Biogeochemistry
https://doi.org/10.1007/s10533-023-01077-0

Rapid denitrification of nitrate-contaminated groundwater in a low-gradient blackwater stream valley

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Received: 28 April 2023 / Accepted: 31 July 2023
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Abstract Leaching of excess nitrogen (N) to groundwater in fertilized landscapes can overwhelm natural biogeochemical processes and cause long-term eutrophication of aquatic systems. We investigated N fate and transport from an intensively managed short-rotation woody crop (*Pinus taeda*) plantation through the riparian zone of an intermittent, low-gradient blackwater stream. Fertilization of the *P. taeda* plantation on the uplands resulted in contamination of groundwater with nitrate concentrations between 0.9 and 1.9 mg N L^{-1}. No corresponding increase in nitrate was observed in stream water or shallow groundwater in the riparian zone. Groundwater travel-time modeling predicted that N from near-stream, upland plantation areas should have reached streams during the monitoring period. Two years of measuring N species in well water in contrasting landscape positions (within the plantation, swale, riparian edge, forested hillslope, and valley), indicated rapid nitrate transformation and denitrification within the forested wetland valleys. Denitrification in the shallow groundwater system within the toeslopes and the riparian zone was estimated to have removed > 90% of nitrate. These results highlight the importance of riparian zones as pathways for the removal of N and for controlling downstream N loads.

Keywords Denitrification · Nitrate · Riparian buffer · Nitrogen removal · Southeastern US · Streamside management zone

This manuscript has been co-authored by UT-Battelle, LLC under Contract No. DE-AC05-00OR22725 with the U.S. Department of Energy. The United States Government retains and the publisher, by accepting the article for publication, acknowledges that the United States Government retains a non-exclusive, paid-up, irrevocable, world-wide license to publish or reproduce the published form of this manuscript, or allow others to do so, for United States Government purposes. The Department of Energy will provide public access to these results of federally sponsored research in accordance with the DOE Public Access Plan (http://energy.gov/downloads/doe-public-access-plan).

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Introduction

Excessive and inefficient fertilization of managed lands is polluting groundwaters and surface waters and contributing to river, lake, and estuarine

Published online: 26 August 2023

eutrophication (Howarth 2008; Robertson and Vitousek 2009; Withers et al. 2014; Wurtsbaugh et al. 2019). Of particular concern is the application of inorganic nitrogen (N) fertilizer, and the subsequent role of highly mobile nitrate in surface and groundwater contamination. In many agricultural settings, N is often a limiting nutrient for crop primary production (Gruber and Galloway 2008). When crop growth is limited due to depleted stocks of reactive nitrogen, N fertilizers can be applied in order to increase crop growth (Galloway et al. 2004; Pepper et al. 2015). When applied in excess, N fertilizer can be transported into surface waterways by groundwater or surface runoff pathways. These elevated N concentrations can lead to eutrophication of waterways, particularly estuaries (Howarth 2008; Vitousek et al. 2022), and lead to health issues for humans if drinking water is contaminated (Fields 2004; Robertson and Vitousek 2009).

The N cycle in terrestrial and aquatic ecosystems includes a denitrification pathway in which heterotrophic bacteria in the presence of organic carbon under anoxic conditions convert dissolved inorganic nitrogen, primarily nitrate, into gaseous forms of N that escape to the atmosphere (Schlesinger and Bernhardt 2013). Promoting denitrification in riparian zones, wetlands, and small streams is a key aspect of strategies to mitigate N pollution from managed lands (Lowe and Keenan 1997; Lowrance et al. 1997; Cooper et al. 2020; Lürling and Mucci 2020; Paerl et al. 2020) and reduce the spatial extent of coastal hypoxia (Rabalais et al. 2002, 2007; Rabalais and Turner 2019). While denitrification provides a way to mitigate elevated nitrate concentrations, when the denitrification process does not come to the end of a full cycle and produce its terminal product (inert N_2 gas), it can release intermediary products, namely nitric oxide (NO) and nitrous oxide (N_2O), which can be hazardous for the environment (Ravishankara et al. 2009; Welsh et al. 2021). A better understanding of riparian denitrification, including identifying spatial "hot spots" and temporal "hot moments", would help in the development of strategies to mitigate N pollution in waterways (McClain et al. 2003; Vidon et al. 2010; Senbayram et al. 2012, 2022; Musolff et al. 2016).

Literature reviewing denitrification in the terrestrial environment is extensive (Hill 2019; Krichels et al. 2022; Sigler et al. 2022; Chang et al. 2022), but there are few studies (Lowrance 1992; Lowrance et al. 1995; Groffman et al. 2006; Jahangir et al. 2013b; McAleer et al. 2017; Popp et al. 2020; Zhang et al. 2022) quantifying groundwater denitrification, especially at a watershed scale. However, we know that denitrification rates are often higher in deep groundwater than in surface water, making groundwater denitrification rates important to quantify when assessing N-cycling processes at a watershed scale (Seitzinger et al. 2006). This gap in the literature is primarily attributed to the difficulty that comes with measuring groundwater denitrification *in-situ*, as the process for attributing observed N_2 concentrations to ambient/background/atmospheric vs. denitrification-derived N_2 is complex (Groh et al. 2019; Lenhart et al. 2021).

Groundwater denitrification is driven by multiple factors that are reflected in measures of water chemistry (dissolved oxygen and dissolved organic carbon especially). Shallow, subsurface flow pathways through C-rich, low-oxygen riparian and hyporheic zones, such as those in blackwater systems, provide bacteria potentially ideal conditions for denitrification to occur. However, *in-situ* studies often find that denitrification rates are site specific (Rivett et al. 2008; Merill and Tonjes 2014), likely due to differing importance of denitrification drivers from site to site. For example, some studies have found that groundwater denitrification mitigated up to 30% of applied N (Jahangir et al. 2013b), while others report a wide range, from 4% to over 70% (Anderson et al. 2014b, a). Future studies that quantify groundwater denitrification rates *in-situ* will help to understand the site-specific conditions that drive denitrification and nitrate removal, and the extent to which denitrification can reduce nitrate concentrations in groundwater before reaching surface waters.

