Improving Water Quality Downstream of Protected Cropping Operations (Hothouses)

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Executive Summary

Recent studies on the Coffs Coast linked water quality issues to agricultural intensification. Runoff from hothouses can contain high levels of nitrogen (N) from fertilisers often draining into streams without any treatment. To manage nutrient pollution from hothouse drainage, we tested whether a new woodchip bioreactor approach can attenuate the highly concentrated N effluent.

The experimental trial was designed and deployed as a collaboration between Southern Cross University, North Coast Local Land Services and Coffs Harbour City Council. Here, we investigate the effectiveness of buried, inline woodchip bioreactors in removing nitrate (NO_3^--N) and the possibility of pollution swapping by producing the greenhouse gas nitrous oxide (N_2O).

Flow rates through the bioreactors across the 5 surveys were 65±29 L hr^{-1}, and residence time was 6.4±2.6 hrs, whilst water temperature was 17±1.0°C. Average dissolved NO_3^--N removal and N_2O gas production were 6.0±5.2 g N m^{-3} hr^{-1} (0.9-12.3 g N m^{-3} hr^{-1}), and 35.1±31.4 mg N m^{-3} hr^{-1} (14-83 mg N m^{-3} hr^{-1}), respectively. Average water depth was 8 cm, and only 21% of the bioreactor woodchips were wet. A significant correlation between dissolved oxygen with NO_3^--N and N_2O-N indicated higher removal occurred in hypoxic to anoxic conditions.

Overall, these inline pipe bioreactors achieved nitrate removal efficiencies (NRE) of 14.5±6.8% (8.2%-25.0%) and N_2O production equivalent to 0.7±0.6% (0.3-1.4 %) of nitrate removal. Our results imply that these trial bioreactors are not significantly swapping NO_3^--N removal with increased N_2O production to the atmosphere. Design improvements are required for better NO_3^--N removal and a review of on-farm fertigation is needed to minimize losses downstream. A suite of measures will be required to achieve treatment levels that meet water quality guidelines.

1. Introduction

Nitrate (NO_3^--N) pollution has become a serious environmental concern for surface water and groundwater ecosystems (Diaz, 2001; Jickells et al., 2017). NO_3^--N release to aquatic ecosystems has increased due to various anthropogenic activities, including the intensive use of fertilisers on agricultural land to fulfil food production demand (Lu and Tian, 2017). Between the years 1860 and 2000, anthropogenic nitrogen (Nr) creation increased from ~15 Tg N per year to ~165 Tg N per year (Galloway and Cowling, 2002) and continues increasing (Galloway et al., 2014). Our region of Australia is no exception to this trend. Ongoing agriculture intensification with fertiliser application in Coffs Coast Catchments are releasing more anthropogenic nitrogen (Nr) to streams, which can ultimately lead to eutrophication, toxic algal blooms, habitat deterioration in river and lakes, hypoxia, and increased N_2O emissions to the atmosphere (Diaz, 2001; Galloway et al., 2003; Wadnerkar et al., 2020).

One potential source of this nitrogen (N) input to streams in the Coffs Coast region is from protected horticulture (hothouse) operations (Wadnerkar et al., 2020). There are obvious benefits of the development of hothouse horticulture. Hothouses can sustain higher yields over shorter growing periods, with the potential for more efficient utilisation of input resources (Singh et al., 2018). Hothouses that utilise innovative fertigation systems are more water and nutrient efficient than traditional field fertigation systems (Bradley and Marulanda, 2000). Ammonium based fertilisers applied in hothouses can be taken up by plants, become bound to soil/media particles, or converted to NO_2^- then to NO_3^- by bacteria, in a process known as nitrification. This NO_3^--N is highly soluble in water and can be leached from plant pots and into streams without any treatment. The volume of fertigation applied may be up to 30% greater than crop requirements to prevent salt accumulation around roots and flush excess salinity (Grewal et al., 2011). The plant nutrient uptake is regularly less than the fertigation rates, and the surplus often exits the system as wastewater runoff containing high nitrogen (N) loads (Grewal et al., 2011).

Among several approaches to address such excessive nitrogen (N) export from agricultural catchments, denitrifying woodchip bioreactors have drawn increasing interest in the last 30 years (Addy et al., 2016; Dougherty, 2019). The earliest known field-scale application of a bioreactor for treating agriculture drainage using woodchips was in the 1990s in Canada (Blowes et al., 1994), then followed by other regions with modifications (Christianson et al., 2012b; Dougherty, 2019; Schipper et al., 2010). A denitrifying woodchip bioreactor is simplistically a ditch filled with a source of carbon (e.g. woodchips, corn husks), which promotes microorganisms to transform NO_3^--N into gaseous forms of nitrogen through the process of denitrification, mostly dinitrogen gas (N_2). Denitrification is the biological reduction of NO_3^--N into dinitrogen gas (N_2), though this natural process can produce (losses of) the greenhouse gas nitrous oxide (N_2O).

The challenge of designing bioreactors to reduce nitrate (NO_3^--N) pollution to waterways also creates the possibility of pollution swapping from a water-bound pollutant (NO_3^-) to an atmospheric pollutant (N_2O). Nitrous oxide (N_2O) is a powerful greenhouse gas and second-largest contributor to global radiative forcing (Yao et al., 2019). Incomplete denitrification in bioreactors can result in nitrous oxide (N_2O) production. Complete denitrification is dependent on many factors; pH, temperature, dissolved oxygen (DO) and carbon to nitrogen ratio (C:N) (Chapin et al., 2002; Warneke et al., 2011). Therefore, the use of bioreactors can produce N_2O as a by-product of the same chemical reactions that remove NO_3^--N. If the NO_3^--N is fully reduced to N_2, then there is no N_2O production.

Here, we hypothesize that the anaerobic conditions created using sealed Poly Vinyl Chloride (PVC) pipes filled with woodchips and installing bioreactors inline would drive complete denitrification with little N_2O production. Our objectives were (1) to quantify nitrate (NO_3^--N) removal efficiency (NRE), nitrate (NO_3^--N) removal rate (NRR) and N_2O potential emissions, and (2) to discuss design factors potentially affecting the bioreactor efficiency in local conditions.

2 Material and Methods

2.1 Study site and pipe bioreactor construction

The hothouse and experimental bioreactor are located northwest of the city of Coffs Harbor, New South Wales, in a subtropical region of eastern Australia (Fig 1). The bioreactor is fed by the drainage of ~22,000 cucumber plants (*Cucumis sativus L.*) from hothouses mounted on ~1.5 Ha land. (Fig. 1). Drainage from the hothouse is captured in 100 mm PVC pipes and is delivered via gravitational flow to 3 inline pipe bioreactors. The bioreactors were installed in November 2019 and modified in September 2020 to prevent leakages and increase pre-treatment capacity. Each bioreactor consists of 1.3 m^3 of woodchips (total 3.8 m^3). Each of the three bioreactors is 18 m long, consisting of 300 mm diameter PVC pipes connected in series, with monitoring pipes for sampling. Two 18 m long, 300 mm PVC pipe holding tanks/basins (2500 L) were added between the hothouse and the bioreactor inlet to attenuate large flows. Troughs (2400 x 520 x 270 mm) were added to the end of each bioreactor to provoke nitrification of NH_4 fertilisers and allow degassing of N_2O-N. Mean flow of 0.07 m^3 hr^{-1}(0.03 - 0.1 m^3 hr^{-1}) encountered during this study equated to a residence time of 6.4 hours (4.1 to 10.2 hrs.).

