxide. Therefore, the best bioreactor for maximizing nitrate removal and minimizing greenhouse gas emissions was #1, which had mid-range HRTs (mean 9.6 h).

The bioreactors in this study generally produced greenhouse gas emissions within the range of constructed wetlands treating wastewater and produced less nitrous oxide than agricultural fields. Compared to constructed wetlands treating wastewater, all the bioreactor surface N_2O fluxes were within the range of reported values from a study in Sweden (up to $1791 \mu\text{g N}_2\text{O-N/m}^2\text{-h}$) (Johansson et al., 2011), and fluxes from bioreactors #2, #4, and #6 were within the range from a study in Estonia (2.4 to $44.7 \mu\text{g N}_2\text{O-N/m}^2\text{-h}$) (Teiter and Mander, 2005). Based on a study of nitrous oxide emissions from agricultural fields (Parkin and Kaspar, 2006), the yearly mass production of nitrous oxide from any of the bioreactors here (-0.07 to $0.8 \text{ kg N}_2\text{O-N/y}$) was orders of magnitude less than that calculated from a 7-ha field planted in corn or soybeans (71 and $19 \text{ kg N}_2\text{O-N/y}$, respectively).

CHAPTER 4: CONCLUSIONS

A limited range of conditions at six full-size denitrifying bioreactors in Illinois, USA precluded drawing conclusions about the effect of a soil cover on greenhouse gas emissions. The cover versus no cover treatments had distinct and not entirely overlapping HRT ranges and flow conditions that limited the ability to confidently evaluate differences in nitrogen dynamics between treatments. Each bioreactor had a unique nitrate and N₂O removal/production response to similar HRTs, temperatures, and water chemistries (nitrate and DO concentrations) when assessed as individual parameters. This suggested a complex combination of inflow characteristics (e.g., temperature, DO, and nitrate load) and bioreactor characteristics (e.g., woodchip material, age, design, and microbial community) could be responsible for these different responses.

Dissolved emissions accounted for most of the mass of N₂O and CH₄ released, especially for methane. Methane contributed almost nothing to emissions at bioreactors #2, #4, and #6, which reiterated the unique nature of each bioreactor. Even though surface N₂O emissions were low, the highest fluxes were generally from nearest the bioreactor outflow, suggesting the presence of denitrification hotspots. Surface and dissolved emissions may not be directionally linked, as there were several instances where a high surface emission occurred during dN₂O or dCH₄ consumption. These data suggested that a soil cover may not force surface gas emissions into the aqueous phase, which argued against the hypothesis that a soil cover would force emissions into the liquid outflow.

Dissolved nitrous oxide had variable production and removal inside these bioreactors. Its removal was observed during nitrate limitation, high nitrate removal efficiencies and/or low DO concentrations but didn't necessarily always coincide with complete nitrate reduction. Bioreactor #4 – Cover always removed dN₂O, which may have been due to its low inflow nitrate loading and young age. Bioreactor #2 – Cover differed from the others in its wide design, low HRTs, and high nitrate loading and seemed to consume and produce nitrous oxide in trends opposite to other bioreactors with dN₂O production during low inflow DO concentrations. Possible management to mitigate nitrous oxide production could take the form of reducing incoming dissolved oxygen levels or controlled drainage.

Bioreactors with properties like bioreactor #1 such as mid-range HRTs (6 - 13 h) could be the best compromise between improving subsurface drainage water quality and achieving

climate smart agriculture. In the future, there should be more long-term greenhouse gas monitoring studies to determine if age is a factor in greenhouse gas emissions. Further work on field-scale bioreactors ensuring a full range of HRTs in cover/no cover treatments would provide a more robust conclusion to this study's research question, although finding enough conveniently located full-scale bioreactors with these conditions will be a challenge as it was in this study. Until further research gives a more conclusive answer to the effects of cover/no cover treatments at the field scale, the conservation practice standard should continue to allow the landowner to make the cover/no cover decision based on maintenance or aesthetic preferences. Although differences in field-scale bioreactors precluded a robust comparison of the cover/no cover treatment, they revealed the distinct uniqueness in response, especially for methane emissions, to environmental variables depending on site, highlighting the interplay of factors that affect greenhouse gas emissions.

