Received: 18 May 2020 | Accepted: 28 June 2020

DOI: 10.1002/ael2.20024

Agricultural & Environmental Letters OPEN ACCESS

RESEARCH LETTER

Nitrate removal and nitrous oxide production from upflow and downflow column woodchip bioreactors

Gary W. Feyereisen[1] | **Kurt A. Spokas**[1] | **Jeffrey S. Strock**[2] | **David J. Mulla**[2] | **Andry Z. Ranaivoson**[2] | **Jeffrey A. Coulter**[3]

[1] USDA-ARS Soil and Water Management Research Unit, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

[2] Dep. of Soil, Water, and Climate, Univ. of Minnesota, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

[3] Dep. of Agronomy and Plant Genetics, Univ. of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA

Correspondence
Gary W. Feyereisen, USDA-ARS Soil and Water Management Research Unit, 439 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA.
Email: gary.feyereisen@usda.gov

Funding information
Minnesota Agricultural Utilization Research Institute

Abstract

Woodchip denitrifying bioreactors (WDBR) reduce off-field tile drainage nitrogen (N) losses from agricultural fields. Limited evaluation exists regarding the influence of flow direction through WDBRs. Changing flow direction could reduce short circuiting. This study evaluated the dependency of nitrate-N removal and dissolved nitrous oxide (dN_2O) production rates on vertical flow direction in triplicate column bioreactors at 12-h (without carbon dosing) and 2-h (with carbon dosing) hydraulic residence times. Results presented demonstrate that there was no significant difference in overall N removal rates from these column bioreactors as a function of flow direction. There was the suggestion of lower N_2O production in the downflow direction, although this was not statistically significant due to the high variability of the N_2O production observed in the upflow direction. Carbon addition led to bioclogging of downflow columns; future work needs to identify dosing rate, placement, and conditions that prevent biofilm formation.

1 | INTRODUCTION

Nitrate-nitrogen (N) loads to the Gulf of Mexico are considered a major contributor to the gulf hypoxic zone (Rabalais, Turner, & Wiseman, 2002). Efforts to curb losses include improving field N management, changing cropping system or land use, and managing tile drainage effluent with structural practices at the edge of field or in drainage ditches (MPCA, 2013). One structural practice is the woodchip denitrifying bioreactor (WDBR), which removes nitrate-N via dissimilatory denitrification (Schipper, Robertson, Gold, Jaynes, & Cameron, 2010). Flow is typically horizontal through WDBRs designed for tile drainage systems, although vertical flow is common in other water treatment systems (Ilyas & Masih, 2018; Tanner, Sukias, Headley, Yates, & Stott, 2012; Vymazal, 2011). Changing WDBR design to vertical flow could reduce short circuiting (Christianson et al., 2020).

Nitrate-nitrogen removal rate (NRR) of WDBRs is strongly dependent on hydraulic residence time (HRT) and temperature (Hoffman, Larsen, & Kjaergaard, 2019;

Abbreviations: cpN_2O, cumulative N_2O production; dN_2O, dissolved N_2O; HRT, hydraulic residence time; NRR, nitrate-N removal rate; rN_2O, relative ratio of cumulative N_2O produced to nitrate-N removed; WDBR, woodchip denitrifying bioreactor

Hoover, Bhandari, Soupir, & Moorman, 2016). Bioreactors typically operate with HRTs from 6 to 20 h (Addy et al., 2016), with longer times required during colder springtime conditions (Hoover et al., 2016) when a substantial portion of annual tile drainage flows in the northern U.S. Corn Belt (Jin & Sands, 2003). Corn (*Zea mays* L.) cobs as a denitrifying medium have been shown to increase NRR compared with woodchips (Cameron & Schipper, 2010), including at low temperatures (2 °C) (Feyereisen et al., 2016). Roser et al. (2018) showed that carbon (C) dosing woodchips with acetate increased nitrate-N load reduction from 5 to 33% at 5 °C and 1.5-h HRT. Shortening HRT by C dosing could reduce WDBR size.