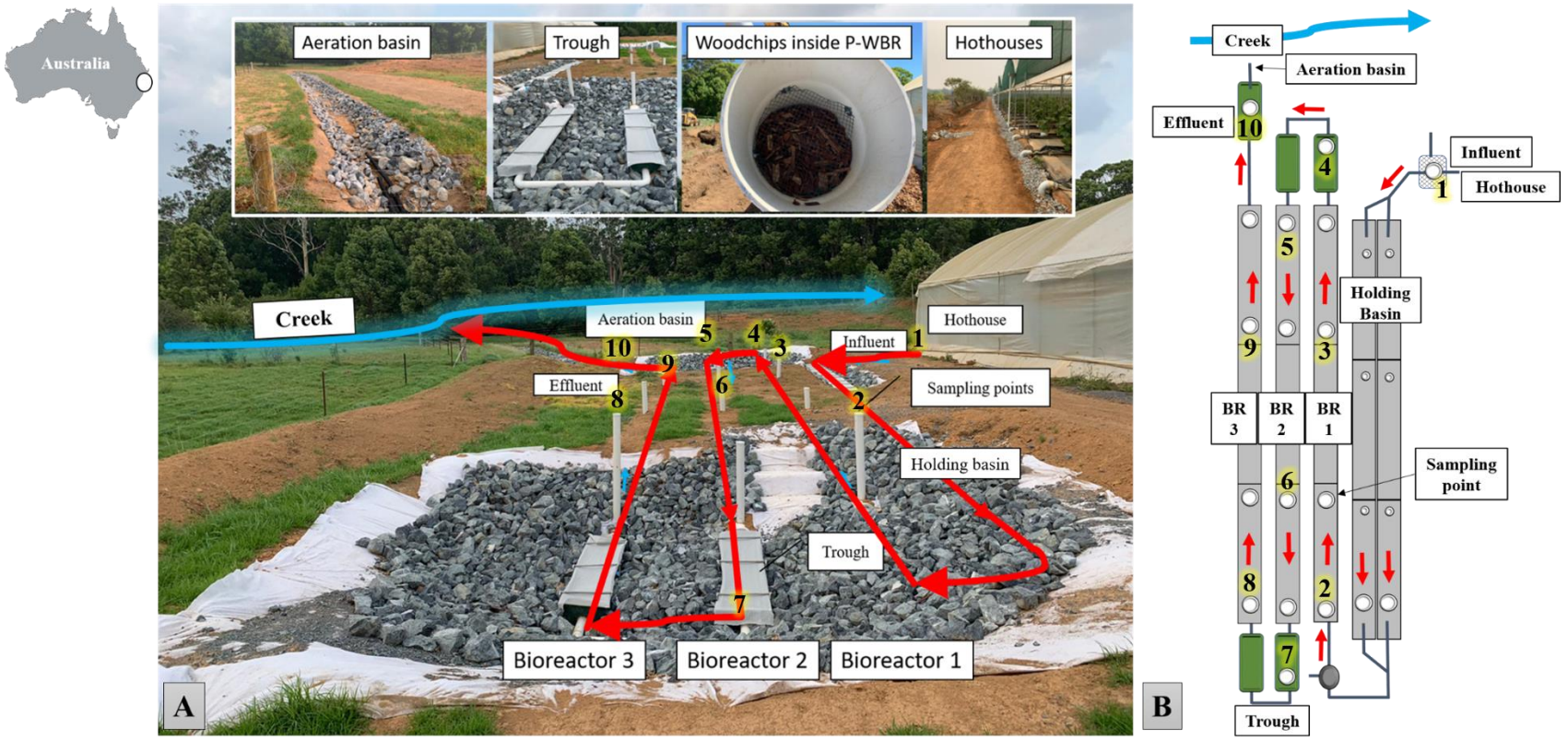


Figure 1: (A) Pipe woodchip bioreactor (P-WBR, photo) installed on drainage of hothouse before entering into the creek. (B) Layout with three inline bioreactors (BR), the holding and aeration basins, troughs, sampling locations, creek and flow path

2.2 Sampling and analysis

Water samples were collected from the 10 sampling points along the woodchip bioreactor for analysis of NO_3^--N and N_2O-N gas over 5 surveys (Fig.1). NO_3^--N samples were filtered on-site using 0.45 μm disposable cellulose acetate syringe filters into 10 mL polyethylene vials, kept on ice for less than 4-hrs, then stored at -18 ºC until analysis. NO_3^--N analysis was determined colourimetrically using a Flow Injection Analyser (Lachat Flow Injection Analyser). Analytical errors were calculated as the average coefficient of variation of replicates. Analytical accuracy for nutrient analysis was determined using certified laboratory standards to be ~2% for NO_3^- -N.

N_2O-N samples were collected by extracting 50 ml of water each into five polyethylene syringes (250 mL) and introducing 100 mL gas with known partial pressures to create a water-air headspace gradient for gas transfer. The samples were then agitated for approximately two minutes to enhance gas equilibration. The equilibrated headspace gas was then injected into 1 L tedlar gas bags (Supelco company) for analysis in a calibrated cavity ring down spectrometer (Picarro G2308) to determine N_2O values in air (Gatland et al., 2014). The partial pressures, concentrations, and percent saturation of the N_2O-N in water were calculated from gas-specific solubility constants as a function of salinity and temperature (Weiss and Price, 1980).

Conductivity, depth and temperature were measured by in-situ automated depth loggers (CTD diver - vanEssen instruments) every 10 minutes in each bioreactor. Dissolved oxygen (DO) loggers (minDOT, Precision Measurement Engineering Inc.) measured DO every 15 minutes. Additional physio-chemical parameters for each discrete sample, including DO, salinity, temperature (ºC), and pH) were measured using a Hach multimeter (40 HQd, Hach, USA). Water flow passing through the bioreactor was quantified at the inflow point with a Unidata Starflow automated logger. NO_3^--N and N_2O loads were estimated by multiplying discharge by the concentrations.

2.3 Calculations and statistical analysis

The hydraulic residence time (HRT) of bioreactor was estimated by dividing wetted woodchip volume by discharge assuming steady-state during 5 surveys:

$$HRT\ (hr) = \frac{Wetted\ woodchip\ volume\ (m^3)}{Discharge\ \left(\frac{m^3}{hr}\right)} \ldots\ldots\ldots\ (Equation\ 1)$$

Nitrogen removal efficiency (NRE, %) is the percentage removal of NO_3^--N, from the bioreactor inlet to outlet, calculated by dividing the difference between influent and effluent concentrations by influent concentration (Christianson et al., 2017; Elgood et al., 2010; Robertson and Merkley, 2009):

$$NRE\ (\%) = \frac{N_{inf} - N_{eff}}{N_{in}f} \times 100 \ldots\ldots\ldots (Equation\ 2)$$

where N_{inf} and N_{eff} are influent and effluent NO_3^--N concentrations (mg N L^{-1}).

Nitrate removal rate (NRR) is another index that measures NO_3^--N removal, expressed as g N removed per m^3 of the wetted woodchip per hour (g N m^{-3} hr^{-1}) similar to Tsukuda et al. (2015) and Warneke et al. (2011):

$$NRR\ (\text{g N m}^{-3}\ \text{of wetted woodchip hr}^{-1}) = \frac{(N_{inf} - N_{eff})Q}{V_{ww}} \ldots\ldots\ldots (Equation\ 3)$$

where V_{ww} is the volume of wetted woodchips, calculated as the average depth in the bioreactor, multiplied by the length, multiplied by the width. Q is flow rate (m^3 hr^{-1}). Similar equations were used to estimate N_2O production. We performed linear regression analysis (using the software SPSS) to determine relationships between parameters in order to explain the factors influencing the performance of the bioreactor.

3 Results and discussion

3.3.1 Bioreactor hydrology

We installed three inline bioreactors downstream of a hothouse feeding ~22000 Cucumber (*Cucumis sativus L.*) plants (Fig 1). During five surveys, total hothouse drainage flows through the bioreactor varied significantly (470-2500 L d^{-1}) (Fig 3/Table 1). Sensors in each pot, which measure soil moisture and hothouse temperature, revealed that irrigation lasted from 3.5 to 7.4 hours. The total volume of daily flow was variable, driven by the hours of irrigation; however, the flow rates through the bioreactors remained constant (0.3-0.4 m^3 hr^{-1}). The hydraulic residence time was generally short, compared to other bed bioreactors (Elgood et al., 2010; Rivas et al., 2020; Rosen and Christianson, 2017), ranging from 4.1 to 10.2 hours (mean 6.4±2.6 hours). Bioreactor was only 8.0±0.03 cm deep and only working with 21% (0.8 m^3 wetted woodchips out of a 3.8 m^3 total volume) of its capacity. The effluent at the outlet of bioreactor was mostly dissolved oxygen (DO) depleted throughout the monitoring period (0.0-0.2 mg L^{-1}, 0.4-1.0 %), indicating that denitrifying conditions were present in bioreactor (Table 1, Fig 5).

