

Project Title: Assessing and Optimizing Wood Chip Bioreactor Denitrification Rates

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Abstract: This study assessed hydraulic retention times, nitrous oxide emissions, nitrate and carbon levels, and relative denitrification rates using three denitrification woodchip bioreactors on the Salinas Valley farms. Using a sodium bromide tracer, we found that the larger bioreactor had a later peak time (41 hours) compared to the bioreactor half its volume (28 hours), despite intended equal hydraulic retention times; the longer retention time indicated by later peak time may have implications for the current nitrate monitoring interval. The tests also confirmed that hydraulic retention time is shorter than expected if woodchip density is not maintained; peak time was 13 hours before woodchip replenishment and 28 hours after. We also found evidence for greater vertical mixing in the bioreactor with a flow rate of 2 gpm, versus the one at 1 gpm. Using a combination of methods, we found relatively high surface emissions and dissolved nitrous oxide concentrations compared to other agricultural, bioreactor, and wetland studies. Additionally, mean surface emissions for each bioreactor near the inlet were 0.03 to 0.25 mg N₂O-N/m²/hour; these values were significantly lower than near-outlet fluxes at both of the two sites sampled, indicating that less denitrification is occurring in the first few feet of the bioreactor (p<0.01). Mid-bed emissions were not significantly different than near-outlet emissions; mean mid-bed and near-outlet fluxes ranged from 1.76 to 3.26 mg N₂O-N/m²/hour. The measured fluxes were higher than from mineral soils studies in the region. Dissolved nitrous oxide levels were higher in the bioreactors than in the nearby drainage ditch and inlet pipe; bioreactor concentrations ranged from 0.58 to 1.86 mg N₂O-N/L water.

Objectives:

Three wood chip bioreactors have been installed in the past year by the Monterey County UC Cooperative Extension. The Cooperative Extension is monitoring NO₃⁻ concentrations of the bioreactor influent and effluent as well as temperature and dissolved oxygen (DO), both of which are important to successful denitrification, the mechanism of nitrate removal. The research objective of this CSU Monterey Bay study is to monitor those bioreactors for additional parameters that can affect denitrification within the bioreactors, and to use this information to optimize woodchip bioreactor NO₃⁻ removal rates and assess greenhouse gas emissions.

Procedures:

1. Tracer Tests

Two full tracers were completed at DBR 3, and a tracer was attempted at DBR 1 but only some of the data was collected due to a pump breaking. The first tracer test at DBR 3 was conducted shortly after 300 lbs of woodchips had been added to improve and increase woodchip density (decrease porosity), while the second test occurred after even more woodchips had been added for a total of 580 lbs (including the original 300 lbs). During the first test, it is likely that the woodchips had not compressed into the bed at that time and that the tracer test results therefore were a hybrid of before the chips were added and after. The last tracer was at a DBR 1, which holds twice the volume of the DBR 3 and DBR 2; four days of data collection occurred before the bioreactor pump stopped working.

We used a sodium bromide (NaBr) tracer to evaluate the hydraulic retention time of the bioreactors and other wetland hydrological parameters. The amount of bromide used for each tracer test was estimated using a model-based approach with the goal of recovering an outlet bromide peak that was 10-50 times the background bromide concentration for each site (Kadlec and Wallace 2009). The total sodium bromide mass for the DBR 3 tracer experiments was 1.5 kg for each run; for DBR 1, 3.0 kg was added due to the larger bioreactor volume.

The bromide tracer, dissolved in water, was injected at the inlet at the same flow rate of the bioreactor influent. We collected outlet samples for bromide analysis for approximately one week. We also collected hourly inlet and outlet nitrate samples for two days during the tracer test. Samples were analyzed for bromide using an ion chromatograph (ICS-2000, Dionex, Sunnyvale, CA), while nitrate samples were run on a flow injection analyzer (QuikChem 8500, Lachat Instruments, Loveland, CO).

Multiple analyses were performed at the end of the test, including firstly the expected/nominal hydraulic retention time so that comparisons could be made to observed retention time. Expected retention time was calculated using the volume of each bioreactor and a woodchip-to-water ratio calculated by submerging woodchips in a measured volume of water in lab. Observed retention time in the bioreactors is presented here as peak time, which is typically shorter than the mean residence time but gives a good frame of reference.

2. Nitrate, Ammonia, Phosphorus, Dissolved Nitrogen (DN), and Dissolved Organic Carbon (DOC)

We collected samples for analysis of nitrate, ammonia, phosphorus, dissolved nitrogen and dissolved organic carbon on a weekly basis from DBR 2, during the summer and fall of 2012. We also collected one set of samples from both DBR 2 and DBR 3 during spring 2014.

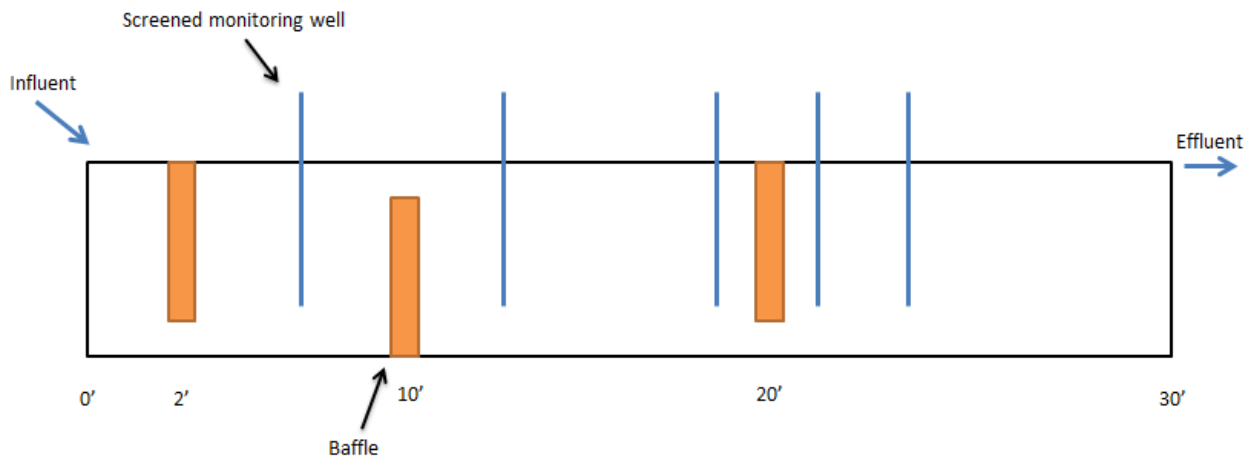
Samples were collected at each sampling event, in random order, from each of the monitoring well ports in the bioreactors at the two-foot depth, three-foot depth, inlet and outlet (Fig. 1). Each well is screened to take samples at four depth intervals, allocating a length of tubing for each depth with aquarium aerators attached to the ends to exclude large particles, i.e. woodchips. Comparisons across the width of the bioreactor were made at approximately 18 feet down the length of the bioreactor, using three monitoring wells that were side-by-side. Samples were not

filtered in the lab but were partially filtered in the field by attaching aquarium bubblers onto the intake tubes of the monitoring well ports. Outlet samples were the one exception that required additional sample filtration, using a 45 μm Whatman filter, due to turbidity. Samples were collected in plastic bottles and frozen until analysis on a flow injection analyzer (QuikChem 8500, Lachat Instruments, Loveland, CO).

Samples were collected using the same method for dissolved nitrogen (DN) and dissolved organic carbon (DOC) analysis but were stored in amber glass bottles, and were acidified and refrigerated until analyses via a Shimadzu Combustion TOC Analyzer.

A comparison of nitrate levels was conducted between two and three foot depths, using a one-sided paired t-test. Similar analysis will be conducted for dissolved organic carbon. Nitrate levels were also compared across the width of the bioreactor at the midway set of monitoring wells by coefficient of variation analysis.