REFERENCES

- Addy, K., Gold, A. J., Christianson, L. E., David, M. B., Schipper, L. A., Ratigan, N. A. (2016). Denitrifying Bioreactors for Nitrate Removal: A Meta-Analysis. *Journal of Environmental Quality*, 45(3), 873–881. <https://doi.org/10.2134/jeq2015.07.0399>
- American Public Health Association. (2017). 2720 C. Gas Chromatographic Method. In R.B. Baird, A.D. Eaton, E.W. Rice, editors, Standard methods for the analysis of water and wastewater. *American Public Health Association*, Washington, DC. P. 223-225.
- ANSI/ASABE. (1992). S424.1: Method of determining and expressing particle size of chopped forage materials by screening. St. Joseph, MI: ASABE
- Audet, J., Jégliot, A., Elsgaard, L., Maagaard, A. L., Sørensen, S. R., Zak, D., Hoffmann, C. C. (2021). Nitrogen removal and nitrous oxide emissions from woodchip bioreactors treating agricultural drainage waters. *Ecological Engineering*, 169(April). <https://doi.org/10.1016/j.ecoleng.2021.106328>
- Bock, E. M., Coleman, B. S. L., Easton, Z. M. (2018). Performance of an under-loaded denitrifying bioreactor with biochar amendment. *Journal of Environmental Management*, 217, 447–455. <https://doi.org/10.1016/j.jenvman.2018.03.111>
- Bruun, J., Hoffmann, C. C., Kjaergaard, C. (2017). Convective transport of dissolved gases determines the fate of the greenhouse gases produced in reactive drainage filters. *Ecological Engineering*, 98(3), 1–10. <https://doi.org/10.1016/j.ecoleng.2016.10.027>
- Cameron, S. G., Schipper, L. A. (2011). Evaluation of passive solar heating and alternative flow regimes on nitrate removal in denitrification beds. *Ecological Engineering*, 37(8), 1195–1204. <https://doi.org/10.1016/j.ecoleng.2011.02.020>
- Christianson, L. E., Bhandari, A., Helmers, M. J., Schilfgaarde, V. (2012). A Practice-Oriented Review of Woodchip Bioreactors for Subsurface Agricultural Drainage. *Applied Engineering in Agriculture*. 28(3), 861–874.
- Christianson, L. E., Christianson, R. D., Lipka, A. E., Bailey, S., Chandrasoma, J., McCoy, C., ... Cooke, R. A. (2019). Calibration of Stainless Steel-edged V-Notch Weir Stop Logs for Water Level Control Structures. *Applied Engineering in Agriculture*, 35(5), 745–749. <https://doi.org/10.13031/aea.13350>

- Christianson, L. E., Cooke, R. A., Hay, C. H., J, H. M., Feyereisen, G. W., Ranaivoson, A. Z., ... Ahnen, M. Von. (2021). Effectiveness of Denitrifying Bioreactors on Water Pollutant Reduction from Agricultural Areas. *Transactions of the ASABE*, 64(2), 641–658.
- Christianson, L, Bhandari, A., Helmers, M., Kult, K., Sutphin, T., Wolf, R. (2012). Performance Evaluation of Four Field-Scale Agricultural Drainage Denitrification Bioreactors in Iowa. *Transactions of the ASABE*. 55(3), 2163–2174.
- Christianson, Laura, Hanly, J., Jha, N., Saggarr, S., Hedley, M. (2013). Denitrification bioreactor nitrous oxide emissions under fluctuating flow conditions. American Society of Agricultural and Biological Engineers Annual International Meeting 2013, ASABE 2013, 4, 2861–2867. <https://doi.org/10.13031/aim.20131597821>
- Chun, J. A., Cooke, R. A. (2008). Calibrating Agridrain Water Level Control Structures using Generalized Weir and Orifice Equations. *Applied Engineering in Agriculture*. 24(5), 595–602.
- David, M. B., Gentry, L. E., Cooke, R. A., Herbstritt, S. M. (2016). Temperature and Substrate Control Woodchip Bioreactor Performance in Reducing Tile Nitrate Loads in East-Central Illinois. *Journal of Environmental Quality*, 45(3), 822–829. <https://doi.org/10.2134/jeq2015.06.0296>
- Davis, M. P., Martin, E. A., Moorman, T. B., Isenhardt, T. M., Soupir, M. L. (2019). Nitrous oxide and methane production from denitrifying woodchip bioreactors at three hydraulic residence times. *Journal of Environmental Management*, 242(May 2019), 290–297. <https://doi.org/10.1016/j.jenvman.2019.04.055>
- Dougherty, H., Cooke, R. A. C., Bhattarai, R., Christianson, L. (2020). Design flow and nitrate removal evaluation of a wide denitrifying bioreactor with baffles. *Ecological Engineering*, 158(September), 106068. <https://doi.org/10.1016/j.ecoleng.2020.106068>
- Dougherty, H. L. (2018). Hydraulic Evaluation of a Denitrifying Bioreactor with Baffles. MS thesis. Urbana, IL: University of Illinois.
- Elgood, Z., Robertson, W. D., Schiff, S. L., Elgood, R. (2010). Nitrate removal and greenhouse gas production in a stream-bed denitrifying bioreactor. *Ecological Engineering*, 36(11), 1575–1580. <https://doi.org/10.1016/j.ecoleng.2010.03.011>
- Friedl, J., Scheer, C., Rowlings, D. W., McIntosh, H. V., Strazzabosco, A., Warner, D. I., Grace, P. R. (2016). Denitrification losses from an intensively managed sub-tropical pasture -