During the denitrification process, nitrous oxide (N_2O), a potent greenhouse gas, is produced (Bremner, 1997). Under typical field conditions, i.e., HRT of 8 h, denitrification of NO_3^- to N_2 gas is nearly complete and release of N_2O is negligible; however, as HRT is shortened, N_2O releases could increase (Davis, Martin, Moorman, Isenhart, & Soupir, 2019). Additionally, there is no information on the direction of water flow in WDBRs on potential N_2O production. Therefore, a laboratory-scale experiment was designed and conducted to examine flow direction on column bioreactor performance by (a) quantifying nitrate-N removal and NRR and (b) investigating production of N_2O at short HRT.

Core Ideas

- Flow direction did not affect nitrate load removal or rate, or N_2O production.
- C dosing at 2-h HRT increased nitrate-N removal rate relative to 12-h HRT without C.
- C dosing at 2-h HRT decreased dissolved N_2O concentration relative to 12-h HRT without C.
- Individual effects of C addition and HRT reduction were not ascertained.
- Biofilms began to impede flow through downflow columns.

2 | MATERIALS AND METHODS

2.1 | Bioreactor design and operation

Upflow and downflow columns were packed in triplicate with 22.9 cm of corn cobs (269 ± 8 g dry) at the inlet, followed by 22.9 cm of wood chips (mixed species, primarily hardwood, 13 × 15 × 5 mm; 336 ± 22 g dry), then a 5.1-cm-thick layer of a woven polymeric mat (Bro-Tex), and finally 7.6 cm of lava rock (10- to 60-mm diam.; Vigoro). The purpose of the mat and lava rock were to provide area for biofilm development and space for processing additional C in effluent prior to exiting the system. The outlets of the downflow columns were plumbed to the height of the perforated plate to maintain media saturation. Peristaltic pumps mixed nutrient and potassium acetate solutions (90%:10% nutrient solution/acetate solution; final concentrations: nitrate-N, 20 mg N L^{-1}; dissolved P, 0.3 mg P L^{-1}; acetate, 93 mg C L^{-1}) prior to the inlet of the upflow columns. For the downflow columns, the mixed solutions were pumped onto a perforated plate at the top of the column, which held media in place and distributed the influent. Gravity effected downflow.

Inoculation was achieved by two means. The first was direct mixing of 10 g of oven-dried (48 h, 60 °C) woodchips taken from an operating field bioreactor 19 mo prior (Willmar, MN) with new corn cobs and woodchips during column packing. The second means was soaking (48 h) the column packing materials with effluent from an operating woodchip bioreactor (Blue Earth, MN). Porosity for HRT calculations was determined by draining saturated columns for 24 h.

Water was circulated through the columns (18 d) to detect leaks and clear detritus from the media (Supplemental Figure S1). Flow rates equivalent to a 12-h HRT (4 ml min^{-1}) were established at 10 °C and the nutrient solution was introduced for 16 d, after which the acetate solution was added (90%:10% nutrient solution/acetate solution). Flow rates were adjusted to 2-h HRT (23.5 ml min^{-1}) during the week (Monday–Friday). Flow rates were reduced to a nominal 12-h HRT on Fridays at 12:00 and acetate additions paused over the weekend because the volume of water needed for the experiment was substantial and weekend oversight of the experiment was limited. Acetate additions and a 2-h HRT were reestablished on Mondays at 13:00 ± 1:00.

Seven days after acetate introduction, the acetate pump failed (Supplemental Figure S1). For the next 7 d, weekend conditions were established: 12-h HRT without acetate addition. The weekday/weekend flow regime was reestablished for the remaining 35 d; data from this period were used for statistical analysis.

2.2 | Sample collection and analysis

Water samples for nutrient analysis were collected on Mondays (12-h HRT) and Thursdays (2-h HRT) from inlets and outlets. Samples for nutrient analysis were filtered (0.45 μm; polyethersulfone), refrigerated (4 °C), and analyzed on Mondays and Thursdays for nitrate-N

(NO_2^--N+NO_3^--N) and ammonium-N, colorimetrically by flow injection (QuickChem 8500; Lachat).

Samples for dissolved gas analysis were collected Mondays and Thursdays for the final 32 d of the experiment with one 3-ml draw with a disposable syringe (BD: model 309604) through stop cocks (Kimble 420163-0000) plumbed into the inlet and outlet lines. The water was injected into a 20-ml vial previously sealed with a butyl rubber septum and then flushed with helium. Samples were equilibrated (22 ± 1 °C) and analyzed following a minimum of 24 h to allow for equilibrium between the dissolved and headspace N_2O concentrations. Samples were analyzed with an automated headspace sampler (Agilent 7694E) plumbed directly to a customized gas chromatographic system (Agilent; HP-5890) with N_2O quantified by an electron capture detector (Spokas, Koskinen, Baker, & Reicosky, 2009). Dissolved N_2O was then estimated through assuming ideal gas law behavior and using Henry's law coefficient for N_2O.