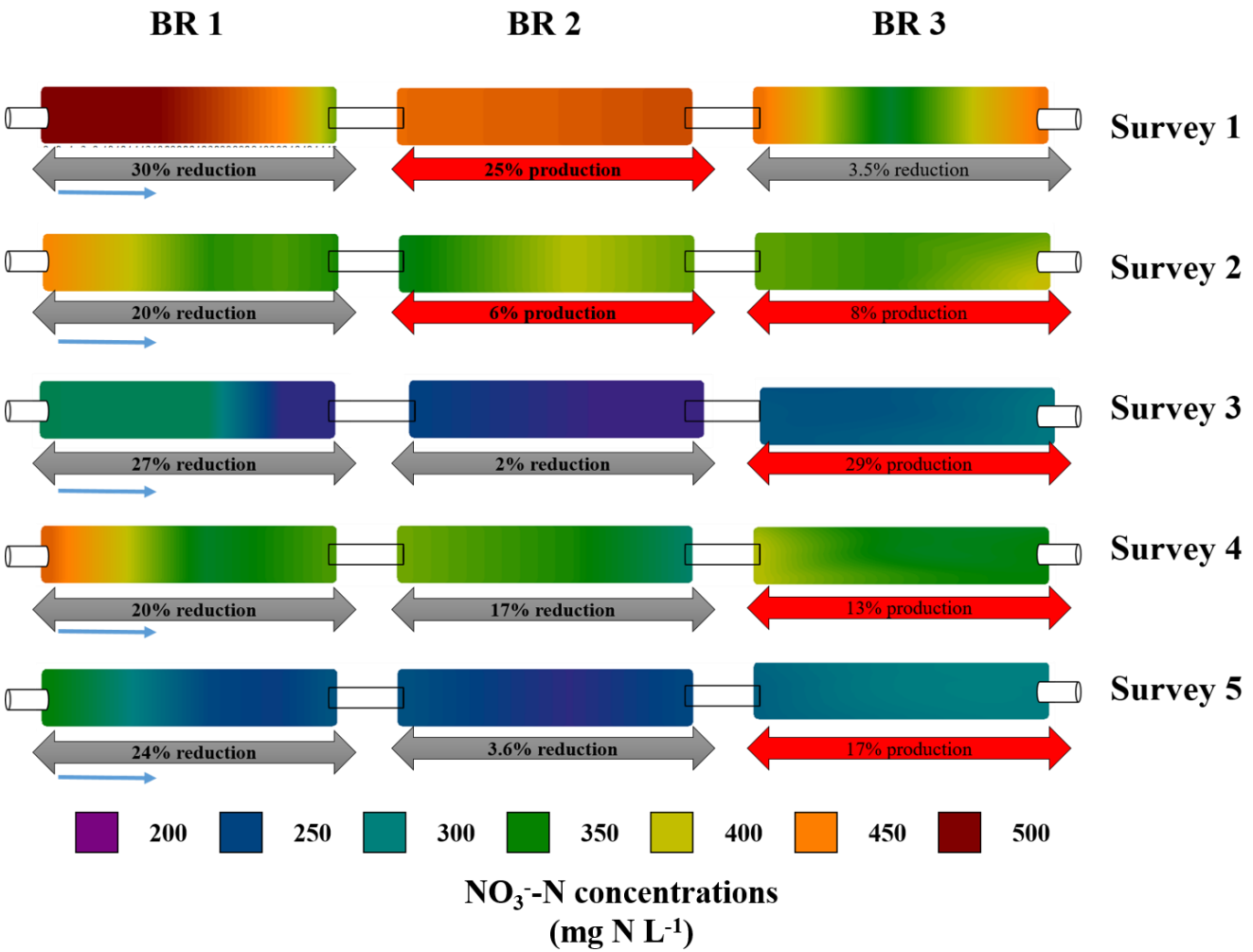


Figure 2: Variation in concentration and % removal or production of NO_3^--N in three inline pipe bioreactors fed by cucumber hothouses drainage on the east coast of subtropical Australia. Bioreactor 1 shows NO_3^--N removal throughout all 5 surveys. Production represents increase in NO_3^--N concentration between inlet and outlet of bioreactor.

3.3.2 NO_3^--N removal

Influent concentrations from the hothouses varied from 317 to 544 mg N L^{-1} (mean 423±91 mg N L^{-1}), whereas effluent concentrations measured at the outlet were in the range of 291 to 455 mg N L^{-1} (average 359±70 mg N L^{-1}). The first bioreactor had a nitrate removal efficiency (NRE) of 24.3±4.5% with maximum NRE of 30.5% over the five surveys. Bioreactor two and three were not consistent in removing NO_3^--N (Fig 2). Overall, calculated NRE for all bioreactors was 14.5±6.8 % and is at the low end of many reports in the literature. For example, Christianson et al. (2012a) reported 55% and 14.0% NRE for bioreactors in Pekin, USA, where influent NO_3^--N concentrations were 4.9 and 2.8 mg N L^{-1} respectively. Verma et al. (2010) reported a range of 42 to 48% NRE for three bioreactors receiving effluent from corn and soybeans, where influent NO_3^--N concentrations were 3 to 16 mg N L^{-1}. From a pilot-scale study carried out in New Zealand on agricultural drainage, bioreactors were estimated to remove 14 to 37% of the NO_3^--N load when effluent NO_3^--N concentrations were 7.7 to 35.6 mg N L^{-1} (Christianson et al., 2011). However, the extremely high influent loads observed in our bioreactor are ~11-140 fold higher than the mean of the other bioreactors mentioned here, demonstrating a large nitrogen loss during fertigation. Consequently, NRE may not be an appropriate metric to analyze the efficiency of this high NO_3 load, and we suggest that nitrogen removal efficiency (NRR, see equation 3 above) is more appropriate in this context.

Overall, NRR from the three inline bioreactors was in the range of 0.9 to 12.3 g N m^{-3} hr^{-1} (6.0±5.2 g N m^{-3} of wetted woodchip hr^{-1}) (Fig 3, Table 1). An in-stream bioreactor installed on agriculture drainage in USA had NRR between 0.02 to 0.3 g N m^{-3} hr^{-1}, where influent concentrations were in the range of 1.2 to 15.1 mg N L^{-1}(Christianson et al., 2012a). A controlled drainage fed bioreactor bed recorded slightly higher NRR of 0.3 g N m^{-3} hr^{-1} where influent concentrations were 14.6-15.0 mg N L^{-1} (Woli et al., 2010). Above ground woodchip bioreactor design for aquaculture wastewater

with influent nitrate concentrations between 20 and 80 mg N L^{-1}, recorded removal rate was >1.6 g N m^{-3} hr^{-1} (Lepine et al., 2016). Similar to our experiment, a bioreactor installed on glasshouse (hothouse) wastewater with high influent nitrate concentrations of 100 to 250 mg N L^{-1} had a removal rate of 0.4 g N m^{-3} hr^{-1} (Warneke et al., 2011). Our extremely high NRR (maximum 12.3 g N m^{-3} hr^{-1}) is at least one order of magnitude higher than the range of NRR reported from the literature (0.2 g N m^{-3} hr^{-1}) (Addy et al., 2016).

Overall, these comparisons to the literature indicate that our bioreactors have a low NRE but a very high NRR. Indeed, the NRR seems to be the highest ever recorded in a field bioreactor. Therefore, the low NRE is likely due to extremely high influent concentrations, and the bioreactor is only operating at 21% capacity due to shallow water depth. With an increase in water depth, we expect that the bioreactor will increase denitrification capacity and subsequently increase both the NRE and NRR (Addy et al., 2016; Christianson et al., 2012b).