Watershed N budgets typically have unaccounted N, in that the amount of N that enters or is added to the system is not reflected in what is measured coming out (Thompson Tew et al. 1986; Meding et al. 2001; Fox et al. 2014; Hester and Fox 2020). This is especially true in watersheds that have been heavily fertilized. Denitrification has long been considered partially responsible, but *in-situ* rates have not been quantified at a watershed scale to assess whether it helps to cl ose the N budget and account for the "missing N" (David and Gentry 2000). To this aim, a recent multi-year, watershed-scale study of the environmental effects of a fertilized short-rotation pine

plantation observed a similar gap in the N budget (Griffiths et al. 2016, 2017), and raised questions as to the importance of denitrification in mediating losses of excess nitrate from the study watersheds.

Here we quantify denitrification rates and monitor water chemistry within an experimental headwater catchment of the Fourmile Watershed at the Savannah River Site (SRS) in the state of South Carolina, USA. This catchment is one of two that were experimentally manipulated to investigate the effects of intensively managed short-rotation pine (*Pinus taeda*) production on water quality (Griffiths et al. 2017). High fertilization rates led to elevated levels of NO_3^--N within the shallow groundwater for at least 7 years after treatment. In contrast, corresponding increases in streamwater or riparian groundwater nitrate were never observed within the treatment watersheds, and no clear changes in groundwater, riparian groundwater, or streamwater nitrate were observed in an adjacent reference (unmanaged) watershed. There are three possible explanations for this lack of increase in stream and riparian groundwater nitrate: (1) longer than expected groundwater travel times; however, previous studies (Jackson et al. 2014; Du et al. 2016; Vache et al. 2021) indicate median groundwater travel times are ~ 8–10 years, with near stream areas closer to 1–2 years, suggesting that sufficient time has passed for the contaminated groundwater to reach the streams; (2) denitrification in groundwater reduced nitrate concentrations before reaching the stream; (3) nitrate resides in the porewater of the vadose zone. Our objectives were to measure concentrations of gaseous and dissolved nitrogen species including N_2, N_2O, NO_3, and total N along the groundwater flow path from the upland plantation, through a topographic swale, to the toeslope, and into the valley, and also compare measurements under an unmanaged forest stand on the other side of the valley.

Methods

Study site

The study hillslope transect is along a first-order, low-gradient, intermittent blackwater stream that is tributary to Upper Fourmile Creek, Fourmile Creek, and the Savannah River. The basin is contained with the Savannah River Site, a National Environmental Research Park located within the sandhills region of the Upper Atlantic Coastal Plain, South Carolina, USA (Fig. 1). The study watershed (Watershed C) is one of 3 adjacent experimental watersheds (Watersheds R, B, and C) that have been extensively studied as part of a paired watershed investigation of intensive woody biomass production (Griffiths et al. 2019; Ferreira et al. 2020; Vache et al. 2021; Ruzol et al. 2022). These streams are groundwater-dominated (Jackson et al. 2014; Klaus et al. 2015; Du et al. 2016) and feature low nutrient concentrations (Griffiths et al. 2017) typical of blackwater streams in the region (Jager et al. 2011). Soils are highly permeable, consisting of loamy sand topsoils overlaying sandy clay loam argillic horizons above unconsolidated sands and clays. Annual precipitation on site ranged from 992 to 1538 mm over the long-term study, with an average of 1225 mm (Kilgo and Blake 2005). Annual evapotranspiration ranged from 304 to 1448 mm over the long-term study, with an average of 866 mm (Kilgo and Blake 2005; Caldwell et al. 2018). Average annual precipitation during this 2-year denitrification study was 995 mm, with the first sampling year receiving 947 mm and second sampling year receiving 1043 mm.

During 2012, approximately 50% of Watersheds C and B were harvested of mature pines, while Watershed R was left undisturbed. Surrounding the intermittent, low-gradients streams, a minimum 12.5 m undisturbed riparian buffer, but usually wider, was left intact on both sides of the stream channels. Buffer boundaries were marked at the transition from planted pines to bottomland hardwoods, so the buffers included a lowland hardwood overstory with a dense woody and herbaceous understory. Hydric soils characterized the riparian buffer.

Following site preparation and planting of loblolly pine seedlings in February—March 2013, the first fertilizer treatment was applied in April 2013. This first application consisted of 281 kg ha^{-1} of diammonium phosphate (50.6 kg N ha^{-1} and 56.2 kg P ha^{-1}). A second fertilizer treatment occurred in March 2014 using 241 kg ha^{-1} of urea (110.9 kg N ha^{-1}). A third treatment of blended urea and diammonium phosphate was applied in February 2015. The blend consisted of urea at 179.2 kg ha^{-1} (82.4 kg N ha^{-1}) and diammonium phosphate at 134.4 kg ha^{-1} (24.2 kg N ha^{-1} and 26.9 kg P ha^{-1}). The final