Flow rates were measured with a bottle, scale, and stopwatch. Loads were calculated by multiplying flow rates by time between flow rate measurements by concentration. Beginning at 33 d after initial addition of acetate, the downflow columns began to overflow as a result of biofilm formation. The overflow was captured and measured. Nitrate-N removal rate (g N m^{-3} d^{-1}) was calculated as:

$$\mathrm{NRR} = \left(\frac{\mathrm{NLoad_{rem}}}{t_i}\right) \Big/ \mathrm{Vol_{med}}$$

where $\mathrm{NLoad_{rem}}$ is nitrate-N load removed between samplings, t_i is time between previous and current samplings, and $\mathrm{Vol_{med}}$ is gross volume of the media. Nitrate-N load reduction (%) was calculated as:

$$\mathrm{NLoadReduction} = \sum_{t=1}^{n} \mathrm{NLoad_{rem}} \Big/ \sum_{t=1}^{n} \mathrm{NLoad_{in}} \times 100$$

where $\mathrm{NLoad_{rem}}$ is as defined above, t is time between samplings, n is the number of samplings, and $\mathrm{NLoad_{in}}$ is the nitrate-N load at the column inlet between samplings. Cumulative N_2O production, cpN_2O, (mg N), was calculated as:

$$cpN_2O = \sum_{t=1}^{n} (dN_2O_{out} \times \mathrm{Vol}_i)$$

where t and n are defined as above, dN_2O_{out} is N_2O concentration at the outlet, and Vol_i is the volume of effluent since the previous sampling. The relative production of N_2O to nitrate-N removed, rN_2O (%), was calculated as:

$$rN_2O = cpN_2O \Big/ \sum_{t=1}^{n} \mathrm{NLoad_{rem}} \times 100$$

where cpN_2O, t, n, and $\mathrm{NLoad_{rem}}$ are as defined above.

Data were analyzed at $P \leq .05$ using the MIXED procedure of SAS v.9.4 (SAS Institute, 2013), with flow direction and nominal HRT as fixed effects, week as a fixed effect and repeated measurement, and replication and interactions with replication as random effects. Outlet dN_2O was logarithm base 10 transformed to meet the requirements of normality and common variance. When main effects or interactions for fixed effects were significant, means were compared with pairwise t tests using the PDIFF option of the MIXED procedure of SAS.

3 | RESULTS AND DISCUSSION

Cumulative nitrate-N load reduction over the 35-d experiment was not significantly different between the upflow and downflow columns, 21.3 and 27.5%, respectively (Table 1; $P = .13$; Supplemental Figure S2). Across the flow direction treatments, a greater percentage of nitrate-N was removed at 12-h HRT (35.1%) than at 2-h HRT (22.2%). The value for the 12-h HRT is identical to the findings of Hoover et al. (2016), 36 ± 4%, for laboratory columns with woodchips at the same HRT, temperature (10 °C), and inlet nitrate-N concentration. Feyereisen et al. (2016) tested columns with woodchips followed by corn cobs at 1.5 and 15.5 °C and reported nitrate-N removal of 15 and 62%, respectively, which brackets the current findings. No ammonium concentrations were above detection limit (0.005 mg N L^{-1}) for downflow samples and only a few were above detection limit for upflow samples (data not shown), suggesting that nitrate-N removal was primarily by denitrification and not dissimilatory nitrate reduction to ammonium.