3.3.3 N_2O-N gas production

Concentrations of dissolved N_2O-N in the inline pipe bioreactor varied from 2.0 to 822.2 µg N_2O-$N_{(aq)}$ L^{-1}. Mean dissolved N_2O-N production (calculated as the difference between effluent and influent concentrations) varied from 228 to 812 µg N_2O-$N_{(aq)}$ L^{-1} indicating that the bioreactor is producing N_2O-N as expected (Table 1). Production of N_2O-N is a by-product of denitrification and is also reported in earlier bioreactor investigations (Christianson et al., 2013; Moorman et al., 2010; Warneke et al., 2011; Woli et al., 2010). For example, an in-stream bed bioreactor installed on agriculture drainage in Canada had N_2O-N concentrations ranging from 1.0 to 36 µg N_2O-$N_{(aq)}$ L^{-1} with N_2O-N production in the range of -5.9 to 22 µg N L^{-1} (Elgood et al., 2010). The highest N_2O-N (83 mg N L^{-1} hr^{-1}) produced in our bioreactors during survey 1 coincided with the highest NO_3^--N influent concentration (544 mg N-L^{-1}). We suspect our higher concentrations and production of N_2O-N are due to

higher NO_3^--N influent concentration and relatively lower influent temperatures (17.2±0.9 °C) in winter. During winter, colder temperatures and slower reaction rates lead to less complete denitrification and more N_2O-N production (David et al., 2016; Elgood et al., 2010; Moorman et al., 2010).

When N_2O-N production is described in relation to NO_3^--N removal, (rN_2O; the percentage of NO_3^--N removed that was produced as N_2O-N) losses are likely small (Table 1, Fig 7). In this study, rN_2O of 0.3-1.4% (mean 0.7±0.6%) was observed. This conversion rate is similar to a bioreactor installed on tile drainage in the USA, where rN_2O was 0.4 to 0.9% of NO_3^--N. In a pilot laboratory study, a smaller bed bioreactor with 6.3 m^3 of woodchips showed rN_2O of 0.3 to 0.5% (Davis et al., 2019). A laboratory column study under variable flow conditions determined that a rN_2O of only 0.03% (Greenan et al., 2009). Average rN_2O in this study (0.7±0.6 %) is less than the IPCC N_2O-N emission factor (0.75) for N released in waterways (De Klein et al., 2006). Overall, our results imply that these trial bioreactors are not significantly swapping NO_3^--N removal with increased N_2O gas production to the atmosphere.

Table 1: Average flow, depth, temperature, residence time, dissolved oxygen (DO), NO_3^--N and N_2O-N influent and effluent concentrations, NO_3^--N removed and N_2O-N produced at three inline pipe bioreactors fed by cucumber farm drainage on the east coast of subtropical Australia over 5 surveys.

Survey	Flow	Depth	Temp.	Residence time	DO	NO_3^--N Influent conc.	NO_3^--N Effluent conc.	Avg. NO_3^--N concentration	NRR	NRE	Average N_2O-N concentration	N_2O-N load produced
	($m^3\ hr^{-1}$)	(cm)	(°C)	(hr)	(%)	mg N L^{-1}	mg N L^{-1}	mg N L^{-1}	g N m^{-3} hr^{-1}	%	µg N L^{-1}	mg N L^{-1} hr^{-1}
1	0.10	8.0	16.8	4.1	1.6	544.4	455.7	449.2±58.3	11.1	16.3	306.6±287.1	83.0
2	0.06	8.0	16.2	5.1	1.7	442.7	404.2	378.1±29.4	2.9	8.7	89.7±84.4	15.5
3	0.03	7.9	18.3	10.2	0.8	317.6	291.7	262.0±35.8	0.9	8.2	165.5±155.6	13.9
4	0.09	8.0	17.9	4.8	0.6	464.1	348.3	365.0±42.6	12.3	25.0	309.3±234.8	51.3
5	0.05	8.0	18.1	8.0	0.4	346.8	297.0	272.0±33.3	3.1	14.4	96.6±77.9	11.5
Avg. ± Std.dev	0.07±0.02	8.0±0.03	17.2±1.0	6.4±2.6	1.0±0.6	423.1±91.8	359.4±70.5	345.3±78.4	6.0±5.2	14.5±6.8	193.5±108.5	35.1±31.4

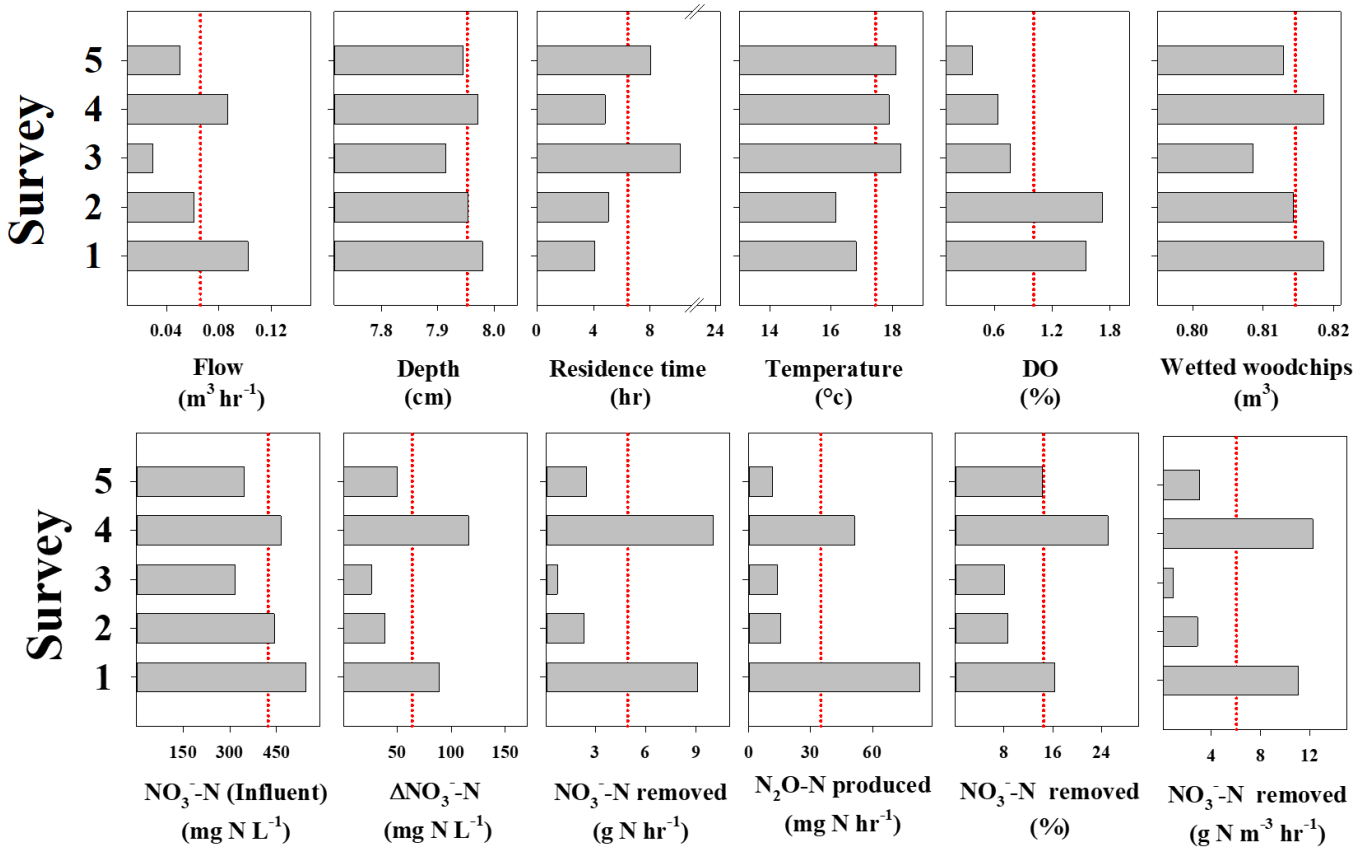


Figure 3: Average flow, depth, hydraulic residence time, temperature, dissolved oxygen, wetted woodchips volume, NO_3-N influent concentrations, NO_3^--N removed and N_2O-N produced in the three inline pipe bioreactors. The red dotted lines represent the average of different parameters over 5 surveys