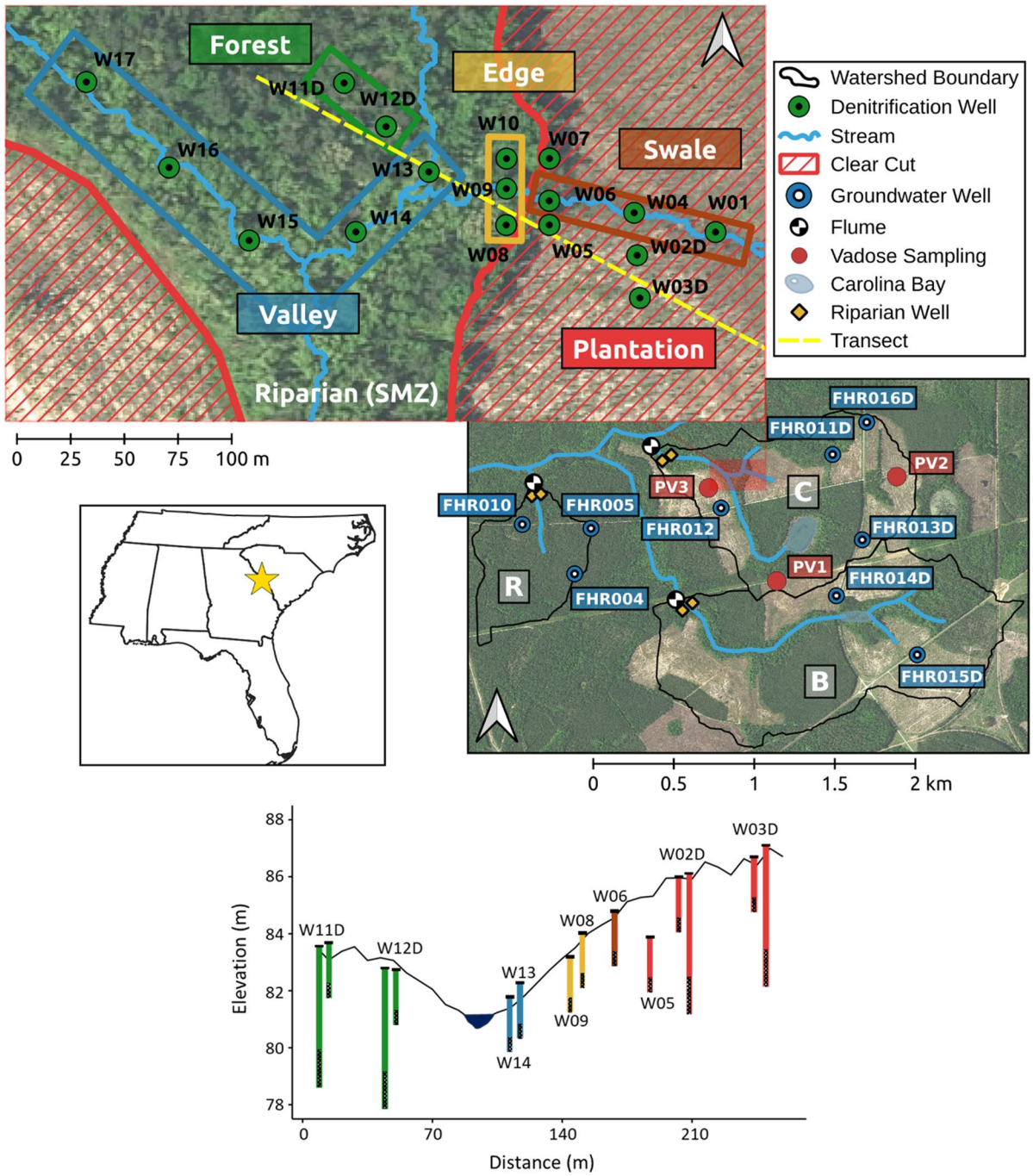


Fig. 1 Location of Savannah River Site, South Carolina, USA (yellow star; middle left); Upper Fourmile Experimental Watersheds (middle right) showing locations of vadose sampling, groundwater wells, Carolina Bay wetlands, stream flumes, and denitrification well transects (red box); and the denitrification study area in Watershed C (upper left) showing the locations of denitrification sampling wells in the plantation and swale, and edge, valley, and unmanaged forest of the riparian zone. Denitrification study transect and locations of denitrification wells within 12 m of the transect (bottom). Aerial imagery shown is April 2015 NAIP orthophotography

Table 1 Fertilizer application rates to Watershed C and Watershed B

Year	Rate of application (kg N ha^{-1})	Total applied (kg N)
2013	50.60	3.173
2014	110.9	6.953
2015	106.6	6.684
2016	196.0	12.289
Total	464.1	29.099

fertilizer treatment occurred in September 2016 and consisted of 425 kg ha^{-1} of urea (196 kg N ha^{-1}). These fertilizer applications were well in excess of timber industry standards and were designed to push the envelope for assessing best management practice (e.g., riparian buffer) effectiveness. In traditional operations, a single application of around 225 kg N ha^{-1} and 28 kg P ha^{-1} would be applied in a mid-rotation, between 5 and 10 years after planting (Fox et al. 2006; Coyle et al. 2013, 2016). Fertilizer application in 2013–2015 occurred via tractor, and in 2016 fertilizer was applied aerially. Fertilizer application is summarized in Table 1.

Instrumentation

Watershed nutrient measurement locations

Starting in 2011, 13 separate groundwater wells were installed across Upper Fourmile Creek (Fig. 1). Watershed R had 3 groundwater wells, Watershed B had 4 groundwater wells, and Watershed C had 6 groundwater wells. These 13 wells were installed in the uplands. Paired shallow (screened Sects. 13.0–19.3 m below soil surface) and deep (screened Sects. 25.6–43.6 m) wells were installed at 2 locations in both Watersheds B (FHR014 and FHR015) and C (FHR013 and FHR016). An additional deep well was installed in Watershed C (FHR011), but without a paired shallow well. Deep wells are identified with a 'D' suffix. All other wells had screening depths between 2.7 and 13.1 m.

Riparian groundwater wells were installed around May 2010. Two wells were installed per watershed near the watershed outlet. Riparian wells were installed in the riparian soils adjacent to stream channels (< 5 m on either side of stream) and screened to collect water 1.7 to 2.0 m below the surface. There were periods where the wells were dry, and not water was collected.

Streams were sampled at the outlet of each watershed. Samples were collected at the H-flumes where water level was measured at 15-min intervals for estimation of discharge.

