Denitrifying bioreactors (also known as woodchip bioreactors) are an agricultural beneficial management practice (BMP) used for the removal of nitrate (NO\textsubscript{3}) in water discharged from farms (Schipper et al., 2010; Addy et al., 2016). Denitrifying bioreactors have been shown to be effective for reducing NO\textsubscript{3}. Previous applications of woodchip bioreactors include subsurface drainage water from crop (Woli et al., 2010; Christianson et al., 2012; David et al., 2016), dairy (Schipper et al., 2010) and hog farm fields (Liu, 2017), aquaculture units (Lepine et al., 2016), and hydroponics operations (Warneke et al., 2011), with reported volumetric removal rates in the range of 0.3 to 16.0 g N m\textsuperscript{-3} d\textsuperscript{-1} (Addy et al., 2016).

A previously reported study conclusively showed that exposing woodchip bioreactors to weekly, 8-h drying–rewetting (DRW) cycles increased NO\textsubscript{3} removal rates by 30 to 80% relative to constantly saturated conditions (Maxwell et al., 2018). Greater dissolved organic carbon (DOC) in the effluent likely explained removal increases, since aerobic breakdown should produce more labile C to be used during denitrification. The previous study only considered an 8-h duration DRW cycle. If periods in which woodchips are unsaturated increase subsequent release of labile C and NO\textsubscript{3} removal once woodchips are rewetted, longer periods of aerobic conditions should increase C release and NO\textsubscript{3} removal rates. The objectives of this study were (i) to determine if the duration of unsaturated conditions affects NO\textsubscript{3} removal rates and (ii) to qualify the relationship of DOC with NO\textsubscript{3} removal rates and its role in increased rates following DRW cycles. These research objectives were explored through a 105-d column experiment as a follow-up experiment to the previously published study (Maxwell et al., 2018).

**Abstract:** A previously reported experiment showed weekly drying–rewetting (DRW) cycles increase nitrate removal rates in woodchip-based denitrifying bioreactors. A follow-up experiment determined the effect of duration of unsaturated conditions on nitrate removal after rewetting. Three different levels of DRW duration were tested in a 105-d column experiment (n = 2), with woodchips left unsaturated once a week for either 2 h, 8 h, or 24 h. Increasing duration of unsaturated conditions significantly increased nitrate removal rates. The longest DRW duration of 24 h resulted in the greatest increase in nitrate removal rates, relative to constantly saturated woodchips, with mean rate increases reaching 172% by the end of the experiment. Results suggest nitrate removal in denitrifying bioreactors is carbon limited, with labile carbon made available during aerobic periods of DRW cycles the most likely cause of observed rate increases. Both studies show DRW cycles dramatically increase the nitrate removal efficiency of denitrifying bioreactors.

**Core Ideas**
- Nitrate removal in woodchips increased linearly with drying–rewetting duration.
- Nitrate removal increased up to 172% in the longest drying–rewetting duration.
- Nitrate removal rates increased proportionally with dissolved organic C leaching.

**Abbreviations:** BMP, beneficial management practice; DOC, dissolved organic carbon; DRW, drying–rewetting; SAT, saturation.
Materials and Methods

The previous 2017 experiment reported the effect of a weekly DRW cycle in which columns receiving a DRW cycle treatment received continuous flow, constant saturation outside of an 8-h period (Maxwell et al., 2018). A follow-up 2018 experiment presented in this study observed varying levels of DRW duration using weekly DRW cycles. Similar to the 2017 experiment, eight experimental units consisted of 15.2-cm-diameter, 96-cm-tall polyvinylchloride (PVC) columns filled with aged, coarse woodchips (2–5 cm diam.) collected from a 6-yr-old field bioreactor with carbon/nitrogen (C/N) ratio 48 to 54. The upflow columns consisted of woodchips above a gravel underbed with water entering the base of the column fed by an eight-channel ISMATEC peristaltic pump (0.68 ± 0.11 L h⁻¹, mean ± SD). Columns received an inflow of dechlorinated tap water spiked with KNO₃ (17.3 ± 0.6 mg NO₃⁻N L⁻¹), with laboratory temperatures varying between 18.7 and 26.6°C. A more complete description of the experimental set-up was previously reported (Maxwell et al., 2018). For comparison, the results of both the 2017 and 2018 experiments are discussed here and are hereafter referred to as DRW 2017 and DRW 2018, respectively.