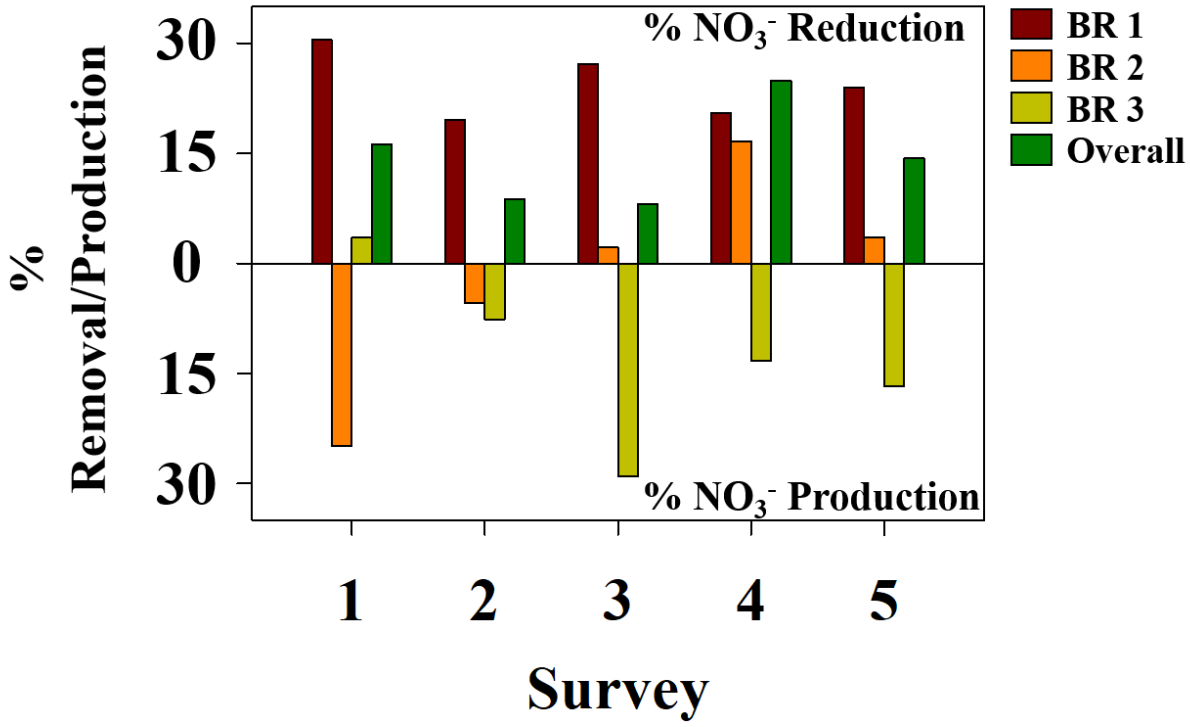


Figure 4: Variation in % removal/production of NO_3^--N in the three inline pipe bioreactors. Bioreactor 1 shows NO_3^--N removal throughout all 5 surveys.

3.3.4 Regulation of NO_3^--N removal and N_2O production

Several factors generally control denitrification including temperature, dissolved oxygen (DO), hydraulic residence time (HRT), design of bioreactor, availability and composition of the carbon source, influent concentrations of NO_3^--N, and abundance of denitrifying bacteria (Addy et al., 2016; Greenan et al., 2009; Hoover et al., 2016; Schipper et al., 2010). In this study, a regression analysis implied that denitrification was limited by dissolved oxygen and temperature (Fig 5, 6 and 7).

Anoxic conditions are critical for denitrification. For complete denitrification to N_2 to occur, DO should be below 0.5 mg L^{-1} (Tchobanoglous et al., 2003). In this study, this was achieved within the first few meters from the inlet of bioreactor 1 (Fig 5), similar to Christianson et al. (2011) and Warneke et al. (2011). A study carried out on a bed bioreactor for treating aquaculture waste found 4.2 mg O_2 L^{-1} of oxygen drop along a 7.8 m long woodchip bed (von Ahnen et al., 2016). Here, there was a drop of 10.8±4.1 mg O_2 L^{-1} (or 96±3.8%) along the 102 m length of the bioreactor. Dissolved oxygen saturation in the influent was relatively high 97.0±4.3% (10.9±4.1 mg L^{-1}), then decreased (consumed) sharply as the influent entered bioreactor 1. At outlet of bioreactor 3, DO saturations were 1.0±0.5%, (0.1±0.05 mg L^{-1}). Higher DO concentrations in the influent can lead to incomplete denitrification (Elgood et al., 2010). Although we observed relatively higher DO at the inlet, we suspect the higher production of N_2O-N compared to other studies (Davis et al., 2019; Weigelhofer and Hein, 2015) is mainly due to higher NO_3^--N influent concentration. Moreover, NO_3-N concentrations decreased with DO, and we observed an increase of dissolved N_2O-N, supporting the assumption that denitrification in anoxic conditions plays a key role in NO_3^--N removal and N_2O-N production (Fig 5, 6).

Nitrogen cycling in bioreactors can also be influenced by pH. The bioreactor pH dropped to 5.4 from 6.4, similar to an in-stream bioreactor built on the agricultural drainage of corn and soybean (7.3 to 6.9) (Robertson and Merkley, 2009) indicative of anaerobic microbial respiration by denitrifying

bacteria (Xu et al., 2009; Zhou et al., 2007). Denitrifying bacteria function efficiently within a pH range from 6.0 to 8.0, and pH in the range of 6.5 to 8.5 does not significantly affect microbial nitrate removal (Kumar and Lin, 2010; Xu et al., 2009). That implies that the lower pH range in this study (5.1-6.9) might be beyond the tolerance level of the denitrifying bacteria and affects the nitrate removal rate. We are uncertain whether higher N_2O formation rates are affecting nitrite reductase (which catalyses the conversion of NO_2-N to N_2O) and nitrate reductase (which catalyses NO_3 to NO_2) enzymes of denitrifying pathways and finally decreasing pH (Kumar and Lin, 2010). The pH of the effluent also depends on the media type (Cameron and Schipper, 2010). Experiments carried out on 9 bioreactors consisting of different filtration media determined that wood has lowest pH compared to other filtration media such as maize cobs (~6), green waste (~6.5), and wheat straw (~6.5). These observations also revealed a pH of 2.5 for hardwood and 4.3 for softwood media (Cameron and Schipper, 2010). Here, we used shredded hardwood in the bioreactor because they are known for better moisture retention (Addy et al., 2016) and are easily available in the local market.

Generally, NRR increases with an increase in temperature (Addy et al., 2016; Schipper et al., 2010). Robertson et al. (2008) recorded NRR increasing from 0.001 to 0.04 g N m^{-3} hr^{-1} at 6 to 10°C to 0.2 to 0.3 g N m^{-3} hr^{-1} at 20 to 22°C. Microbial activity is stimulated by warmer temperatures, potentially providing more dissolved carbon by partial decomposition of wood material. Similar to this study, a bioreactor installed on glasshouse (hothouse) wastewater had NRR of 0.4 g N m^{-3} hr^{-1} where influent drainage has temperature of 15 to 23.7°C (Warneke et al., 2011). One New Zealand maize cob bioreactor used heated beds to increase NRR; however, there was no significant increase in NRR (0.97 to 0.98 g N m^{-3} hr^{-1}) when temperature increased by 3.4°C (Cameron and Schipper, 2011). In our trial, the temperature of the influent varied between 16.2°C and 18.1°C and there was no significant correlation between change in temperature and NO_3^--N removal (Fig 6), perhaps due to a small

temperature range. Nevertheless, we found a negative trend between temperature change with NO_3-N concentration and N_2O-N production, respectively (Fig 4, 5). The lowest NRR of 0.9 g N m^{-3} hr^{-1} (observed when the lowest effluent recorded temperature was 16.2°C) indicates colder conditions may minimize NRR (Christianson et al., 2011; Robertson et al., 2000).

With a mean flow of 0.2±0.1 m^3 hr^{-1} (0.03 to 0.10 m^3 hr^{-1}) and mean depth of 8.0±0.03 cm (7.9 to 8.0 cm), the mean residence time was 6.4±2.5 hrs. A meta-analysis incorporating 26 published studies on bioreactors found denitrifying bioreactors with residence time longer than 6 hours had significantly higher NRE than bioreactors with shorter residence times (Addy et al., 2016). Here, the lowest NRR (0.9 g N m^{-3} hr^{-1}) and NRE (8.2 %) were observed when hydraulic residence time was longest (10.2 hours) during survey 3. We suspect a blockage in the flow path occurred during survey 3, affecting NRR and NRE. Bioreactors installed on agriculture drainage in the USA had a NRE of 30-70% at 4-8 hours of residence time, substantially higher than the NRE observed (8.2-25%) in this study with a residence time of 4-10 hours. For denitrifying bioreactor associated with a controlled drainage system, NRE for several high-flow events was greater for the year (50%) compared to NRE for low flow years (23%), likely due to dry periods immediately preceding each of these events (Woli et al., 2010). Here, we observed lowest NRE (8.2%) when flow rates were lowest (0.03 m^3 h^{-1}). Flow rates are known to influence denitrification in bioreactors (Greenan et al., 2009), where higher flow generally positively influences NRE and NRR. For example, a bioreactor installed on agriculture drainage demonstrated that the nitrate mass removed (49 to 194 mg m^{-2} hr^{-1}) generally increased with increasing flow rates (0.6 to 2.7 m^3 h^{-1}) (Chun et al., 2010). Overall, this illustrates that flow rates control the removal efficiency of the bioreactor.