Denitrification wells

From October 2016 through March 2017, 21 shallow wells were installed at 17 locations in Watershed C for water and gas sampling to estimate denitrification rates (Fig. 1). Shallow wells consisted of 0.6 m screens attached to a 1.5 m riser and deep wells consisted of a 1.5 m screen attached to 3 m risers. All wells were backfilled with filter packs and the top 20 cm sealed with bentonite. Paired deep wells were installed at 4 locations: W02, W03, W11, and W12. Depth to groundwater and groundwater elevation was recorded at each sampling event (DTG, GW_Z, respectively), and the change in depth to groundwater between sampling events was calculated (ΔDTG).

Wells were placed in transects along different landscape positions of a likely groundwater flow pathway (Fig. 1). These locations included: on a hillslope within a 4-year-old (at the time of initial sampling) short-rotation pine stand ('plantation'), along a drainage swale ('swale'), the boundary edge between the streamside management zone (SMZ) and plantation ('edge'), within the hyporheic zone of an intermittent stream valley ('valley'), and along a forested hillslope within the SMZ ('forest') (Fig. 1).

Collection and measurement of dissolved gases

The 17 denitrification wells were sampled monthly, except when dry, from March 2017 to March 2019 for water chemistry (temperature, pH, specific conductivity (SPC), dissolved oxygen (DO), oxidation–reduction potential (ORP)), dissolved gas concentration (N_2, Ar, and N_2O), and nutrients (total nitrogen (TN), nitrate (NO_3^--N), dissolved organic carbon (DOC), and ammonium (NH_4^+-N)). Wells were purged 24–48 h before each sampling event to ensure fresh and representative groundwater samples.

Collection and measurement of dissolved gases followed the protocol of comparable studies measuring denitrification (Groffman et al. 2006; Weymann

et al. 2008; Jahangir et al. 2013a; McAleer et al. 2017; Zhou et al. 2018; Zhang et al. 2022). N_2 concentrations were determined using the N_2:Ar method for measuring dissolved N_2 in solution using high-precision Membrane-Inlet Mass Spectrometry (MIMS). N_2O concentrations were determined using gas chromatography.

All dissolved gas samples were taken using a peristaltic pump at a rate of 90 mL min^{-1} to minimize ebullition. N_2 and Ar samples were collected by overflowing 12 mL exetainers with sampled groundwater, preserved with 0.2 mL of 50% $ZnCl_2$ solution, and capped with zero headspace. These vials were then inverted in 50 mL centrifuge tubes and stored in ice until returned to the lab. N_2 and Ar samples required no lab processing and were refrigerated between 4 and 7 °C until shipped for analysis.

N_2O samples were collected by overflowing 160 mL glass serum bottles. Serum bottles were then capped with butyl rubber septa and aluminum crimp caps, with zero headspace and stored on ice until sample processing. All N_2O samples were processed in preparation for analysis the next day following each sampling event. An inert headspace equilibration technique (3:1, He:H_2O) as outlined by Jahangir et al. (2012) was used to extract representative headspace samples for each sample to be analyzed for N_2O. Two, 2-inch hypodermic needles with stopcocks and silicone tubing attached, were inserted through a rubber septa into the serum bottle. Helium (He) was injected through one needle at 15–20 psi, while sample water flowed out the second needle and tubing into a graduated cylinder. As soon as a headspace formed in the bottles, stopcocks were closed and the serum bottles were then placed on a horizontal shaker (140 oscillations per minute) and left for 13 min. The sample bottles were then removed from the shaker and left to stand at room temperature for 60 min. 15 mL samples of the headspace were then extracted with a 20 mL syringe and injected into evacuated 12 mL exetainers. Until shipment to the analytical lab, headspace samples were stored at room temperature.

Hydrogeochemistry

Following the sample collection for dissolved gases in the denitrification wells, a 500 mL water sample was taken with the peristaltic pump for nutrient analysis. These samples were stored in acid-washed, HDPE bottles and placed on ice for processing and filtration. Immediately after sample collection and prior to placing samples on ice, all water chemistry parameters (temperature, pH, SPC, DO, and ORP) were measured *in-situ* using a handheld probe (YSI Quatro Cable ProPlus).

Deep and shallow groundwater wells across each watershed were sampled for nutrients and DOC monthly beginning in September 2011 and riparian wells monthly beginning in May 2010. A bailer was used to fill 500 mL acid-washed HDPE bottles from both the groundwater and riparian wells and immediately placed on ice for processing and filtration. In contrast to other sampling regimes, stream water was sampled weekly for nutrient analysis starting in January 2010. 500 mL acid-washed HDPE bottles were used to sample stream water.

Three samples were taken from each 500 mL aggregate sample: 60 mL sample stored in a HDPE bottle for total nitrogen (TN); 60 mL sample for NO_3^--N and NH_4^+-N stored in an HDPE bottle; and a 40 mL sample for DOC stored in an amber glass vial. The sample for TN analysis was left unfiltered. The NO_3^--N and NH_4^+-N sample was vacuum filtered (0.7 µm filter pore size; 45 mm GF/F GE Healthcare Whatman). The DOC sample was filtered and then preserved with 0.1 mL (2 drops) of 6N hydrochloric acid (HCl). TN, NO_3^--N, and NH_4^+-N samples were frozen (− 20 °C) until analysis, and the DOC sample was refrigerated until analysis (4—7 °C).

Plantation vadose water

At 3 locations (PV1—PV3; Fig. 1) within the plantations of Watershed C, soil sampling below the argillic Bt horizon every meter down to 5.1 m was conducted for the sampling of plantation vadose zone water from December 2019 to January 2020. Soil samples were collected using a combination of hand augers with extensions and through the use of a Giddings Machine 15-SCT GSRPST drill rig with a bucket auger attachment. Soil samples were immediately placed into Ziploc® double zipper freezer bags and stored in ice-filled coolers. In the lab, 12 mL Exetainer® vials (Labco Ltd, Lampeter, UK) were filled with the stored soil samples. Depending on water content, between 7 and 19 vials were filled for each depth sample to ensure enough water was

cryogenically extracted for nutrient analysis. Cryogenic vacuum distillation of the residual pore water was completed using an extraction setup based on the design of Koeniger et al. (2011) and Millar et al. (2018). Samples were placed in an aluminum heating block and connected to a second Exetainer vial via a stainless-steel capillary (2.00×0.95 mm). This second vial was set in a liquid nitrogen cold trap, and the aluminum block heated to 200 °C, keeping the samples under a baseline vacuum pressure of between 75 and 100 mTorr. Residual pore water was vaporized out of the sample vials and distilled into the collection vial within the cold trap. Samples were then placed back in the freezer until analysis for TN and NO_3^--N.