There were no significant differences in rN_2O or dN_2O between upflow and downflow treatments across HRTs, although variability tended to be lower for the downflow treatment, particularly at 2-h HRT (Table 1; Figure 1). Across flow directions, rN_2O was not significantly different ($P = .14$) between 2- and 12-h HRT with high variability; means (SE) were 0.15 (0.09) and 0.39 (0.06)%, respectively. Dissolved N_2O concentrations at the outlet, averaged across HRTs and weeks, were significantly greater for 12-h than for 2-h HRTs ($P = .01$); back-transformed means were 23.2 and 5.3 μg N L^{-1}, respectively. There were significant dN_2O differences among weeks with dN_2O declining until the third week and then stabilizing (Figure 1). Dissolved N_2O was greater for upflow through Week 3;

TABLE 1 Cumulative nitrate-N load reduction, relative N_2O production (rN_2O), dissolved N_2O outlet concentrations (dN_2O), and nitrate-N removal rate (NRR) for bioreactor columns operated in two flow directions at two hydraulic residence times (HRT)

HRT (h)	Flow direction		
	Up and Down[a]	Up	Down
	Cumulative NO_3-N load reduction, %		
2 & 12[b]		21.3 (2.2) A[c]	27.5 (2.4) A
2		19.1 (2.0)[d]	25.4 (2.4)
12		32.2 (3.7)	38.1 (5.6)
2	22.2 (2.0) a		
12	35.1 (3.3) b		
	rN_2O, %		
2 & 12		0.28 (0.12) A	0.12 (0.02) A
2		0.22 (0.12)	0.08 (0.02)
12		0.49 (0.23)	0.29 (0.13)
2	0.15 (0.06) a		
12	0.39 (0.13) a		
	dN_2O, μg N L^{-1}		
2 & 12		16.7 (16.8) A	11.7 (12.0) A
2		6.4 (4.1)	4.2 (2.5)
12		27.1 (22.2)	19.3 (15.9)
2	5.3 (2.4) a		
12	23.2 (13.5) b		
	NRR, g N m^{-3} d^{-1}		
2		25.8 (5.5)	34.5 (7.5)
12		10.7 (2.8)	12.8 (4.9)
2	30.1 (4.9) b		
12	11.8 (2.8) a		

[a]Values in column "Up and Down" represent mean (SE) across flow directions ($n = 6$).
[b]Values in rows with "2 & 12" in HRT column represent mean (SE) of treatment columns ($n = 3$) throughout the experiment across HRTs.
[c]Means (SE) within a row for each variable followed by the same uppercase letter are not significantly different at $P \leq .05$; means within a column for each variable followed by the same lowercase letter are not significantly different at $P \leq .05$.
[d]Means (SE) within a row and without a letter were not compared statistically because the ANOVA p value for the interaction between HRT and flow direction was not significant at $P \leq .05$.

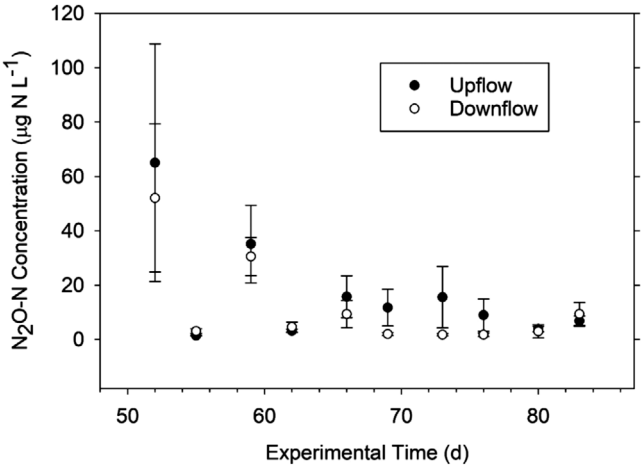

FIGURE 1 Time plot of dissolved N_2O concentration

dN_2O for the final two weeks was not significantly different between flow directions (Figure 1).

Feyereisen et al. (2016) reported average rN_2O of 0.92% across 1.5 and 15.5 °C with dN_2O from 2 to 164 μg N L^{-1}. In another column experiment, Feyereisen, Christianson, Moorman, Venterea, and Coulter (2017) found that rN_2O averaged across treatments of corn cobs and corn cobs followed by a layer of plastic biofilm carrier was 0.3 and 1.6% at 15.5 and 1.5 °C, respectively. Davis et al. (2019) measured dN_2O and N_2O emissions from the surface of uncapped field pilot-scale WDBRs at 2-, 8-, and 16-h HRTs. Dissolved N_2O comprised >97% of N_2O fluxes, with total ratios of dN_2O-to-NO_3^- removed of 5.19, 0.35, and 0.52%, for 2-, 8-, and 16-h HRTs.