The DRW 2017 experiment consisted of two treatments (n = 4): continuous flow, constant saturation (SAT), serving as the control, or continuous flow outside of weekly 8-h aerobic periods where columns were drained then refilled once flow was restarted (8-h DRW). In DRW 2018, the same eight experimental columns were assigned one of four treatments (n = 2): continuous flow, constant saturation (SAT) or one of three DRW treatments receiving a weekly DRW cycle in which DRW duration was 2, 8, or 24 h. Columns receiving the DRW treatment were left unsaturated only once a week for the respective duration, after which they were resaturated and given continuous flow, constant saturation until the next weekly DRW cycle. The 2 and 24 h DRW time periods were selected to compare the effects of shorter or longer DRW duration.

Stock tank and column outflow NO₃ and DOC concentrations were measured using a small volume multiplexed pumping system (MPS) and spectrophotometer (s::can spectro::lyser, Austria). As was previously reported (Maxwell et al., 2018), measurements of NO₃ and DOC concentrations were made on each column every 2 h continuously over the experiment, with over 21,000 data points collected in both studies. Laboratory samples were collected and analyzed for NO₃ concentration (USEPA Method 353.2, BAE Environmental Analysis Laboratory, North Carolina State University) and DOC concentration (USEPA Method 415.1, Teledyne Tekmar Apollo 9000, Mason, OH; 0.45-µm filter) at the beginning and end of each experiment to calibrate the spectrophotometer. Flow rates were measured by measuring volume of column outflow in a graduated cylinder two to three times per day.

Volumetric NO₃ removal rates (R_NO₃) for each column were the measured response variable and were calculated as the difference between column inlet NO₃–N concentrations and outlet NO₃–N concentrations multiplied by the flow rate and divided by the woodchip-filled column volume (0.009 m³), yielding units in grams of N per cubic meter per day. Emission or leaching rates of DOC (L_DOC) were calculated using the same method. In the results, mean R_NO₃ are aggregated into weekly periods according to number of previous DRW cycles (i.e., Period 3 includes the week following the third weekly DRW cycle and before the fourth DRW cycle.) Statistical significance of DRW duration effect was tested using repeated measures ANOVA analysis in SAS v. 9.3 (SAS Institute, 2011) using instantaneous measurements of R_NO₃ at 2-h intervals as the response variable (full SAS code is found in the Supplemental Material).

Results

The DRW 2018 experiment showed R_NO₃ increases positively with duration of the DRW cycle (Fig. 1). Volumetric NO₃ removal rates, R_NO₃, in DRW 2018 were lowest in SAT and 2-h DRW treatments and highest in the 24-h DRW treatment. Weekly mean R_NO₃ in the 33-d baseline period before initiating DRW cycles were 16.5 ± 3.7, 12.1 ± 3.8, 15.8 ± 3.7, 18.8 ± 4.4 g N m⁻³ d⁻¹ for SAT, 2-, 8-, and 24-h DRW groups, respectively. At the end of the experiment, during Period 11, weekly mean R_NO₃ for all groups were 7.6 ± 1.1,
9.4 ± 1.7, 12.9 ± 1.4, and 24.4 ± 4.0 g N m⁻³ d⁻¹. The ANOVA analysis showed that the effect of 8-h (p = 0.001) and 24-h (p < 0.0001) DRW cycles was statistically significant, relative to constantly saturated columns (Supplemental Table S1). The effect of 2-h DRW cycles was not significant (p = 0.389), suggesting 2 h is not sufficient time of aerobicisation to substantially influence NO₃ removal rates.

Nitrate removal rates in the 8-h DRW columns were 45 ± 13% greater relative to SAT columns in DRW 2018. This may be a consistent range of increase for this DRW duration under these conditions, considering Rₙo₃ was increased by 34 ± 8% in 8-h DRW columns in DRW 2017. Tripling the DRW duration in 24-h columns in DRW 2018 increased Rₙo₃ by a similar order, with mean Rₙo₃ over the entire experiment more than double that of SAT columns (113 ± 29% increase). During the week following the final DRW cycle (Period 11), weekly mean NO₃ removal in 24-h DRW columns was greater by 172%. Percentage increases in Rₙo₃, caused by DRW cycles at the end of the DRW 2018 experiment may be more reflective of long-term trends, since the ratio of weekly mean Rₙo₃ in DRW treatment columns, relative to SAT columns, increased up until 100 to 105 d after the first DRW cycle in both the 2017 and 2018 experiments.