(a)



(b)



Figure 1. (a) Location of baffles and monitoring wells, according to the dimensions of DBR 3; and (b) view of the bed with two narrow PVC monitoring wells tops sticking out several inches above the surface and one larger pipe used by the UC Cooperative Extension also apparent. The inlet pipe is to the far left of the photograph.

3. Nitrous Oxide (surface and dissolved)

We collected samples weekly from July 10 through September 20, 2013, at DBR 2 and DBR 3. The intent of nitrous oxide sampling was to quantify emissions during the summer season, when emissions were likely to be the highest due to the temperature dependence of denitrification. Surface and dissolved nitrous oxide emission samples were collected during the same sampling event.

Surface

We measured surface nitrous oxide emissions from the top of the bioreactors at three chamber locations per site. The chamber locations were “near-inlet” i.e. within six feet of the bioreactor inlet; “mid-bed” i.e. approximately 18 feet down the length of the bed; and “near-outlet” i.e. within six feet of the bioreactor outlet (Fig. 2). Chambers were placed at least one and a half feet away from the edge of the bioreactor, but close enough to the edge of the bioreactor so that sampling could be conducted without standing on the bioreactor and potentially disturbing the chamber flux.

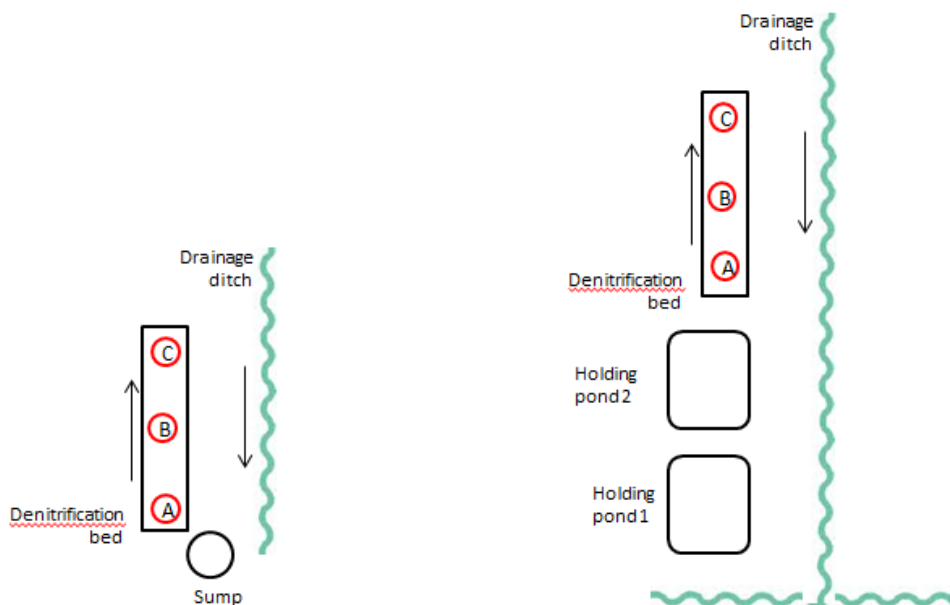


Figure 2. Placement of chambers at each field site, DBR 2 (left) and DBR 3 (right). Chamber A was located within the first six feet of the bioreactor near the *inlet*, chamber B about 18 feet down (*middle*), and chamber C over 30 feet down near the *outlet*.

Plastic bases (0.05 m^2) and static, vented chambers were used during sampling per the methodology of Parkin et al. (2003) and Rochette and Eriksen-Hammel (2008). We installed the bases at least a day before sampling; bases were removed and re-installed several times during the study to maintain integrity of the bases (i.e. when woodchips were added). Bases were inserted to a depth of 8 cm. Chambers were wrapped in gold reflective tape to minimize the effect of elevated temperatures on internal chamber conditions (Parkin et al. 2003).

During the sampling event, five gas samples were taken at eight-minute intervals, for a total sampling time of 32 minutes. Samples were drawn from chambers using 12 mL pre-evacuated

glass Exetainer vials (Labco Ltd., High Wycombe, UK) fitted with a needle. Vials were then processed on a Shimadzu GC 2014 gas chromatograph (Shimadzu Corporation, Pleasanton, CA) and calibrated using a standard curve. Quality control samples were run every 20 samples. The data were analyzed using the HMR package in R Statistical Software to create a flux diagram and average flux per m² per second (Pederson 2013; R Core Team 2013).

Dissolved

Samples for dissolved nitrous oxide were collected at the inlet, outlet, and midpoint of the bed as well as from the adjacent drainage ditch at both sites, at a consistent location both upstream and downstream of the bed outlet. The inlet and outlet samples differed from the surface nitrous oxide sampling locations in that the inlet sample was taken before entering the bioreactor from the inlet pipe, and the outlet sample was taken from the free-flowing water just before the outlet pipe. At the midpoint of the bioreactor, samples were collected at the surface of the bioreactor and also at a two-foot depth. The runoff water in the drainage ditch at DBR 2 was usually stagnant, and therefore other factors such as plant and algal growth in the ditch may have affected nitrous oxide content. In contrast, the runoff water in the drainage ditch at DBR 3 was never stagnant during sampling events. Two replicates were sampled per location, and the average result of these replicates was used as the final sample value.

Sample collection was based on vapor-liquid equilibrium principles. First, a 25-mL water sample was drawn from the bed with the 50-mL glass syringe; then, the remaining headspace was filled with an equal volume of argon, an inert gas. Immediately after, the syringe was shaken vigorously for one minute. Lastly, a 20-mL gas sample was injected from the syringe into a glass vial, which was stored at room temperature until analysis on a Gas Chromatograph (Shimadzu) (Kazunori et al. 2010). Air and water temperatures during the sampling period were also measured for correlation analysis.

Analysis was conducted by, firstly, computing the concentration of dissolved nitrous oxide per volume of water in the sample using the following equation, which is also used in determining volume of nitrous oxide during basal denitrification:

$$V_{N20} = C_t [V_h + (V_{water}\alpha)] \times \frac{1 L}{1000 mL}$$

where V_{N20} (μL) is the volume of N₂O emitted at time t , C_t is the N₂O gas concentration in the gas phase at time t , V_h (mL) is the volume of the headspace, V_{water} (mL) is the volume of water, and α (mL N₂O per mL water) is the Bunsen absorption coefficient (Carter and Gregorich 2008).

Dissolved nitrous oxide-to-nitrate ratios were calculated using the mean of bed emissions for whole sampling period for each site for dissolved nitrous oxide and a rough estimate of nitrate levels observed at each site. Hourly export of nitrous oxide from the bioreactors was calculated using the outlet flow rates measured during tracer tests and mean dissolved nitrous oxide levels in the bed.

Statistics

The data was non-parametric, so Mann-Whitney tests were used for data comparisons with similar format to t-tests; Spearman's rank sum test was used for correlation analyses.

4. Denitrification Enzyme Activity (DEA) Assay

We collected samples from the DBR 2 and DBR 3 bioreactor on May 4 and August 23, 2013 and from the DBR 3 bioreactor on August 23, 2014. Wood chips were sampled from four 8-inch-deep holes along the bioreactor with the aid of a hand spade, and we homogenized all the samples from different holes in one container. To ensure microbial viability, we made sure to analyze the samples no later than 24 hours after collection, and when left overnight, we did so in a cold room in a container loosely capped. The woodchip samples were processed and analyzed in CSUMB laboratories following the methodology described by Groffman et al. (1999), adapted for quantifying denitrification in woodchips. Synthesis of new denitrifying enzymes is inhibited by the addition of chloramphenicol, and the nitrogen gas produced is measured as the accumulation of nitrous oxide (N_2O) which represents the total complete and incomplete denitrification since acetylene inhibits the reduction of N_2O to N_2 .