Sample analysis

Unfiltered TN samples were analyzed using the combustion oxidation and chemiluminescence detection method (Shimadzu TOC-L CHS/CSN analyzer and Shimadzu TOC-V). Filtered samples were analyzed for NO_3^--N using the cadmium-reduction method (SEAL Analytical AA3 autoanalyzer and Astoria Pacific AA2), and for NH_4^+-N using the phenol-hypochlorite method (SEAL Analytical AA3 autoanalyzer). Filtered and preserved samples were analyzed for DOC concentrations using the high-temperature combustion catalytic oxidation method (Shimadzu TOC-L CSH/CSN analyzer). N_2:Ar samples were analyzed using MIMS as described in Kana et al. (1994) and N_2O samples were analyzed using automated gas chromatography (GC) on a Shimadzu GC-14 GC system for greenhouse gas analysis.

N_2O and excess N_2 calculations

Dinitrogen produced from denitrification was calculated as N_2 in excess of the expected solubility of N_2 in water equilibrated with the atmosphere (Weymann et al. 2008). Dissolved N_2 in excess of water equilibrated with the atmosphere was assumed to be from denitrification occurring in groundwater (Weymann et al. 2008; Jahangir et al. 2013b; McAleer et al. 2017). This formulation of $Excess_{N_2}$ (Eq. 1) was originally derived by (Weymann et al. 2008) and has been used in previous *in-situ* denitrification studies.

$$Excess_{N_2} = Total_{N_2} - EA_{N_2} + EQ_{N_2} \quad (1)$$

In Eq. 1, $Excess_{N_2}$ reflects N_2 in excess of water in equilibrium with the atmosphere, $Total_{N_2}$ reflects the total amount of N_2 that was within the sample, EA_{N_2} reflects the N_2 from the dissolution of excess air, and EQ_{N_2} reflects the amount of N_2 in equilibrium with the atmosphere. Excess N_2 and Ar can dissolve into infiltrating water through the entrapment and dissolution of gas bubbles as water percolates through the unsaturated zone into the saturated zone (Heaton and Vogel 1981). We estimated the excess N_2:Ar using the known ratios of N_2:Ar present in the atmosphere, compared that with the Ar concentrations within our sample, and then compared that with the measured Ar concentration with water in equilibrium with the atmosphere at the sampling temperature (Eq. 2) (Heaton and Vogel 1981; Weymann et al. 2008). EA_{N_2} is calculated from Eq. 2:

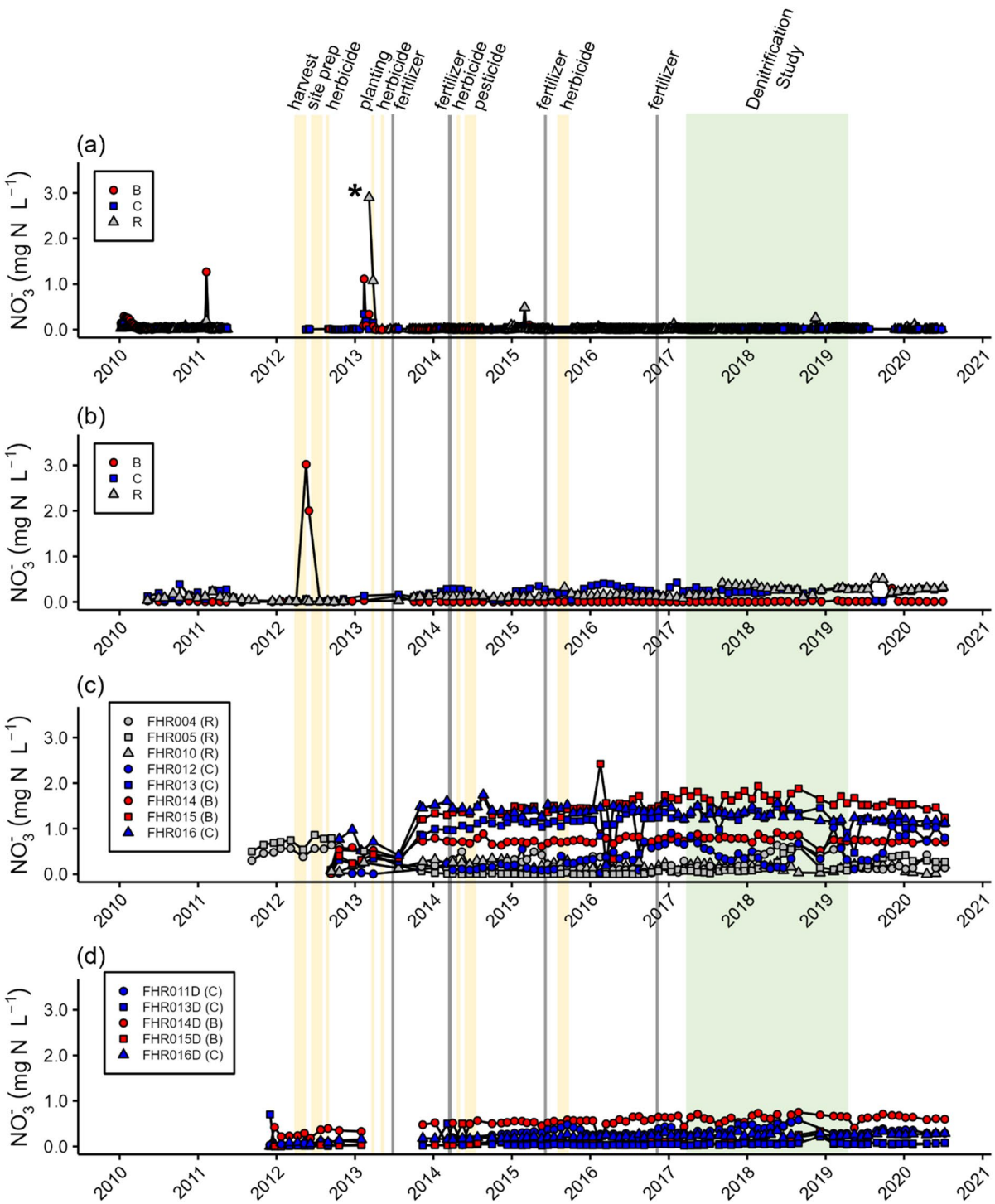
$$EA_{N_2} = \left(Total_{Ar} - EQ_{Ar}\right) * \frac{Atm_{N_2}}{Atm_{Ar}} \quad (2)$$