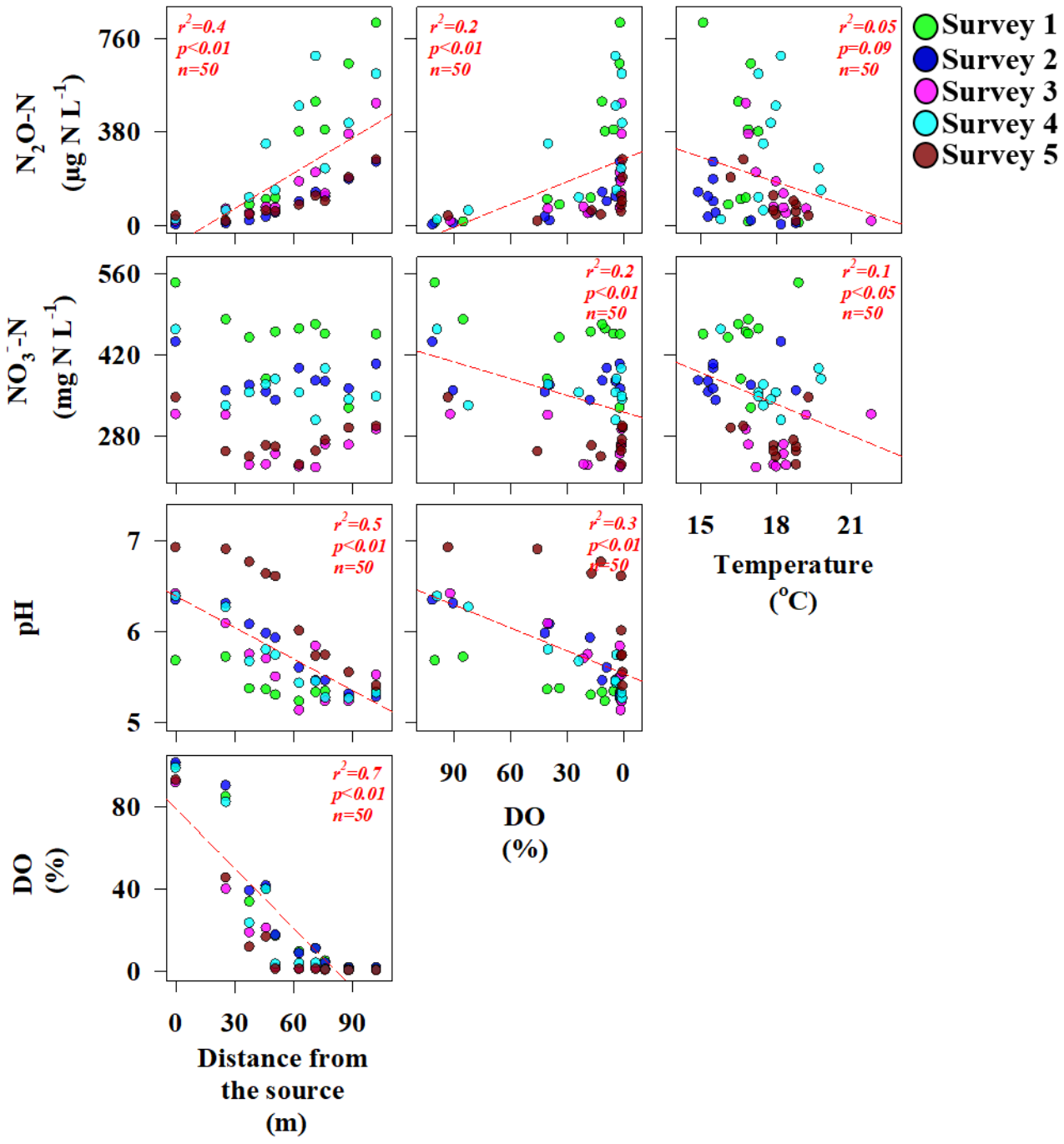


Figure 5: Scatter plot of distance from the source, DO (%) and temperature versus NO_3^--N and N_2O-N gas concentration in three inline pipe bioreactors during five surveys. DO (%) and temperature correlated with NO_3^--N.

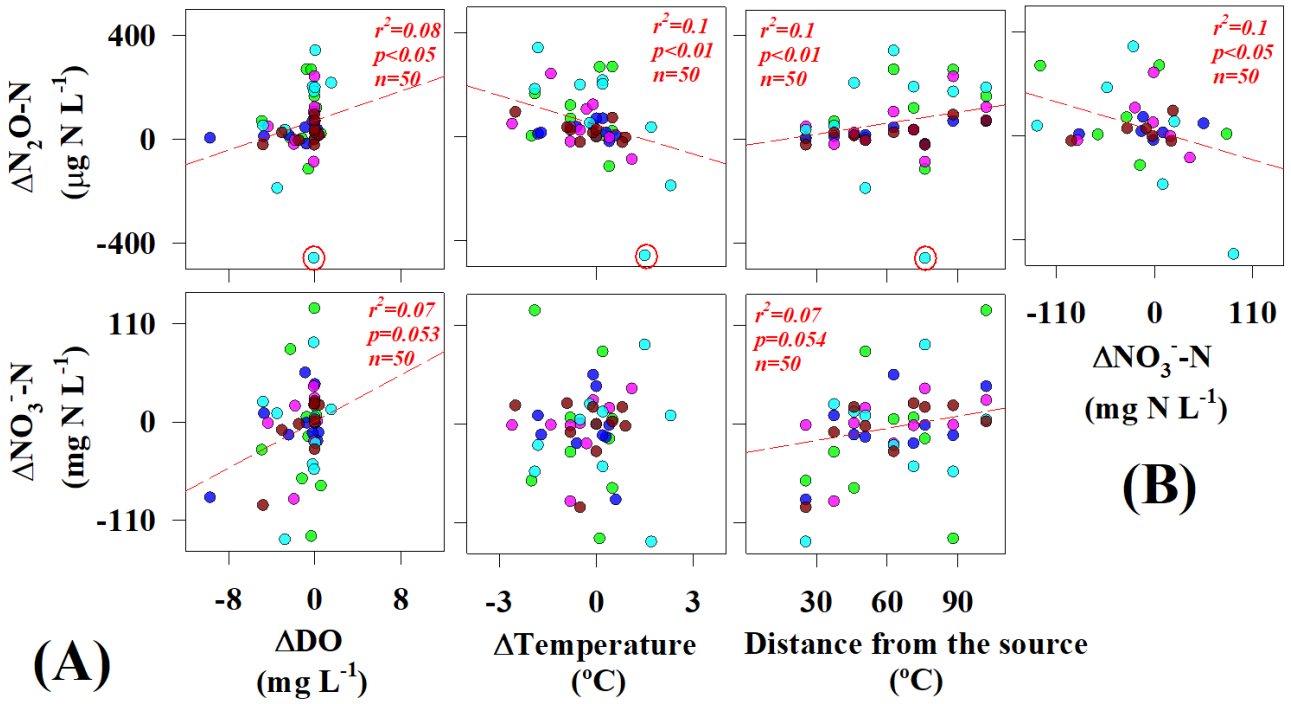


Figure 6: (A) Scatter plot of delta dissolved oxygen (DO), temperature and distance from the source versus delta concentration of NO_3^--N and N_2O-N gas. **(B)** Scatter plot of delta concentration of NO_3^--N and N_2O-N gas in the three inline pipe bioreactors. Delta represents differences between two adjacent observations of parameters. The dashed red line indicates trend between parameters and observations marked in red circles indicate outliers. Negative values of NO_3^--N and N_2O-N indicates removal. Relationships show the effect of change in DO (%) and temperature on NO_3^--N removal and N_2O-N gas production.