We prepared three DEA media in one liter volumetric flasks with different amounts of carbon (glucose) and a constant, non-limiting level of nitrate, dissolved in deionized water:

1. DEA Media 1 (no carbon): 0.72 g KNO_3 , 0.125g of Chloramphenicol,
2. DEA Media 2 (0.5 g glucose): 0.72 g KNO_3 , 0.5 g glucose, and 0.125g Chloramphenicol
3. DEA Media 3 (1.5 g glucose): 0.72 g KNO_3 , 1.5 g glucose, and 0.125g Chloramphenicol

For each of sample, we placed 10g of woodchips into Erlenmeyer flasks, added 10mL of DEA media 1, 2, or 3 and capped it tightly with a red stopper cap. To ensure anaerobic conditions, we evacuated each flask at 700 mmHg for 10 minutes and followed by flushing with argon for 3 minutes. Once all the flasks containing woodchips and DEA media had been evacuated and flushed, we began the incubation and gas sample extraction. Flasks were placed on the shaker table at 125 rpm, which was paused in between gas sample extractions. Before starting sample collection, we removed 20 mL of argon from each flask and replaced it with the same volume of acetylene. The T=0 sample was collected shortly after by collecting one 20 mL gas sample per each flask and injecting it into an evacuated vial. We collected subsequent samples with the same procedure at T=30 minutes, T=60 minutes, and T=90 minutes.

We analyzed the gas samples using gas chromatography to determine the amount of N_2O present in each sample. We report denitrification enzyme activity as ppb per hour per gram of dry woodchips, i.e. excluding water weight.

Results and Discussion:

Tracer testing and bioreactor hydrology

Five tracer tests were attempted between June and October 2014, with two at DBR 3 and half of one at DBR 1 deemed successful. Only the results of these three "successful" tracers will be discussed, with minimal mention of the first DBR 3 tracer since woodchips had not been fully

replenished and therefore, hydraulic retention time was shown to be shorter than intended, as would be expected due to less flow impediment from fewer woodchips.

Nominal detention times were calculated to be approximately 24 hours for both DBR 1 and DBR 3, since DBR 1 is twice the volume but also has twice the flow rate of DBR 3. These nominal detention time calculations used a porosity value of 0.447 based on a rough lab calculation method. However, the UC Cooperative Extension stated that nominal detention times should be closer to two days; this would indicate a porosity of 0.89, nearly double what we estimated.

The peak time for DBR 1, also with freshly-replenished chips, was 41 hours. Peak times are usually slightly shorter than the actual hydraulic retention time, so this site's hydraulic retention time is likely to be somewhere around the UC Cooperative Extension-expected retention time. The peak of the tracer conducted at DBR 3 after woodchip replenishment, however, was 28 hours; this is much shorter than expected by the UC Cooperative Extension, though closer to our estimate. It also shows that despite planning for identical retention times between sites, this was not the case.

The DBR 3 tracer curve appears to have a sharp peak, followed by a plateau and two more fluctuations indicating elevated pulses of tracer (Fig. 3). The DBR 1 slope of the curve is less steep than for DBR 3, although the larger bed volume of DBR 1 may account for the less steep slope due to greater propensity for dispersion. The DBR 1 tracer curve may represent a more evenly-mixed tracer, as indicated by internal bed monitoring (Fig. 4).

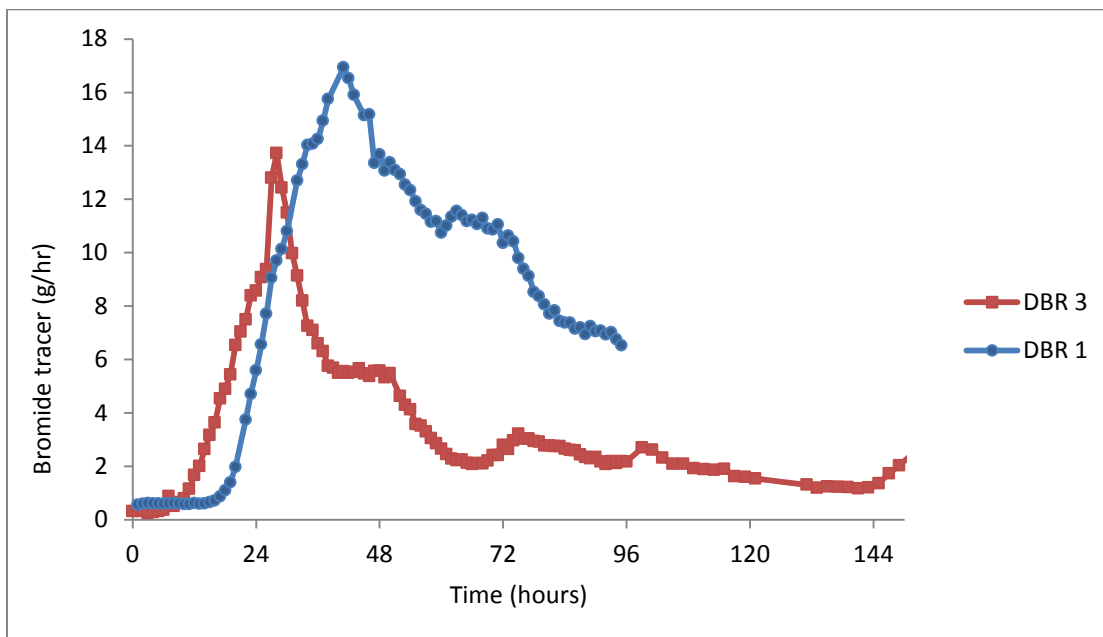


Figure 3. Bromide tracer curves based on outlet samples collected hourly. Different site characteristics such as flow rate and bioreactor volume are likely reasons for the difference in peak times; these characteristics also played a role in the behavior of the tracer. The DBR 1 tracer may have been much less vertically-stratified, while the DBR 3 tracer was likely more confined to at or below a 3-foot depth.

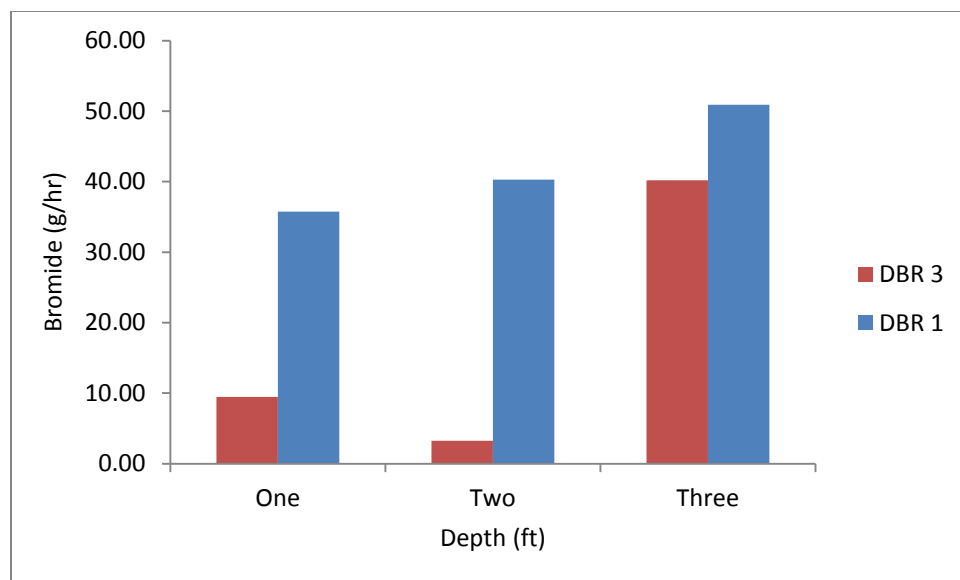


Figure 4. Samples taken from each bioreactor at 18-20 feet down the length from three depths (one, two, and three feet), 24 hours after each test was started, show that DBR 3 had greater depth stratification than DBR 1. It should be noted that although samples were taken from the same distance down the bioreactor, flow rate and baffle placement differ between bioreactors.