where $Total_{Ar}$ reflects the total Ar concentration in the sample, EQ_{Ar} reflects the Ar concentrations of water in equilibrium with the atmosphere at a given sampling temperature, and the ratio of Atm_{N_2} to Atm_{Ar} reflects the known ratio of N_2 to Ar in the atmosphere. Units for all parameters in Eqs. 1 and 2 are molar concentrations. $Excess_{N_2}$ was converted to mg N L^{-1} for all subsequent equations.

All forms of N presented below (Eqs. 3–6) are in units of mg N L^{-1}. There are many N cycling processes important in this system (DNRA, nitrification), but we focus in this paper on denitrification. We use the combined intermediary (N_2O) and terminal ($Excess_{N_2}$) end products of denitrification (EP) (Eq. 3), plus the measured amount of NO_3^- in groundwater to estimate the initial amount of nitrate in the system available for denitrification as outlined by Böhlke et al. (2002) and Weymann et al. (2008) (Eq. 4).

$$EP = Excess_{N_2} + N_2O \quad (3)$$

$$N_{initial} = NO_3^- + EP \quad (4)$$


harvest
site prep
herbicide
planting
herbicide
fertilizer
fertilizer
herbicide
pesticide
fertilizer
herbicide
fertilizer
Denitrification
Study
(a)
(b)
(c)
(d)
B
C
R
*
FHR004 (R)
FHR005 (R)
FHR010 (R)
FHR012 (C)
FHR013 (C)
FHR014 (B)
FHR015 (B)
FHR016 (C)
FHR011D (C)
FHR013D (C)
FHR014D (B)
FHR015D (B)
FHR016D (C)
NO3- (mg N L-1)
0.0
1.0
2.0
3.0
2010
2011
2012
2013
2014
2015
2016
2017
2018
2019
2020
2021

◂**Fig. 2** Nitrate (NO_3^-) concentrations over time in the study watershed (C) and in an adjacent treatment watershed (B) and reference (unmanaged) watershed (R). Nitrate concentrations are shown for: **a** streamwater; **b** riparian groundwater; **c** shallow groundwater; and (d) deep groundwater. The mean nitrate concentrations from the two riparian groundwater wells on each date are shown in (**b**). Yellow vertical bars show the timing of various silvicultural practices in Watersheds C and B. Grey vertical bars show the timing of fertilizer applications in Watersheds C and B. The green vertical bar shows the measurement period for the denitrification study. Watersheds are identified by color, and individual wells within a watershed are identified by marker shape. The asterisk (*) in **a** reflects a single streamwater sample taken in the reference watershed (R) that measured off the figure scale at 8.4 mg N L^{-1}

Reaction progress is a measure of the progress of the denitrification reaction or the extent to which nitrate is lost via denitrification and is estimated by comparing the products of denitrification to the estimate of initial nitrogen concentrations ($N_{initial}$) (Weymann et al. 2008; Jahangir et al. 2013b; McAleer et al. 2017). This formulation of RP is outlined by Weymann et al. (2008) and Zhou et al. (2018), and is based on Böhlke et al. (2002).

$$RP = \frac{EP}{N_{initial}} \tag{5}$$

Finally, we estimated the amount of incomplete denitrification using Eq. 6. We compared the ratio of the intermediary denitrification product (N_2O), with the sum of the combined end products of denitrification (EP; Eq. 3) (Weymann et al. 2008; Jahangir et al. 2013b; McAleer et al. 2017).

$$Ratio_{N_2O} = \frac{N_2O}{EP} \tag{6}$$

Statistics

We analyzed for differences in nutrients, dissolved gases, and denitrification parameters by landscape position and by sampling event (month). All collected data were tested for normality using a Shapiro-Wilks test. All datasets violated the assumptions of normality ($p > 0.05$), and as a result, only non-parametric statistics were used. Seasonality of dissolved gas, nutrients, and denitrification parameters was tested using a Kruskal–Wallis H test. Seasons were defined as Fall (September—November), Winter (December—February), Spring (March—May), and Summer (June—August). We compared the ranked sums between each landscape position using a Kruskal–Wallis H test. Annual changes of dissolved gases, nutrients, and denitrification parameters were tested using a Kruskal–Wallis H test. In cases where a statistically significant difference occurred ($\alpha = 0.05$), we further analyzed relationships using a Dunn test for multiple comparisons.

To analyze relationships between water chemistry parameters, nutrient concentrations, dissolved gas concentrations, denitrification measures, landscape characteristics, and hydrological characteristics, a Spearman correlation analysis was completed. Water chemistry parameters (temperature, ORP, pH, SPC, and DO), nutrient concentrations (DOC, NO_3^-, NH_4^+, TN), and hydrological plus landscape characteristics (DTG, ΔDTG, and GW_Z) were defined as site-specific environmental factors. Denitrification measures consisted of dissolved gas concentrations ($Excess_{N_2}$ and N_2O) and denitrification parameters (EP, RP, and $Ratio_{N_2O}$).