Based on previous findings, such as Davis et al. (2019), and the temperature and step-based nature of denitrification wherein the last step to be mediated is from N_2O to

N_2 (Wallenstein, Myrold, Firestone, & Voytek, 2006), we expected N_2O production for the 2-h HRT to increase. In this respect, our findings were unexpected. Apparently, the addition of readily available C via acetate addition provided ample electron donor capacity to maintain nearly complete denitrification (Hanaki, Hong, & Matsuo, 1992). Although not significantly different, the suggestion of lower N_2O production for downflow could be a result of gas diffusion gradient counter to water flow direction (Bruun, Hoffmann, & Kjaergaard, 2017) or additional aeration at the tops of the downflow columns, which were open to atmosphere (Pijuan et al., 2014). The lower variability in the downflow columns is most evident in the standard errors in rN_2O (Table 1).

For NRR, neither the interaction of flow direction × HRT ($P = 0.36$) nor the main effect of flow direction ($P = .16$) were significant (Table 1). Averaged across flow directions, NRR at 2-h HRT was greater than at 12-h HRT, 30.1 vs. 11.8 g N $m^{-3}d^{-1}$, respectively (Table 1). Averaged across flow directions, NRR at 12 h was slightly greater than that reported by Hoover et al. (2016), 6.9±0.3 g N $m^{-3}d^{-1}$, and again bracketed by values from Feyereisen et al. (2016) at 1.5 and 15.5 °C, 6.8 and 29.3 g N $m^{-3}d^{-1}$, respectively. Two factors explain the 2.6-fold increase in NRR at the shorter HRT. First, as input loading into a WDBR is increased by greater flow rate, NRR tends to increase (Greenan, Moorman, Parkin, Kaspar, & Jaynes, 2009; Pluer, Geohring, Steenhuis, & Walter, 2016). Second, the addition of readily available C increases electron donor availability for denitrification (Lemaire et al., 2006). Roser et al. (2018) reported a 2.4- and 3.1-fold increase in NRR with C dosing and woodchips at 12-h HRT at 5 °C.

Addition of C poses the challenge of bioclogging of woodchip bioreactors (Anderson, Jang, Venterea, Feyereisen, & Ishii, 2020). Given the limited gravity head gradient driving flow, the downflow columns were susceptible to bioclogging at the ratio of C/N of this experiment (Fig. S3). The issue of bioclogging in denitrification bioreactors has been noted by others (Inês, Soares, Braester, Belkin, & Abeliovich, 1991) and remains an issue to be solved. Solutions may include reducing C/N or adjusting the location of C delivery. However, the benefits in dramatically increasing NRR at high flow and low temperatures continue to be worth further study.

4 | CONCLUSIONS

The overall purpose of this laboratory experiment was to evaluate the influence of vertical column flow direction on bioreactor performance. There was no significant difference observed in nitrate-N removal rates as a function of column flow direction. Additionally, the data collected here confirm a lack of significant difference in N_2O production potentials, although the downflow direction did result in numerically lower values. Production of N_2O was reduced with C additions at short HRTs, although individual effects of C addition and HRT were uncertain from this experiment. A necessary consideration for downflow bioreactors is the microbial clogging of water flow. This biofilm production must be further evaluated prior to adding supplemental C in downflow field bioreactors.

AUTHOR CONTRIBUTIONS STATEMENT

Conceptualization, GWF, KAS, JSS, DJM, and AZR; Methodology, GWF, KAS, JSS, and JAC; Validation, GWF, KAS, and JAC; Formal Analysis, JAC, KAS, and GWF; Investigation, GWF, KAS, JSS, DJM, AZR, and JAC; Resources, JSS, KAS, and GWF; Data Curation, GWF, KAS, and JAC; Writing–Original Draft Preparation, GWF, KAS, and JAC; Writing–Review & Editing, GWF, KAS, JSS, DJM, AZR, and JAC; Visualization, GWF, KAS, and JAC; Supervision, GWF, KAS, and JSS; Project Administration, JSS; Funding Acquisition, JSS.

ACKNOWLEDGMENTS

We express gratitude to Todd Schumacher, Marta Roser, Lizzy Selvik, Olivia Richter, Martin DuSaire, and Sam Okkema for their technical assistance. We acknowledge and thank the Minnesota Agricultural Utilization Research Institute for partially funding this work. We also thank the two peer reviewers whose constructive comments contributed to a stronger manuscript. Use of manufacturers' names does not represent endorsement by the USDA. The USDA is an equal opportunity provider and employer.