- Impact of soil moisture on the partitioning of N₂ and N₂O emissions. *Soil Biology and Biochemistry*, 92(2), 58–66. <https://doi.org/10.1016/j.soilbio.2015.09.016>
- Goeller, B. C., Burbery, L. F., Febria, C. M., Collins, K. E., Burrows, N. J., Simon, K. S., ... McIntosh, A. R. (2019). Capacity for bioreactors and riparian rehabilitation to enhance nitrate attenuation in agricultural streams. *Ecological Engineering*, 134(December 2018), 65–77. <https://doi.org/10.1016/j.ecoleng.2019.03.014>
- Grießmeier, V., Gescher, J. (2018). Influence of the potential carbon sources for field denitrification beds on their microbial diversity and the fate of carbon and nitrate. *Frontiers in Microbiology*, 9(June 2018), 1–12. <https://doi.org/10.3389/fmicb.2018.01313>
- Healy, M. G., Barrett, M., Lanigan, G. J., João Serrenho, A., Ibrahim, T. G., Thornton, S. F., ... Fenton, O. (2015). Optimizing nitrate removal and evaluating pollution swapping trade-offs from laboratory denitrification bioreactors. *Ecological Engineering*, 74(3), 290–301. <https://doi.org/10.1016/j.ecoleng.2014.10.005>
- Healy, Mark G., Ibrahim, T. G., Lanigan, G. J., Serrenho, A. J., Fenton, O. (2012). Nitrate removal rate, efficiency and pollution swapping potential of different organic carbon media in laboratory denitrification bioreactors. *Ecological Engineering*, 40, 198–209. <https://doi.org/10.1016/j.ecoleng.2011.12.010>
- Hertzberger, A., Pittelkow, C. M., Harmel, R. D., Christianson, L. E. (2019). Analysis of the MANAGE Drain Concentration Database to Evaluate Agricultural Management Effects on Drainage Water Nutrient Concentrations. *Transactions of the ASABE*, 62(4), 929–939.
- IEPA, IDOA. (2015). Illinois Nutrient Loss Reduction Strategy. Illinois Environmental Protection Agency.
- IPCC. (2007). *Climate Change 2007: Synthesis Report. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Core Writing Team, Pachauri, R.K and Reisinger, A. (eds.)]. IPCC, Geneva, Switzerland, 104 pp.
- Jahangir, M. M. R., Johnston, P., Khalil, M. I., Grant, J., Somers, C., Richards, K. G. (2012). Evaluation of headspace equilibration methods for quantifying greenhouse gases in groundwater. *Journal of Environmental Management*, 111, 208–212. <https://doi.org/10.1016/j.jenvman.2012.06.033>