A principal component analysis (PCA) was conducted to investigate the different drivers of denitrification and how these varied by landscape position. We used all measured and calculated parameters in this analysis and ran separate PCAs by year.

We report adjusted *p*-values for any post-hoc analysis (i.e., Dunn test). Only complete observations where there were records of all measured parameters were used in statistical tests.

Results

Watershed nitrate

Prior to the fertilization, a regional drought caused the streams in the reference and both treatment watersheds to go dry. Treatment Watershed B and C were dry from mid-2011 through the summer of 2012 and reference Watershed R was dry from mid-2011 through the spring of 2013. Following the first application of fertilizer in April 2013, nitrate increased in shallow groundwater wells down slope of the application sites in both treatment Watersheds C and B (Fig. 2). In contrast, groundwater nitrate concentrations in the unmanaged Watershed R decreased or remained at background levels of < 0.5 mg N L^{-1}.

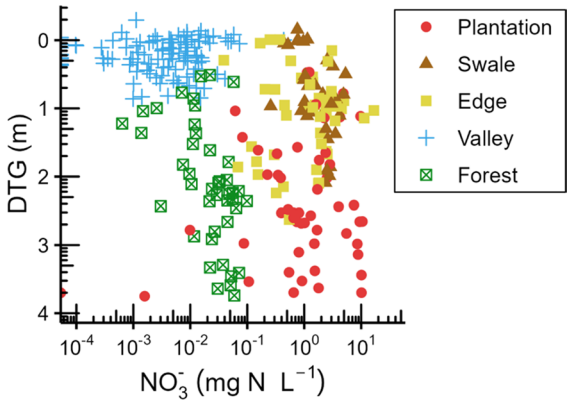


Fig. 3 Plot of depth to groundwater (DTG) in meters (m) vs. the log of nitrate (NO_3^-) concentrations (mg N L^{-1}). Marker shape and color identify landscape position

In the treatment watersheds, nitrate concentrations increased one to two orders of magnitude to just under 2.0 mg N L^{-1}, still under the EPA drinking water limit of 10 mg N L^{-1}. In contrast, there was no increase in nitrate concentration in riparian groundwater or in stream water in the treatment watersheds over the 7-year period after fertilization (Fig. 2).

Hydrology and hydrogeochemistry in denitrification wells

Water levels in the denitrification wells were at or near the surface within the valley for most of the study (Fig. 3). Depth to groundwater and variability thereof increased upslope (Fig. 3). The temperature of shallow groundwater across all landscape positions varied seasonally and ranged from 9.9 to 22.2 °C, with a median temperature of 16 °C. Groundwater in the swale was cooler than the other positions (Table 2). The forest, the only south-facing landscape position, had the highest measured groundwater temperatures. Groundwater in the catchment was relatively acidic, with the pH ranging from 3.7 to 6.9, with a median of 4.54. There was no significant variation in pH by landscape position ($p > 0.05$).

Groundwater dissolved oxygen varied substantially throughout the year, ranging from < 1 mg L^{-1} to 10.18 mg L^{-1}, with a median of 2.03 mg L^{-1}. Median concentrations in the valley (0.92 mg L^{-1}), were significantly lower than concentrations in all other landscape positions ($p < 0.001$). The highest median DO occurred in the plantation (3.43 mg L^{-1}). Specific conductivity ranged from < 10 µS cm^{-1} to as high as 140.7 µS cm^{-1} with a median of 31.6 µS cm^{-1}. Median specific conductivity was highest in the swale (39.4 µS cm^{-1}) and plantation (33.4 µS cm^{-1}). Oxygen reduction potential (ORP) ranged from 49.6 to 412 mV with a median of 266.9 mV. ORP was significantly lower (median of 198.2 mV) in the valley than any other landscape position ($p < 0.001$; Table 2).

Nutrient chemistry in denitrification wells

Groundwater NO_3^--N varied seasonally ($\chi^2 = 8.39$, $p = 0.039$, df = 3). Concentrations during the Spring were higher than the Fall ($p = 0.08$), and significantly higher than Winter ($p = 0.04$).

NO_3^--N concentrations varied significantly across landscape positions ($\chi^2 = 144.55$, $p < 0.001$, df = 4). NO_3^--N concentrations in the stream side management zone (SMZ) (valley + forest) were significantly lower than any of the other locations (Fig. 4a). Median NO_3^--N concentrations in the valley and forest were relatively similar (0.005 and 0.014 mg L^{-1}, respectively) (Table 2). Similarly, NO_3^--N concentrations in groundwater in the plantation, edge, and swale were similar as well (0.807, 1.261, and 1.123 mg L^{-1}, respectively). However, NO_3^--N concentrations varied from month to month and well

Table 2 Median values of measured parameters within the groundwater of the shallow denitrification wells by landscape position

	DTG (m)	DO (mg L^{-1})	ORP (mV)	pH	Sp. conductivity (µS cm^{-1})	Temperature (C)	DOC (mg L^{-1})	TN (mg N L^{-1})	NH_4^+ (mg N L^{-1})	NO_3^- (mg N L^{-1})
Plantation	2.19	3.43	296.1	4.55	33.4	16.2	1.34	0.964	0.034	0.807
Swale	0.58	2.27	301.1	4.75	39.4	13.6	0.98	1.124	0.018	1.123
Edge	0.99	2.20	317.5	4.40	32.0	16.3	1.02	1.188	0.021	1.261
Valley	0.16	0.92	198.2	4.58	29.8	15.5	2.87	0.134	0.034	0.005
Forest	1.89	2.89	256.8	4.50	29.6	17.5	1.24	0.096	0.015	0.014