DATA AVAILABILITY STATEMENT

The data used for the analyses herein are available at Feyereisen (2020), https://doi.org/10.5061/dryad.hqbzkh1d2.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ORCID

Gary W. Feyereisen https://orcid.org/0000-0003-2785-4594
Kurt A. Spokas https://orcid.org/0000-0002-5049-5959
Jeffrey S. Strock https://orcid.org/0000-0001-5357-0638
David J. Mulla https://orcid.org/0000-0001-7040-5888
Andry Z. Ranaivoson https://orcid.org/0000-0002-4717-5065
Jeffrey A. Coulter https://orcid.org/0000-0003-4609-4551

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*, 873–881. https://doi.org/10.2134/jeq2015.07.0399

Anderson, E. L., Jang, J., Venterea, R. T., Feyereisen, G. W., & Ishii, S. (2020). Isolation and characterization of denitrifiers from woodchip bioreactors for bioaugmentation application. *Journal of Applied Microbiology*. https://doi.org/10.1111/jam.14655

Bremner, J. M. (1997). Sources of nitrous oxide in soils. *Nutrient Cycling in Agroecosystems*, *49*, 7–16. https://doi.org/10.1023/A:1009798022569

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*, 1–10. https://doi.org/10.1016/j.ecoleng.2016.10.027

Cameron, S. G., & Schipper, L. A. (2010). Nitrate removal and hydraulic performance of organic carbon for use in denitrification beds. *Ecological Engineering*, *36*, 1588–1595. https://doi.org/10.1016/j.ecoleng.2010.03.010

Christianson, L. E., Feyereisen, G. W., Hay, C. H., Tschirner, U. W., Kult, K. J., Soupir, M. L., & Hoover, N. L. (2020). Denitrifying bioreactor woodchip recharge: Media properties after a 9-year design life. *Transactions of the ASABE*, *63*, 407–416.

Davis, M. P., Martin, E. A., Moorman, T. B., Isenhart, 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*, 290–297. https://doi.org/10.1016/j.jenvman.2019.04.055

Feyereisen, G. W., Christianson, L. E., Moorman, T. B., Venterea, R. T., & Coulter, J. A. (2017). Plastic biofilm carrier after corn cobs reduces nitrate loading in laboratory denitrifying bioreactors. *Journal of Environmental Quality*, *46*(4), 915–920. https://doi.org/10.2134/jeq2017.02.0060.

Feyereisen, G. W., Moorman, T. B., Christianson, L. E., Venterea, R. T., Coulter, J. A., & Tschirner, U. W. (2016). Performance of agricultural residue media in laboratory denitrifying bioreactors at low temperatures. *Journal of Environmental Quality*, *45*, 779–787. https://doi.org/10.2134/jeq2015.07.0407

Feyereisen, G., Spokas, K. A., Strock, J. S., Mulla, D. J., Ranaivoson, A. Z., & Coulter, J. A. (2020). Data from: Nitrate removal and N_2O production from upflow and downflow column woodchip bioreactors. Dryad Digital Repository. https://doi.org/10.5061/dryad.hqbzkh1d2

Greenan, C. M., Moorman, T. B., Parkin, T. B., Kaspar, T. C., & Jaynes, D. B. (2009). Denitrification in wood chip bioreactors at different water flows. *Journal of Environmental Quality*, *38*, 1664–1671. https://doi.org/10.2134/jeq2008.0413

Hanaki, K., Hong, Z., & Matsuo, T. (1992). Production of nitrous oxide gas during denitrification of wastewater. *Water Science & Technology*, *26*(5–6), 1027–1036. https://doi.org/10.2166/wst.1992.0544

Hoffman, C. C., Larsen, S. E., & Kjaergaard, C. (2019). Nitrogen removal in woodchip-based biofilters of variable designs treating agricultural drainage discharges. *Journal of Environmental Quality*, *48*, 1881–1889. https://doi.org/10.2134/jeq2018.12.0442

Hoover, N. L., Bhandari, A., Soupir, M. L., & Moorman, T. B. (2016). Woodchip denitrification bioreactors: Impact of temperature and hydraulic retention time on nitrate removal. *Journal of Environmental Quality*, *45*, 803–812. https://doi.org/10.2134/jeq2015.03.0161