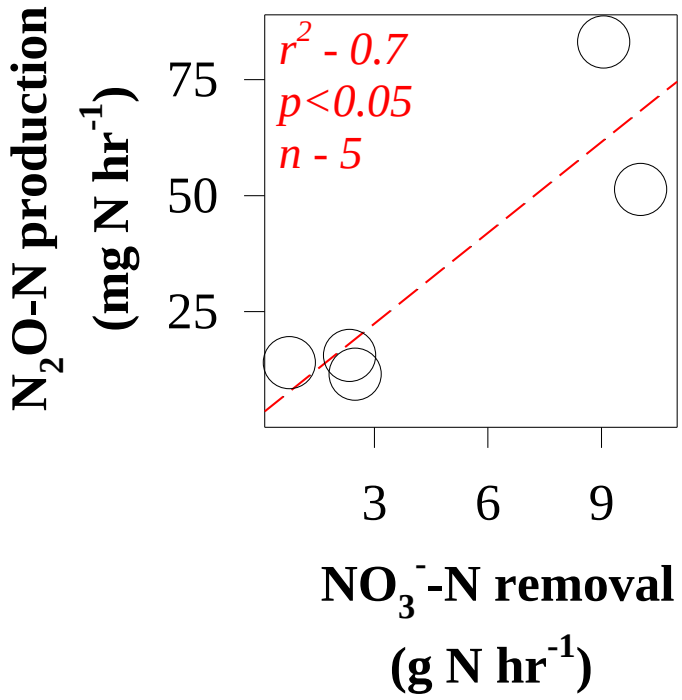


Figure 7: Scatter plot of nitrate load removal vs N_2O-N production three inline pipe bioreactors fed by cucumber farm drainage.

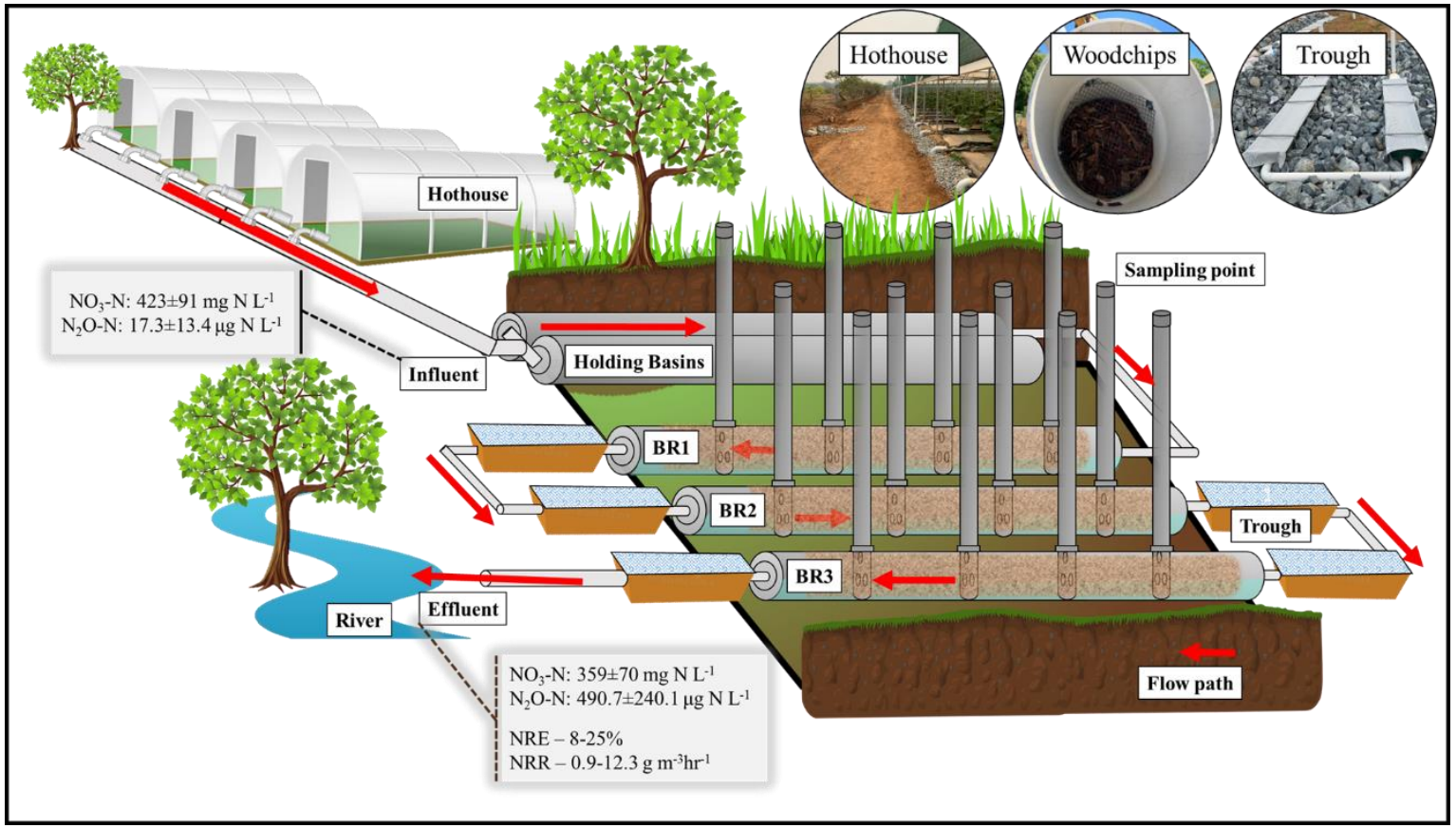


Figure 8: Schematic of pipe woodchip bioreactor which achieved mean nitrate removal efficiencies (NRE) of 14.5±6.8%, a nitrate removal rate (NRR) of 6.0±5.2 g N m^{-3} hr^{-1}.

4 Cost evaluation, future prospects and recommendations

The installation cost for the 3 inline bioreactors was ~A$25000. The most expensive installation component for these bioreactors was the contractor labour and machinery. As this was a prototype and many days of trial and error were encountered, the installation costs of a final design upscaled to other hothouses will likely reduce. Using the average total influent nitrate load fed to the bioreactor (0.7±0.4 kg N per day), the nitrate removed by the bioreactor would be 0.02 to 0.22 kg per day. Assuming a ten-year-long expected lifetime for the bioreactor, the cost required to remove 1 kg of NO_3-N is in the range of A$28 to A$378. Because these costs are based on winter samples and low flow rate when NRE was presumably the lowest, it is likely that the value is overestimated.

Though our NRR during (6.0±5.2 g N m^{-3} hr^{-1}) experiment was at least one order of magnitude higher than reported elsewhere, the depth of influent within the bioreactor was very shallow (~8 cm). Therefore, the active woodchip volume used for denitrification was just 21% of total woodchip capacity (0.8 m^3 of the total 3.8 m^3), minimizing the denitrification potential. A few simple modifications may increase the denitrification potential of this bioreactor:

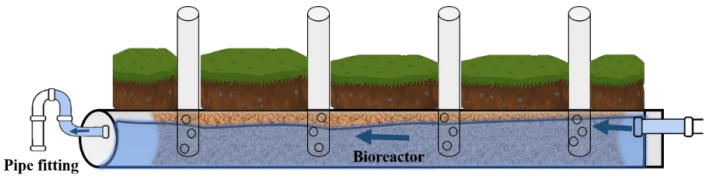
1. The bioreactor outlet position was 5 cm above the bottom of the pipe to keep woodchips wet and prevent backflow; however, we noticed this arrangement keeps the influent's depth in the bioreactor low. A simple water-regulating valve or structure at the outlet of a bioreactor can be added to control water depth (Figure 9). This would increase the wetted volume of woodchips, increase residence time and likely NRR.
2. 

Figure 9: Schematic of potential design modification. Pipe fitting at the outlet will help to regulate flow in the bioreactor and keep it wet most of the time.