As mentioned, woodchip density was shown to play a large role in hydraulic retention time by comparing the first and second tracer at DBR 3. The first tracer at DBR 3 was conducted the same day that a 300-lb. load of woodchips were added to the bioreactor but probably did not have time to fully compact within the bed. The bromide peak occurred at 13.5 ± 2 hours for this test. Therefore, the greater woodchip density resulted in more than a two-fold increase in tracer peak time.

Thirty-five percent of the tracer was recovered during the unfinished DBR 1 tracer, for the incomplete test. The mass recovery of bromide for the two tests at DBR 3 ranged from 44% to 54%. Mass recovery of salts is usually calculated for tracer tests, where over 80% is generally considered acceptable (Kadlec and Wallace 2009). Sampling duration should not have been an issue, since samples were collected for three to four times the nominal retention time as recommended (Kadlec and Wallace 2009). Internal monitoring in the beds during the tracer indicates that for DBR 3, the bromide concentration at the lower depths of the bed are reasonable (25 ppm to 40 ppm) at halfway down the length of the bioreactor after one to two days of sampling (during and slightly after the peak). However, the internal monitoring concentrations shoot up after the peak at the outlet has already passed (> 2 days) as shown in the first DBR 3 tracer, which indicates, in conjunction with the presence of a second peak during the second DBR 3 tracer, that some of the tracer could be sinking at the inlet and leaving the inlet later.

The DBR 1 peak time of 41 hours could have implications for how nitrate removal is calculated at this site. Mean residence time calculations usually result in values greater than peak time; although mean residence time was not calculated in this study for several reasons, a hydraulic residence time of at least 2 days is likely. Therefore, any composite nitrate samples collected from the outlet would need to be offset by two days from the inlet for calculations to be accurate.

DBR 1's peak time was 1.5 times later than DBR 3's. The difference in peak times could be more complicated than it appears if there was differential mixing between depths in the two bioreactors. A combination of a slower flow rate compared to DBR 1 and downward-forcing action by a baffle in the first several feet of the bioreactor is likely to have caused the unwanted tracer depth stratification at DBR 3.

Porosity calculations in this study led to a much shorter nominal hydraulic retention time (24 hours) than stated by the UC Cooperative extension (48 hours). It is unlikely that porosity was overestimated in the lab estimation method; however, a greater look at the discrepancy between the two nominal retention time estimates is recommended.

Graphs showing all three full tracer curves are found in Appendix A.

Nutrient and carbon analyses

Nitrate concentrations did not vary across the width (perpendicular to the flow) of the bioreactor, for either the two- or three-foot depth, at the set of wells that were located halfway down the length of the bioreactor as shown by a coefficient of variation visualization (Fig. 5). The uniformity of nitrate levels across the width indicates that flows at that section are relatively uniform, since complete denitrification would likely have occurred if water was getting caught up, and that was not the case.

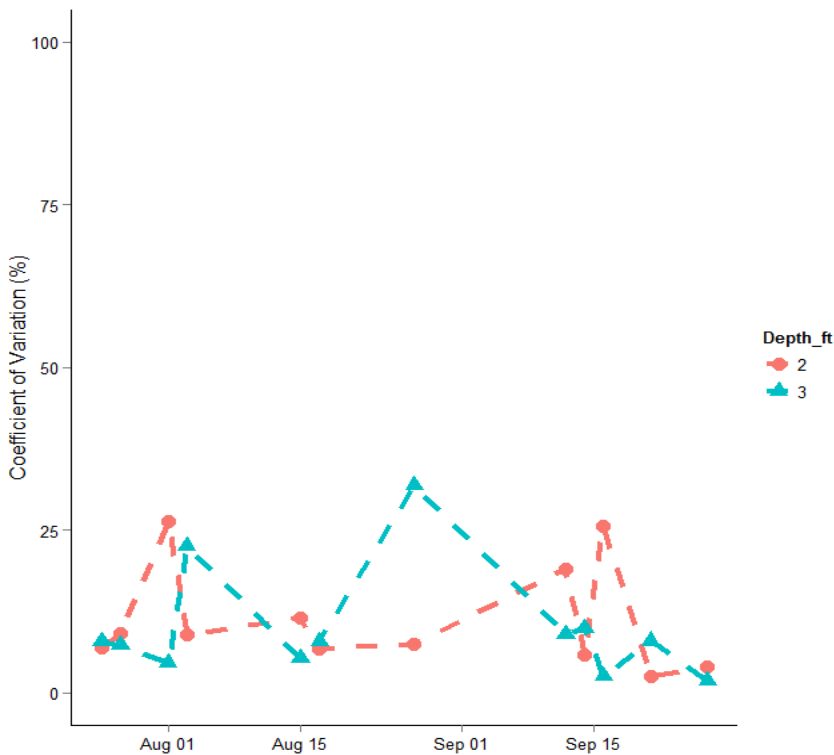


Figure 5. Coefficient of variation (as a percent) for nitrate levels across the width of a section of the bioreactor, with values ranging from 2 to 32 percent. Each point represents the variation from the mean of the three well locations for one sampling date. The bioreactor is approximately four feet wide, so distance between wells is approximately 1.5 feet.

A one-sided, paired t-test was conducted to assess if a difference exists between nitrate levels at two-foot and three-foot depths within DBR 2. The t-test supports a mean difference of 4.3 ppm between depths, with the two-foot depth having lower nitrate values ($p=0.03$).

Nitrous Oxide

Surface nitrous oxide flux

A total of eight nitrous oxide sampling events were completed for DBR 2 and seven sampling events for DBR 3. Results are presented in Figure 6; however, it is important to note that DBR 3 (to the right in the figure), especially the mid-bed chamber, is likely to have been affected by a layer of several inches of unsaturated woodchips on the surface of the bed that may have disrupted the ability to capture the true flux from the saturated bed underneath. The DBR 2 site was more frequently observed to be visibly saturated at the surface. Since the surface nitrous oxide sampling method was potentially greatly affected by these conditions at one site and not the other, no comparisons between sites for this data will be made.

DBR 2 had a mean surface nitrous oxide flux of 0.25 mg/m²/hour near the inlet, 3.26 mg/m²/hour at 18 feet down the length of the bioreactor, and 2.75 mg/m²/hour near the outlet. DBR 3 had a mean flux of 0.03 mg/m²/hour near the inlet and 1.76 mg/m²/hour near the outlet. The sampling events for chamber 18 feet down the length captured more ambient emissions than fluxes, and was therefore not included in the dataset.