The effect of DRW duration on Rₙo₃ was roughly linear ($R^2 = 0.70$) and followed the equation $R_{\text{NO}_3,\text{inc}} = 0.54 \times T_{\text{DRW}} + 11.74$, where $R_{\text{NO}_3,\text{inc}}$ is the increase in removal rates (g N m⁻³ d⁻¹) relative to SAT columns and $T_{\text{DRW}}$ is the length of the unsaturated period in hours. The DRW duration had a similarly linear effect on $L_{\text{DOC}}$ ($L_{\text{DOC},\text{inc}} = 1.12 \times T_{\text{DRW}} + 0.09$). It would be anticipated, however, that the relationship is only linear up to a certain limit of DRW duration; at sufficiently long conditions of drying, there may be insufficient water for woodchip breakdown or oversaturation of released DOC.

In all treatments (including SAT) in both studies, $R_{\text{NO}_3}$ was strongly correlated with $L_{\text{DOC}}$ (Fig. 2). In the DRW 2018 study, linear regressions between individual measurements of the two variables had $R^2$ of 0.97, 0.99, 0.84, and 0.83 for SAT, 2-, 8-, and 24-h DRW columns, respectively. Slopes of the linear regression across treatment groups in both DRW 2018 and DRW 2017 studies were 7.0 to 8.6. These results show that increased DRW duration led to greater DOC export, supporting the hypothesis that these aerobic periods release more labile DOC and help overcome the C limitation of denitrification believed to occur in woodchip bioreactors with recalcitrant, lignin-heavy media (Saliling et al., 2007; Cameron and Schipper, 2010; Feyereisen et al., 2016; Roser et al., 2018). Degradation of lignin by anaerobic respiration is negligible (Zeikus et al., 1982; Holt and Jones, 1983; Odier and Monties, 1983), with lignin breakdown primarily accomplished through aerobic microbes or fungi (Kirk and Farrell, 1987). By-products of aerobic breakdown of lignin can be more readily used during anaerobic respiration (Healy and Young, 1979; Colberg and Young, 1985). Increased time of aerobicisation led to greater labile DOC availability and leaching, with subsequently higher NO₃ removal. This is consistent with previous studies showing DRW cycles increased N₂O production and C mineralization (Groffman and Tiedje, 1988; Beare et al., 2009).

**Conclusions**

The data from this follow-up study give strong evidence that increased DRW duration increases NO₃ removal rates in denitrifying bioreactors, with removal rates in 24-h DRW columns nearly tripled by the end of the experiment. This challenges the traditional assumption that woodchip bioreactor design should maximize the time of saturation (within a desired range of hydraulic retention time) for greater NO₃ removal efficiency. To the authors’ knowledge, a method yielding such a dramatic increase in NO₃ removal rates in woodchip bioreactors without requiring additional energy or chemical inputs (e.g., C dosing, heating, electrical stimulation, etc.) has not been reported (Cameron and Schipper, 2011; Law et al., 2018; Roser et al., 2018). Further research is required to quantify total C loss from woodchips since DRW cycles will decrease woodchip lifespan. The results of this study pertain only to denitrifying bioreactors with woodchip fill media; it is possible that DRW cycles would not have as large an effect on NO₃ removal in bioreactors with more labile media (e.g., corn cobs, leaf litter) or fresh woodchips.

![Fig. 2. Nitrate removal rates ($R_{\text{NO}_3}$) plotted against dissolved organic C leaching rates ($L_{\text{DOC}}$) in all treatment groups in drying–rewetting (DRW) experiments in 2017 and 2018 (DRW 2017 and DRW 2018). Nitrate removal was strongly correlated with $L_{\text{DOC}}$ even in saturated (SAT) columns not undergoing DRW cycles.](image-url)
Supplemental Material
Supplemental material includes a table of the ANOVA output from SAS, testing the significance of the DRW treatment in each group.

Conflict of Interest
The authors declare no conflict of interest.

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