- Johansson, A. E., Klemetsson, Å. K., Klemetsson, L., Svensson, B. H. (2011). Nitrous oxide exchanges with the atmosphere of a constructed wetland treating wastewater. *Tellus B: Chemical and Physical Meteorology*, 55(3), 737–750.
<https://doi.org/10.3402/tellusb.v55i3.16363>
- Johnson, G. M. (2021). Range of and Relationships between Physical and Hydraulic Properties of Commonly Available Denitrifying Bioreactor Woodchips. MS thesis. Urbana, IL: University of Illinois.
- Johnson, G.M., Christianson, R.D., Cooke, R.A.C., Díaz-García, C., Christianson, L.E. *Under review* 2022. Denitrifying bioreactor woodchip sourcing guidance based on physical and hydraulic properties. Submitted to *Ecological Engineering*.
- Kampschreur, M. J., Temmink, H., Kleerebezem, R., Jetten, M. S. M., van Loosdrecht, M. C. M. (2009). Nitrous oxide emission during wastewater treatment. *Water Research*, 43(17), 4093–4103. <https://doi.org/10.1016/j.watres.2009.03.001>
- Knowles, R. (1982). Denitrification. *Microbiological Reviews*, 46(1), 43–70.
- Lepine, C., Christianson, L., Sharrer, K., Summerfelt, S. (2015). Optimizing Hydraulic Retention Times in Denitrifying Woodchip Bioreactors Treating Recirculating Aquaculture System Wastewater. *Journal of Environmental Quality*, 45(3), 813–821.
<https://doi.org/10.2134/jeq2015.05.0242>
- Le Mer, J., Roger, P. (2001). Production, oxidation, emission and consumption of methane by soils: A review. *European Journal of Soil Biology*, 37(1), 25–50.
[https://doi.org/10.1016/S1164-5563\(01\)01067-6](https://doi.org/10.1016/S1164-5563(01)01067-6)
- Manca, F., De Rosa, D., Reading, L. P., Rowlings, D. W., Scheer, C., Schipper, L. A., Grace, P. R. (2021). Effect of soil cap and nitrate inflow on nitrous oxide emissions from woodchip bioreactors. *Ecological Engineering*, 166(April), 106235.
<https://doi.org/10.1016/j.ecoleng.2021.106235>
- Martin, E. A., Davis, M. P., Moorman, T. B., Isenhardt, T. M., Soupir, M. L. (2019). Impact of hydraulic residence time on nitrate removal in pilot-scale woodchip bioreactors. *Journal of Environmental Management*, 237(May 2018), 424–432.
<https://doi.org/10.1016/j.jenvman.2019.01.025>

- Maxwell, B. M., Cooke, R. A., Christianson, R. D., Christianson, L. E. (2021). Stage-discharge Relationships of Drawdown Plates for Denitrifying Woodchip Bioreactors. *Applied Engineering in Agriculture*, 37(6), 1023–1029.
- McGuire, P. M., Reid, M. C. (2019). Nitrous Oxide and Methane Dynamics in Woodchip Bioreactors: Effects of Water Level Fluctuations on Partitioning into Trapped Gas Phases. *Environmental Science and Technology*, 53(24), 14348–14356. <https://doi.org/10.1021/acs.est.9b04829>
- Moorman, T. B., Parkin, T. B., Kaspar, T. C., Jaynes, D. B. (2010). Denitrification activity, wood loss, and N₂O emissions over 9 years from a wood chip bioreactor. *Ecological Engineering*, 36(11), 1567–1574. <https://doi.org/10.1016/j.ecoleng.2010.03.012>
- Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestad, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Takemura, T., Zhang, H., 2013. Anthropogenic and natural radiative forcing. *Clim. Chang.* 2013 Phys. Sci. Basis. Contrib. Work. Gr. I to Fifth Assess. Rep. Intergov. Panel Clim. Chang. 659–740. <https://doi.org/10.1017/CBO9781107415324.018>.
- Natural Resources Conservation Service. Conservation Practice Standard: Denitrifying Bioreactor (2020).
- Nordström, A., Herbert, R. B. (2018). Determination of major biogeochemical processes in a denitrifying woodchip bioreactor for treating mine drainage. *Ecological Engineering*, 110(June 2017), 54–66. <https://doi.org/10.1016/j.ecoleng.2017.09.018>
- Parkin, T. B., Venterea, R. T., Hargreaves, S. K. (2012). Calculating the Detection Limits of Chamber-based Soil Greenhouse Gas Flux Measurements. *Journal of Environmental Quality*, 41(3), 705–715. <https://doi.org/10.2134/jeq2011.0394>
- Parkin, T.B. and Venterea, R.T. (2010). Sampling Protocols. Chapter 3. Chamber-Based Trace Gas Flux Measurements. IN Sampling Protocols. R.F. Follett, editor. p. 3-1 to 3-39.
- Randall, G. W., Goss, M. J. (2008). Nitrate Losses to Surface Water Through Subsurface, Tile Drainage. *Nitrogen in the Environment*, (1999), 145–175. <https://doi.org/10.1016/B978-0-12-374347-3.00006-8>
- Rendall, T. (2015). Effect of passive and active heating on the performance of denitrifying bioreactors. MS thesis. Urbana, IL: University of Illinois.