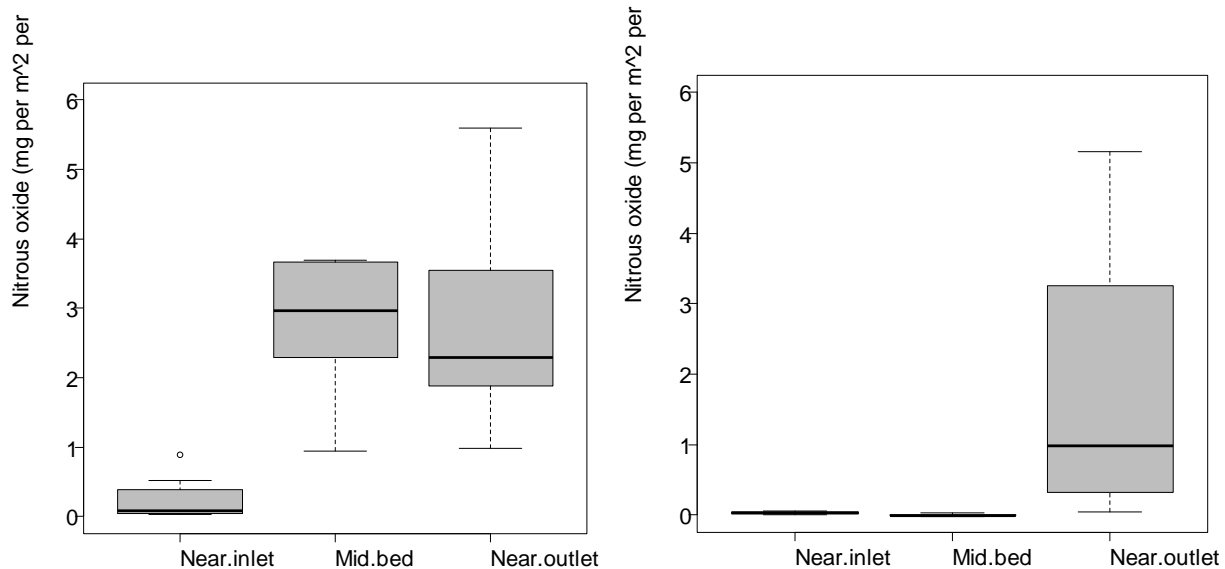


Figure 6. Surface nitrous oxide emissions at DBR 2 (left) and DBR 3 (right), from weekly data collected July 16 through September 20, 2013.*

*An outlier point of 10.17 mg N₂O-N m⁻² h⁻¹ at DBR 2 is not shown here so that the y-axis scale could be comparable between graphs, but was part of the dataset for the mid-bed chamber.

At both sites, near-inlet surface fluxes were significantly lower than near-outlet fluxes ($p<0.01$ for both sites). The fluxes at 18 feet down the length at DBR 2 were not significantly different than the near-outlet fluxes ($p=0.96$).

There was no significant correlation between bed temperature and surface flux, using DBR 2 data ($p=0.28$). A table presenting air and bed temperatures during nitrous oxide sampling events can be found in Appendix D. The mean bed temperature of DBR 3 was 2.2 °C warmer than at mean DBR 2 bed temperature.

pH in the denitrification beds ranged from 6.9 to 7.3. Given the low variability of values, all around neutral, no further assessment was deemed necessary.

Nitrous oxide emissions were significantly different between near-inlet and near-outlet chamber locations on the surface of the beds, approximately one to two orders of magnitude less at the inlet of both sites versus the near-outlet. Even though nitrous oxide emissions are a disfavorable product of the denitrification beds, they are also an indicator that denitrification is occurring. Further research as to why this could be the case, and potentially alternative influent injection designs, are recommended. These low emissions could be due to the hydrology in the bed below where the sampling chamber was placed. One possibility is that flows are not as uniform at the inlet as the rest of the bed. Another possibility, based on field observations, is that woodchip density is lower at the inlet sometimes. Before 580 lbs were added mid-August to the DBR 3 site, the bed was very compressible when stepped on. Nitrous oxide emissions may be lower with complete nitrate removal, which was not achieved by either bioreactor.

The 10.2 mg per m² per hour flux at DBR 2, mid-bed is 1.8 times higher than even the next highest flux for the whole study, and appears to be an outlier. This was around the same time period that around 400 lbs of fresh woodchips were added to the DBR 2 bioreactor. The additional carbon provided by the new woodchips could have played a role in this unusually high flux, but other explanations may exist such as hydrology based on pump rate, whether the pump had broken within the prior week, and installation of the base for sampling.

Surface emission levels from mid-bed and near-outlet were quite high compared to nitrous oxide emissions from farmland in Salinas and Davis studies (see Appendix B for summary of several surface flux studies in California). Bioreactor mean emissions in this study, measured during summer months, were 12 to 19 times higher than the highest-measured fluxes in the Salinas soils study. However, it should be taken into account that while an apples-to-apples comparison to agriculture is startling, the amount of acreage that would be designated to denitrification beds is much less than that designated to growing crops.

Carbon dioxide and methane data were collected and analyzed by a gas chromatograph during this study, but these data are still in raw form.

Dissolved nitrous oxide

Mean dissolved nitrous oxide values for each site are presented in Table 1. Samples taken from within the bioreactors (mid-bed, outlet) contained dissolved nitrous oxide levels that were considerably higher than samples taken from outside of the bioreactor (inlet, nearby drainage ditch) at both sites, DBR 2 and DBR 3. The inlet and drainage ditch dissolved nitrous oxide levels at DBR 2 were approximately 1 percent of the outlet levels, and 1.6 to 3.4 percent of mid-bed levels. While an insufficient number of samples for the DBR 3 inlet, the drainage ditch

sample sampled upstream of the bioreactor was equivalent to only 0.1% of the highest dissolved nitrous oxide level measured from within that bioreactor.

Table 1. Mean dissolved nitrous oxide measurements (mg N₂O-N per liter water) for all the sampling dates, July through September 2013, at the inlet, in-bed, outlet, and nearby drainage ditches of each DBR 2 and DBR 3. No data is available when there were not at least three viable samples analyzed (ND = no data).

	DBR 2	DBR 3
Inlet	0.0233	ND
Middle	1.26	0.812
Middle- 2' depth	0.580	1.42
Outlet	1.86	0.932
Drainage ditch- above bioreactor	0.017	0.0013
Drainage ditch- below bioreactor	0.0233	ND

Dissolved nitrous oxide load estimations and ratios of dissolved nitrous oxide to nitrate are provided in Table 2.

Table 2. Hourly export of dissolved nitrous oxide from outlet (mg N₂O-N h⁻¹) and nitrous oxide to nitrate ratio for each site.

*** Both calculations use the average of mid-bed and outlet calculations for bioreactor dissolved nitrous oxide levels. Flow rate for the hourly export was 1 gpm.**

	DBR 2	DBR 3
Hourly dissolved nitrous oxide export (mg)	162	252
Dissolved nitrous oxide-to- nitrate ratio (% , approx.)	1.5	3.5

The dissolved nitrous oxide levels within the bioreactor, except for at the inlet, were much higher than in nearby drainage ditches. This result contrasts one woodchip denitrification wall study, conducted over 9 years, which found dissolved nitrous oxide levels from walls installed in agricultural fields in Iowa were not significantly higher than levels from the tile drainage without denitrification walls. Influent nitrate levels in that study were of similar magnitude to DBR 3 (Moorman et al. 2010). They compared the ratio of nitrous oxide to nitrate, reporting 0.0062 kg nitrous oxide per kg nitrate, or 0.62%. However, their reported dissolved nitrous oxide levels were about an order of magnitude smaller than our near-outlet and mid-bed levels.

A comparison of surface versus dissolved emissions in this study shows approximately equal emissions from both sources, by estimating hourly fluxes from the bed surface area versus from dissolved levels in the outlet flow. Hourly surface fluxes from the DBR 2 and DBR 3 were estimated at 230 and 134 mg, respectively, whereas hourly dissolved nitrous oxide export from

the same bioreactors was estimated at 162 and 252 mg.

A study conducted in Japan showed that dissolved nitrous oxide emissions account for 50.3 to 67.3% of aboveground emissions for wheat, soybean and rice plots, with highest concentrations where leached nitrogen was also high (Minamikawa et al. 2010). Further comparisons between groundwater and soil-derived emissions have been made by Sawamoto et al. (2003).