Ilyas, H., & Masih, I. (2018). The performance of the intensified constructed wetlands for organic matter and nitrogen removal: A review. *Journal of Environmental Management*, *198*, 372–383. https://doi.org/10.1016/j.jenvman.2017.04.098

Inês, M., Soares, M., Braester, C., Belkin, A., & Abeliovich, A. (1991). Denitrification in laboratory sand columns: Carbon regime, gas accumulation, and hydraulic properties. *Water Research*, *25*(3), 325–332. https://doi.org/10.1016/0043-1354(91)90013-G

Jin, C.-X., & Sands, G. R. (2003). The long-term field-scale hydrology of subsurface drainage systems in a cold climate. *Transactions of the ASAE*, *46*, 1011–1021.

Lemaire, R., Meyer, R., Taske, A., Crocetti, G. R., Keller, J., & Yuan, Z. (2006). Identifying causes for N_2O accumulation in a lab-scale sequencing batch reactor performing simultaneous nitrification, denitrification and phosphorus removal. *Journal of Biotechnology*, *122*, 62–72. https://doi.org/10.1016/j.jbiotec.2005.08.024

MPCA. (2013). *Nitrogen in Minnesota surface waters*. Retrieved from https://www.pca.state.mn.us/sites/default/files/wq-s6-26a.pdf

Pijuan, M., Torà, J., Rodríguez-Caballero, A., César, E., Carrera, J., & Perez, J. (2014). Effect of process parameters and operational mode on nitrous oxide emissions from a nitritation reactor treating reject wastewater. *Water Research*, *49*, 23–33. https://doi.org/10.1016/j.watres.2013.11.009

Pluer, W. T., Geohring, L. D., Steenhuis, T. S., & Walter, M. T. (2016). Controls influencing the treatment of excess agricultural nitrate with denitrifying bioreactors. *Journal of Environmental Quality*, *45*, 772–778. https://doi.org/10.2134/jeq2015.06.0271

Rabalais, N. N., Turner, R. E., & Wiseman, W. J., Jr. (2002). Gulf of Mexico hypoxia, aka "the dead zone." *Annual Review of Ecology and Systematics*, *33*, 235–263. https://doi.org/10.1146/annurev.ecolsys.33.010802.150513

Roser, M. B., Feyereisen, G. W., Spokas, K. A., Mulla, D. J., Strock, J. S., & Gutknecht, J. (2018). Carbon dosing increases nitrate removal rates in denitrifying bioreactors at low-temperature high-flow conditions. *Journal of Environmental Quality*, *47*, 856–864. https://doi.org/10.2134/jeq2018.02.0082

SAS Institute. (2013). The SAS system for Windows. Version 9.4. Cary, NC: SAS Institute.

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*, 1532–1543. https://doi.org/10.1016/j.ecoleng.2010.04.008

Spokas, K. A., Koskinen, W. C., Baker, J. M., & Reicosky, D. C. (2009). Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. *Chemosphere*, *77*, 574–581. https://doi.org/10.1016/j.chemosphere.2009.06.053.

Tanner, C., Sukias, J. P.S., Headley, Tom R., Yates, C. R., & Stott, R. (2012). Constructed wetlands and denitrifying bioreactors for

on-site and decentralized wastewater treatment: Comparison of five alternative configurations. *Ecological Engineering*, *42*, 112–123. https://doi.org/10.1016/j.ecoleng.2012.01.022

Vymazal, J. (2011). Constructed wetlands for wastewater treatment: Five decades of experience. *Environmental Science & Technology*, *45*, 61–69.

Wallenstein, M. D., Myrold, D. D., Firestone, M., & Voytek, M. (2006). Environmental controls on denitrifying communities and denitrification rates: Insights from molecular methods. *Ecological Applications*, *16*, 2143–2152. https://doi.org/10.1890/1051-0761(2006)016%5b2143:ECODCA%5d2.0.CO;2

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Feyereisen GW, Spokas KA, Strock JS, et al. Nitrate removal and nitrous oxide production from upflow and downflow column woodchip bioreactors. *Agric Environ Lett*. 2020;5:e20024. https://doi.org/10.1002/ael2.20024