- Rivas, A., Barkle, G., Stenger, R., Moorhead, B., Clague, J. (2020). Nitrate removal and secondary effects of a woodchip bioreactor for the treatment of subsurface drainage with dynamic flows under pastoral agriculture. *Ecological Engineering*, 148(August 2019), 105786. <https://doi.org/10.1016/j.ecoleng.2020.105786>
- Sanchez Bustamante Bailon, A. P. (2020). Dissolved Phosphorous Removal in Denitrifying Bioreactors: Field and Laboratory Studies. MS thesis. Urbana, IL: University of Illinois.
- Schipper, L. A., Robertson, W. D., Gold, A. J., Jaynes, D. B., Cameron, S. C. (2010). Denitrifying bioreactors-An approach for reducing nitrate loads to receiving waters. *Ecological Engineering*, 36(11), 1532–1543. <https://doi.org/10.1016/j.ecoleng.2010.04.008>
- Shih, R., Robertson, W. D., Schiff, S. L., Rudolph, D. L. (2011). Nitrate Controls Methyl Mercury Production in a Streambed Bioreactor. *Journal of Environmental Quality*, 40(5), 1586–1592. <https://doi.org/10.2134/jeq2011.0072>
- Teiter, S., Mander, Ü. (2005). Emission of N₂O, N₂, CH₄, and CO₂ from constructed wetlands for wastewater treatment and from riparian buffer zones. *Ecological Engineering*, 25(5), 528–541. <https://doi.org/10.1016/j.ecoleng.2005.07.011>
- US EPA. (2021). Hypoxia 101: What is hypoxia and what causes it? EPA Hypoxia Task Force. <https://www.epa.gov/ms-htf/hypoxia-101>
- Warneke, S., Schipper, L. A., Bruesewitz, D. A., Baisden, W. T. (2011). A comparison of different approaches for measuring denitrification rates in a nitrate removing bioreactor. *Water Research*, 45(14), 4141–4151. <https://doi.org/10.1016/j.watres.2011.05.027>
- Warneke, S., Schipper, L. A., Bruesewitz, D. A., McDonald, I., Cameron, S. (2011). Rates, controls and potential adverse effects of nitrate removal in a denitrification bed. *Ecological Engineering*, 37(3), 511–522. <https://doi.org/10.1016/j.ecoleng.2010.12.006>
- Warneke, S., Schipper, L. A., Matiassek, M. G., Scow, K. M., Cameron, S., Bruesewitz, D. A., McDonald, I. R. (2011). Nitrate removal, communities of denitrifiers and adverse effects in different carbon substrates for use in denitrification beds. *Water Research*, 45(17), 5463–5475. <https://doi.org/10.1016/j.watres.2011.08.007>
- Weiss, R. F. (1974). Carbon Dioxide in Water and Seawater: The Solubility of a Non-Ideal Gas. *Marine Chemistry*, 2, 203–215. [https://doi.org/https://doi.org/10.1016/0304-4203\(74\)90015-2](https://doi.org/https://doi.org/10.1016/0304-4203(74)90015-2)

- Wickramarathne, N. M., Cooke, R. A., Book, R., Christianson, L. E. (2020). Denitrifying Woodchips Bioreactor Leachate Tannic Acid and True Color: Lab and Field Studies. *Transactions of the ASABE*, 63(6), 1747–1757.
- Wiesenburg, D. A., Guinasso, N. L. (1979). Equilibrium Solubilities of Methane, Carbon Monoxide, and Hydrogen in Water and Sea Water. *Journal of Chemical and Engineering Data*, 24(4), 356–360. <https://doi.org/10.1021/je60083a006>
- Young, C. L. (1981). Solubility Data Series. *Chemistry International -- Newsmagazine for IUPAC* (Vol. 8). Parkville, Victoria, Australia. <https://doi.org/10.1515/ci.2008.30.6.19>