The dissolved nitrous oxide to nitrate ratios for DBR 2 and DBR 3 were 1.54 and 3.54%, respectively. Dissolved nitrous oxide emissions may be lower with complete nitrate removal; a stream-bed denitrifying bioreactor study supported lower dissolved nitrous oxide to nitrate ratios when nitrate was removed to a level below 5 $\mu\text{g NO}_3\text{-N/liter}$ (Elgood et al. 2010). A summary of the above studies and other dissolved nitrous oxide studies are found in Appendix C, although several report much lower nitrate levels, and therefore it is difficult to make direct comparisons.

Surface versus dissolved levels

There did appear to be a positive correlation between surface and dissolved nitrous oxide data, although not a significant one ($p=0.24$, $\rho=0.35$). The lack of stronger evidence of connection between the two datasets may be due to minor sampling errors or hydrology related to release of dissolved nitrous oxide from the water matrix.

Denitrification Enzyme Activity

Preliminary data analysis suggests an increase in denitrification enzyme activity (DEA) with additional labile carbon (glucose) additions, however the trend is not statistically significant ($p = 0.11$). Moreover, we were surprised that the enzymatic activity did not respond to high level of carbon addition relative to the low levels of glucose addition (Fig. 7). One possible explanation is that carbon limitations in the bioreactors may not play as large a role as suspected if the microbe populations are not abundant. However, this conclusion is only pertinent to the shallow depth from which we drew samples (eight inches) for the DEA assay. There may be other confounding factors as well, such as a decline in the microbial presence in our samples during the time period between sample collection and performing the assay. We also recommend that future studies include a higher carbon ratio in case using three times as much carbon as the 1C treatment was not sufficient to stimulate a relative increase in DEA.

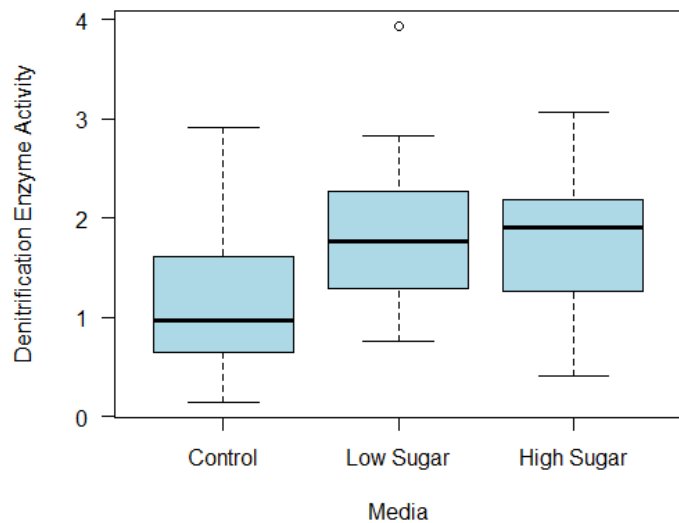


Figure 7. DEA results from three media tested.

Nitrate data assessment

Note: The time lag between inlet and outlet is not accounted for in these graphs, i.e. inlet/outlet values collected at the same time are not indicative of the nitrate removal value. Actual nitrate values are not presented for confidentiality.

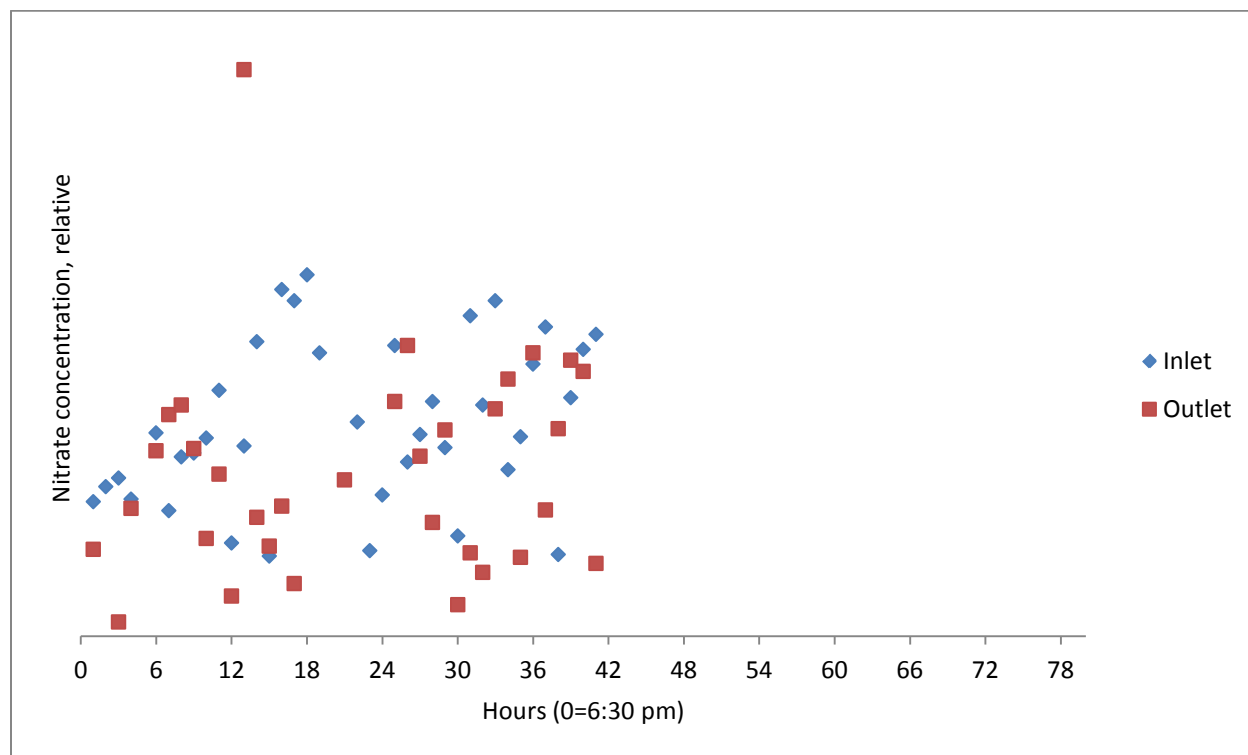


Figure 8. Nitrate data for DBR 1, collected 17–18 October 2013.

Inlet and outlet concentrations fluctuate more so due to tile drain input source compared to the surface flow/holding pond input source. The difference between minimum and maximum outlet concentrations for the 42-hour data collection interval is 100% of the mean outlet concentration.