2. Troughs were added to the end of each bioreactor to promote nitrification of NH_4 fertilizers and degassing of N_2O-N; however, they are also introducing oxygen in the anoxic bioreactor, which minimizes denitrification. We recommend the removal of troughs. Instead, an aeration ditch or tank can be added before the influent feed the bioreactor (Figure 10). This will allow nitrification of NH_4 fertilizers before it feeds the bioreactor, collect sediment and perhaps keep the bioreactor anoxic throughout.

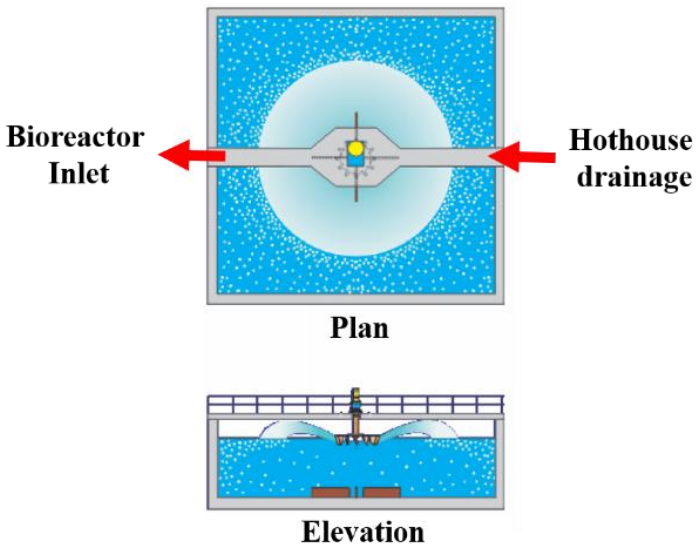


Figure 10: Plan and elevation of the simple aeration tank.

3. Influent NO_3-N concentrations from hothouses were extremely high at 423±91 mg N L^{-1}, indicating huge losses of nitrogen that represents an environmental and economic issue, likely occurring at other similar farms. We recommend a review of on-farm fertigation and nutrient doses to plants to minimize nutrient losses.

5 Conclusions

Pipe woodchip bioreactors can attenuate highly concentrated nitrogen (N) drainage leaving a hothouse and improve water quality downstream. Our trial bioreactor and general observations provide insights into how effective these bioreactors may be in minimizing pollution.

- Pipe bioreactors achieved nitrate removal efficiencies (NRE) of 14.5±6.8%, a nitrate removal rate (NRR) of 6.0±5.2 g N m^{-3} hr^{-1} and N_2O production equivalent to 0.7±0.6% (0.3-1.4 %) of nitrate removal.
- The nitrate removal rate (NRR), i.e. 12.3 g N m^{-3} hr^{-1} (on survey 4) was the highest ever recorded NRR in a field bioreactor trial. Lower nitrate removal efficiencies (NRE) (14.5%) are likely due to extremely high influent concentrations (423±91 mg N L^{-1}), and the bioreactor's operation at only 21% capacity due to shallow water (~8 cm). Simple modifications should enhance the nitrate removal efficiency and nitrate removal rate.
- Improvements for better NO_3^--N removal performance could include:

  1) Installation of a water level regulating device at the outlet of the bioreactor;

  2) Redesign of nitrification structures, i.e. trough;

  3) A review of on-farm fertigation inputs aiming at improving fertiliser management to prevent losses and lower effluent nitrogen concentrations.
- Overall the installation cost for the three inline bioreactors was ~A$25000 but is likely to be reduced if upscaled and deployed in many locations.

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Appendix

Survey 1								
Sampling station	**Depth (cm)**	**Temp. (°C)**	**DO (%)**	**DO (mg/N/L)**	**pH**	**Conductivity (µS)**	**NOx (mg N/L)**	**N_2O (µg N/L)**
1	34.0	18.9	100.0	9.4	5.7	6190	544.3	9.5
2	7.3	16.9	85.0	8.2	5.7	6140	481.1	12.9
3	7.3	16.1	33.8	3.3	5.4	6080	449.9	82.4
4	7.3	16.6	40.3	3.9	5.4	6060	378.5	104.4
5	7.3	16.8	17.1	1.7	5.3	6070	459.6	110.1
6	7.3	17.3	9.6	0.9	5.2	6060	465.2	380.1
7	7.3	16.5	11.1	1.1	5.3	6120	472.4	501.2
8	9.3	16.9	5.0	0.5	5.3	6110	456.3	387.0
9	9.3	17.0	1.8	0.2	5.3	6130	328.6	655.8
10	9.3	15.1	1.6	0.1	5.3	6140	455.7	822.2
Survey 2								
1	34.0	18.2	101.3	18.2	6.4	5130	442.8	1.9
2	7.3	18.8	90.3	8.5	6.3	5320	358.4	6.9
3	7.3	17	39.1	3.8	6.1	5270	368.0	18.0
4	7.3	15.3	41.5	4.1	6.0	5320	356.2	33.4
5	7.3	15.6	17.6	1.7	5.9	5280	341.8	49.6
6	7.3	15.5	8.7	0.9	5.6	5410	397.0	95.7
7	7.3	14.9	11.1	1.1	5.5	5420	375.6	133.4
8	9.3	15.3	4.0	0.4	5.5	5390	374.5	115.4
9	9.3	15.5	1.6	0.2	5.3	5450	362.0	186.1
10	9.3	15.5	1.7	0.2	5.3	5470	404.2	256.3

Survey 3								
Sampling station	**Depth (cm)**	**Temp. (ºC)**	**DO (%)**	**DO (mg/N/L)**	**pH**	**Conductivity (µS)**	**NOx (mg N/L)**	**N_2O (µg N/L)**
1	32.0	21.8	91.8	8.0	6.4	4630	317.6	16.0
2	7.3	19.2	40.0	3.7	6.1	2250	316.3	66.0
3	7.3	18.4	18.8	1.7	5.8	3542	230.1	47.4
4	7.3	17.9	21.0	2.0	5.7	3427	231.1	72.9
5	7.3	18.3	1.8	0.2	5.5	4246	249.2	69.9
6	7.3	18	1.2	0.1	5.1	3739	227.5	176.9
7	7.3	17.2	1.7	0.2	5.8	3654	226.0	214.0
8	9.3	18.3	0.9	0.1	5.2	3963	265.8	127.5
9	9.3	16.9	0.7	0.1	5.2	3807	264.9	370.1
10	9.3	16.8	0.8	0.1	5.5	3940	291.7	494.5
Survey 4								
1	32.0	15.8	98.8	9.8	6.4	5880	464.2	22.0
2	7.3	17.5	82.2	7.0	6.3	4726	332.7	58.7
3	7.3	17.3	23.5	2.3	5.7	5390	355.1	111.9
4	7.3	17.5	39.8	3.8	5.8	4987	369.0	329.8
5	7.3	19.8	3.5	0.3	5.7	5230	378.4	141.3
6	7.3	18	3.8	0.4	5.4	4904	354.9	483.9
7	7.3	18.2	4.0	0.2	5.5	4711	307.5	686.9
8	9.3	19.7	1.0	0.1	5.3	5160	396.3	229.6
9	9.3	17.8	0.5	0.0	5.3	4879	343.3	414.1
10	9.3	17.3	0.6	0.1	5.3	4724	348.3	614.4

Survey 5								
Sampling station	**Depth (cm)**	**Temp. (ºC)**	**DO (%)**	**DO (mg/N/L)**	**pH**	**Conductivity (µS)**	**NOx (mg N/L)**	**N_2O (µg N/L)**
1	35.0	19.3	93.0	9.0	6.9	4609	346.8	37.2
2	7.3	18.8	45.5	4.2	6.9	4035	253.7	16.3
3	7.3	18	11.8	1.2	6.8	4300	244.7	42.6
4	7.3	17.9	16.7	1.6	6.6	4337	263.7	58.3
5	7.3	18.8	1.1	0.1	6.6	4302	261.5	54.9
6	7.3	18.8	0.9	0.1	6.0	4318	230.9	82.2
7	7.3	17.9	1.0	0.1	5.7	4302	254.3	118.8
8	9.3	18.7	0.6	0.1	5.7	4318	273.2	97.4
9	9.3	16.2	0.3	0.0	5.6	4390	294.0	192.7
10	9.3	16.7	0.4	0.0	5.4	4412	297.0	266.0