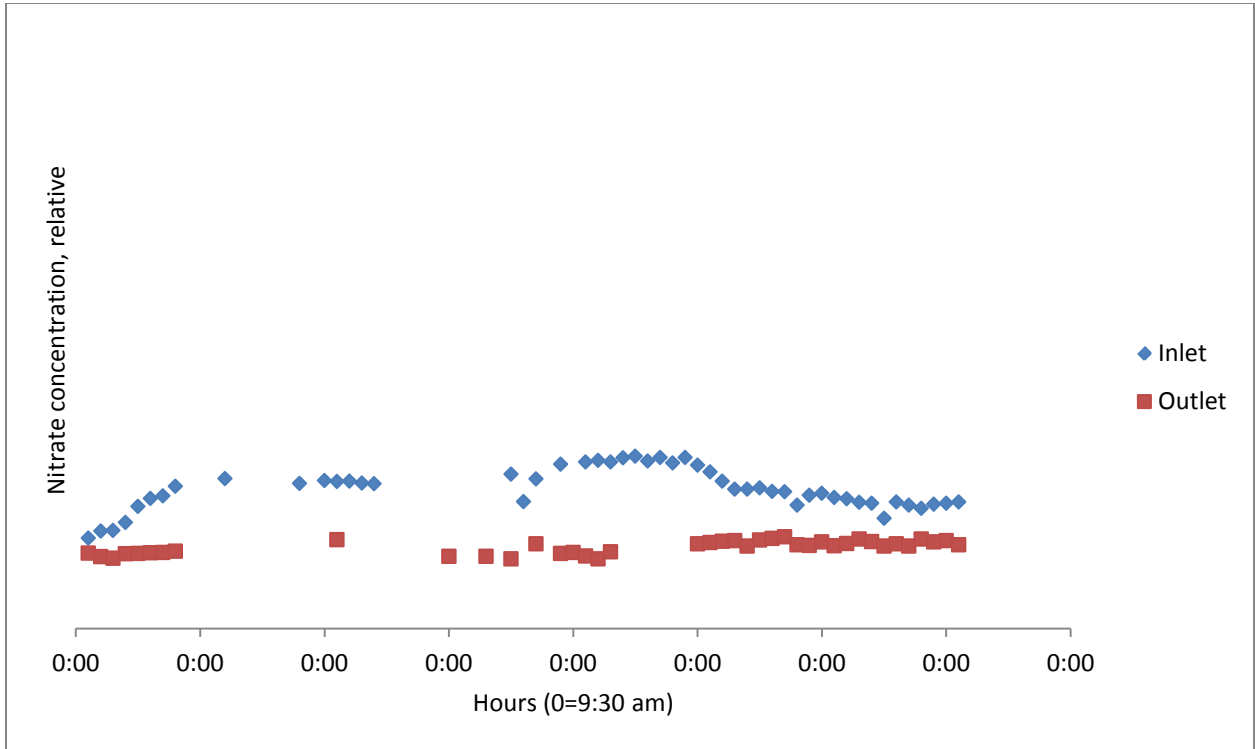


Figure 9. Nitrate data for DBR 3, collected 3-5 October 2013.

Nitrate values increase and decrease slowly and smoothly, without hardly any fluctuation that does not fit with the several hours' trend of concentration change. There is a maximum 28% change for the mean outlet concentration observed over for the three day data collection interval.

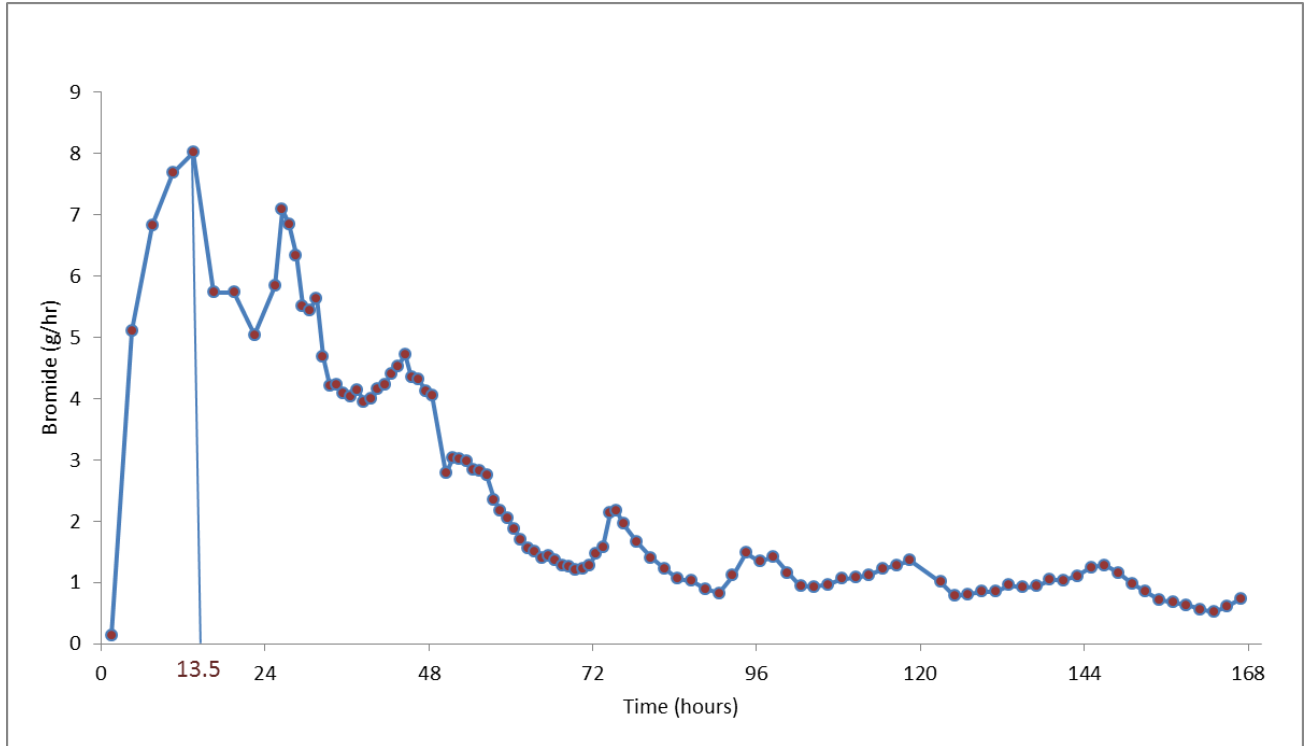
More collection days would be needed to determine if time of day is an important factor in inlet nitrate concentration, and additionally if a greater nitrate removal rate is observed during daytime (warmer) bed temperatures as expected.

Future work to be completed:

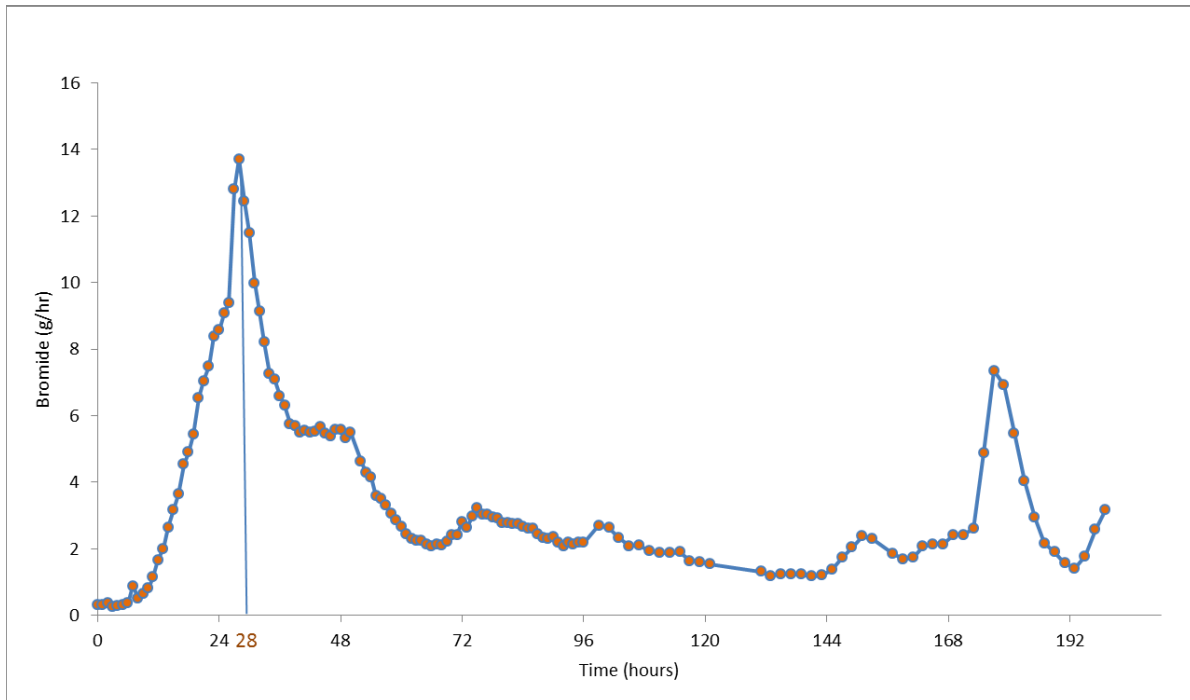
Dissolved organic carbon levels

A t-test for dissolved organic carbon data has not yet been conducted. The purpose of this test would be to show whether lower nitrate values correspond to higher dissolved carbon, and at which depth this was occurring.

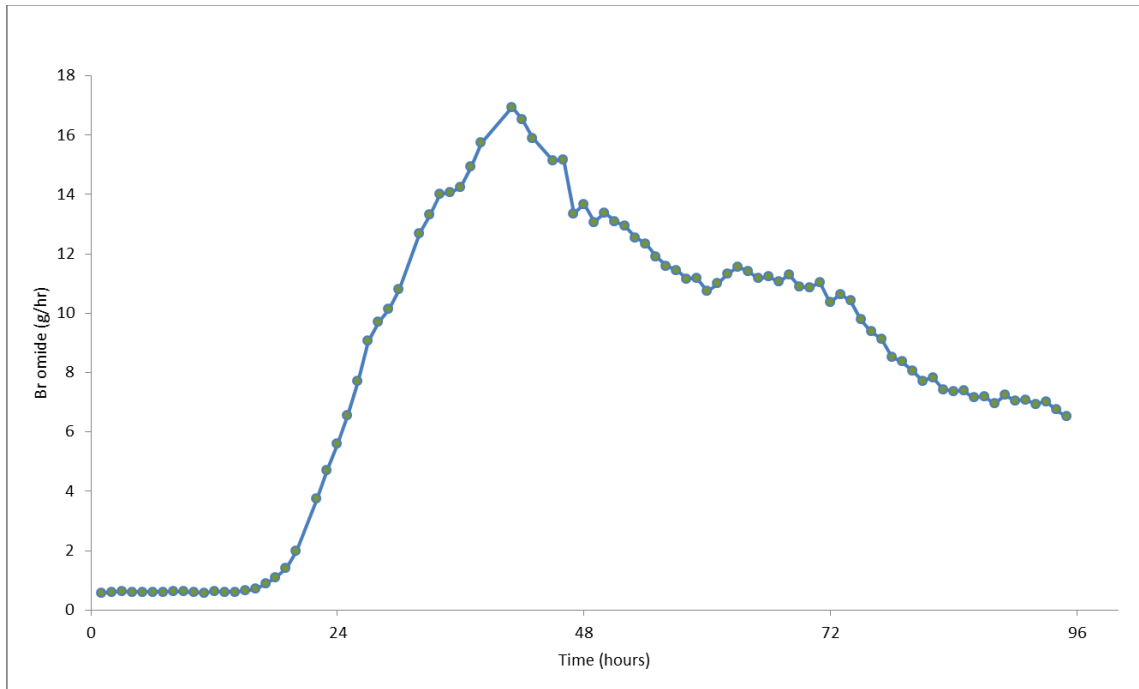
Appendix A: Tracer test bromide curves



(a) First bromide tracer at DBR 3. Tracer peak at 13.5 hours.



(b) Second bromide tracer at DBR 3.



(c) Tracer test at DBR 1, incomplete due to complications with the bioreactor pump stopping four days into the test. Tracer peak at 41 hours.

Appendix B: Summary of surface nitrous oxide emission levels from several California farms and some non-local wetland studies

Location	Category	Site description and season	Emissions	Nitrate level	Comments/ Author
Salinas Co.	Farm	Lettuce at the Hartnell east campus; Chular loam; sampled several times per week when soil moisture was elevated, less frequently under dry conditions	0.58 to 1.51 kg N ₂ O-N ha ⁻¹ (0.0066 to 0.17 mg per m ² per hour)	Max. nitrate for 2009 was 60-100 mg N per kg soil, for 2011 was 40-60 mg N per kg soil	Interpolated daily flux measurements to get annual flux; sampling at 0, 20 40 or 0, 15, 30 if expected high; 2-year study/ Berger and Horwath, ARB report (2012)
Yolo Co.	Farm	Cover crop, furrow irrigated plot had highest emissions; Reiff loam and Yolo silt loam; Fall/winter; reports that highest emissions occurred at beginning of rainy season	5 mg per m ² per hour was max daily value; mean during growing season <0.1 mg and during winter <0.2 mg per m ² per hour		Cover crops conserve carbon but release nitrous oxide/ Kallenbach et al. (2010)
Yolo Co.	Farm	One field standard tillage and one field recently converted (5 years) to min. tillage; Multiple seasons	0 to 23.7 g N ha ⁻¹ day ⁻¹ (0.099 mg per m ² per hour)		Lee et al. (2008)
East-central Illinois	Woodchip denitrification bed	Lined subsurface bed; April to June; soil temperature in April reported as 25 °C at 5 cm from the surface	0.01 to 0.13 mg per m ² per hour		Same chamber set up (inlet, middle, outlet) as this study but chambers were smaller and possibly less accurate/ Woli et al. (2010)
Columbus, Ohio	Surface wetlands, created	Surface flow riverine wetlands; multiple sub-sites were tested including high marsh, edge marsh, low marsh, and variable flow pulses; Year-round; highest fluxes recorded during the summer when soil temp. was ≥ 20 °C	7.0 ± 4.8 µg-N m ⁻² h ⁻¹ for low marsh plots; 12.6 ± 2.5 µg-N m ⁻² h ⁻¹ for edge plots (equal to 0.007 mg per m ² per hour and 0.0126 mg per m ² per hour, respectively)	-	- Nitrous oxide emissions were lowest in permanently flooded plots without vegetation - Emissions increased in edge zones during and after flooding/ Hernandez and Mitsch (2006)

Norway	Subsurface wetlands (HSSF)	Summer	890 to 6,900 $\mu\text{g N}$ per m^2 per day (equal to 0.037 to 0.288 mg N per m^2 per day)		- <i>Greenhouse gas emissions from treatment wetlands</i> summary table, p. 145/ Kadlec and Wallace (2009) book, <i>Treatment Wetlands</i> 2 nd ed.
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Appendix C: Summary of several woodchip bioreactor dissolved nitrous oxide studies, two on bioreactors constructed in the field and two laboratory column studies.

Location	Study site	Dissolved nitrous oxide	Nitrate	Comments/Author
Boone Co., Iowa	Woodchip denitrification wall	15.1 g N ₂ O -N per ha over 3 months; 0.0062 kg nitrous oxide per kg nitrate	20 – 25 ppm average in influent	Moorman et al. (2010)
Southern Ontario, Canada	Stream-bed pinechip bioreactor	Bioreactor effluent concentration range over period of study: <1 to 36 µg NL ⁻¹ Mean monthly dissolved N ₂ O production (difference of influent and effluent concentrations): -5.9 to 22 µg N per L	6 ppm max over 1 year span	Summer effluent temperature range: 16.7 to 19.2 °C/ Elgood et al. (2010)
Laboratory column study	In lab with variable water flow rates of 2.9 to 13.6 cm per day in respective columns	0.003 to 0.028% production of total N denitrified		Complete denitrification is stated to be occurring/ Greenan et al. (2009)
Laboratory column study	Columns with hardwood chips, softwood chips, sawdust, greenwaste, and wheat straw	200 to 300 µg per L for the warm incubation (27 °C) of hardwood (eucalyptus) chips; between 50 and 100 µg per L for cold treatment (16.8 °C)	14.4 and 17.2 ppm at inlet of barrels	Warneke et al. (2011)

Appendix D: Summary of weekly temperature readings during nitrous oxide sampling events at each site (July 16 to September 20).

Site	Mean bed temp. (°C)	Bed temp. range (°C)	Mean air temp. (°C)	Air temp. range (°C)
DBR 2	18.4	17.1 to 19.4	20.6	17.1 to 26.1
DBR 3	20.6	19.7 to 21.8	22	19.5 to 25.3