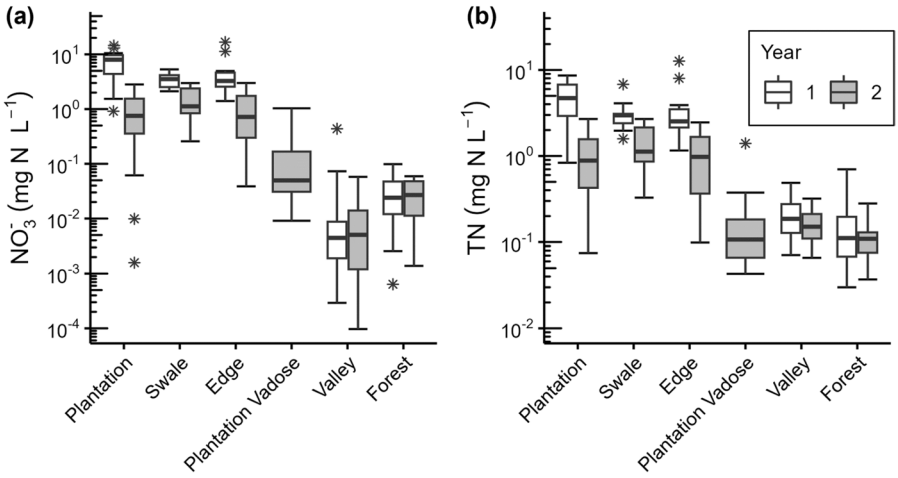


Fig. 4 **a** NO_3^--N concentrations by landscape position and sampling year. The y-axis is on a log scale. **b** Total nitrogen (TN) concentrations by landscape position and sampling year. The y-axis is on a log scale. Boxes represent the interquartile range (IQR) for each landscape position and year, with the edges representing the 25th and 75th percentiles. Whiskers represent 1.5 times the upper and lower limits of the IQR. Median values are represented by the bar across the IQR. Outliers are defined as values outside 1.5 times the limits of the IQR. Outliers are marked with an asterisk (*)

to well, and NO_3^--N concentrations were observed above the US EPA standard of 10 mg N L^{-1} three times in the plantation (10.5, 10.1, and 10.1 mg L^{-1}) and twice in the edge location (16.7 and 11.3 mg L^{-1}). There was a statistically significant reduction ($p<0.001$) in NO_3^--N concentrations from Year 1 to Year 2 of monitoring in all topographic locations outside of the SMZ (Fig. 4a). Median concentrations of NO_3^--N in the plantation dropped almost an order of magnitude, from 8.61 to 0.76 mg L^{-1}. Concentrations in the swale were reduced from 3.19 to 1.02 mg L^{-1}, and in the edge from 2.85 to 0.89 mg L^{-1}. NO_3^--N concentrations of depth-integrated samples of plantation vadose water during Year 2 plotted between NO_3^--N concentrations in groundwater in the edge and valley locations (Fig. 4a).

Groundwater TN concentrations also varied seasonally ($\chi^2 = 7.884$, $p=0.0485$, df = 3). TN concentrations during the Spring were higher ($p=0.1$) than in the Winter.

TN concentrations within the forest and valley were significantly lower than all upslope locations ($\chi^2 = 134.62$, $p<0.001$, df = 4) (Fig. 4b). Median concentrations in the valley (0.134 mg N/L) and forest (0.096 mg N/L) did not differ. There was a significant reduction ($p<0.001$) in TN concentrations from Year 1 to Year 2 in all locations outside of the SMZ, as was the case for NO_3^--N (Fig. 4b). Like NO_3^--N, plantation vadose water TN concentrations plotted lower than the edge wells, but more similar to valley TN concentrations than was the case with NO_3^--N.

Groundwater NH_4^+-N varied seasonally ($\chi^2 = 10.06$, $p=0.018$, and df = 3), with concentrations in Spring lower than in the Fall ($p=0.07$) and Summer ($p=0.07$).

NH_4^+-N concentrations varied significantly across landscape positions ($\chi^2 = 20.65$, $p<0.001$, df = 4). NH_4^+-N concentrations in the plantation and valley were significantly greater than in the swale or forest (Table 2). NH_4^+-N concentrations were significantly greater in Year 2 than in Year 1 of monitoring at all locations.

Groundwater DOC varied seasonally ($\chi^2 = 12.78$, $p=0.005$, df = 3). DOC concentrations in the Fall were significantly higher than in the Summer or Spring. DOC concentrations also varied significantly across landscape positions ($\chi^2 = 90.51$, $p<0.001$, df = 4). DOC was significantly higher in the valley (2.87 mg/L) than in any other location (Table 2). There was no significant change in shallow groundwater DOC from Year 1 to Year 2.

Table 3 Median, minimum, and maximum measured values for denitrification reaction terminal ($Excess_{N_2}$) and intermediate (N_2O) end products, end product ratio ($Ratio_{N_2O}$), and reaction progress (RP)

Landscape position	*n*	$Excess_{N_2}$(mg N L^{-1})			N_2O (mg N L^{-1})			$Ratio_{N_2O}$			RP		
		Median	Min	Max	Median	Min	Max	Median	Min	Max	Median	Min	Max
Plantation	37	0.23	0.02	0.74	1.92	0.39	22.13	0.92	0.60	0.99	0.75	0.27	0.97
Swale	23	0.26	0.09	0.88	1.25	0.55	6.54	0.85	0.54	0.97	0.58	0.24	0.80
Edge	39	0.32	0.09	0.81	1.95	0.28	33.80	0.90	0.31	0.98	0.69	0.26	0.97
Valley	67	0.14	0.01	0.64	0.23	0.10	4.02	0.64	0.16	0.97	0.99	0.89	1.00
Forest	24	0.24	0.02	0.50	0.25	0.14	1.33	0.56	0.33	0.93	0.97	0.80	0.99

The number of samples '*n*' varied by landscape position based on the number of sampling wells (Fig. 1) and variation in the number of wells that were dry over time

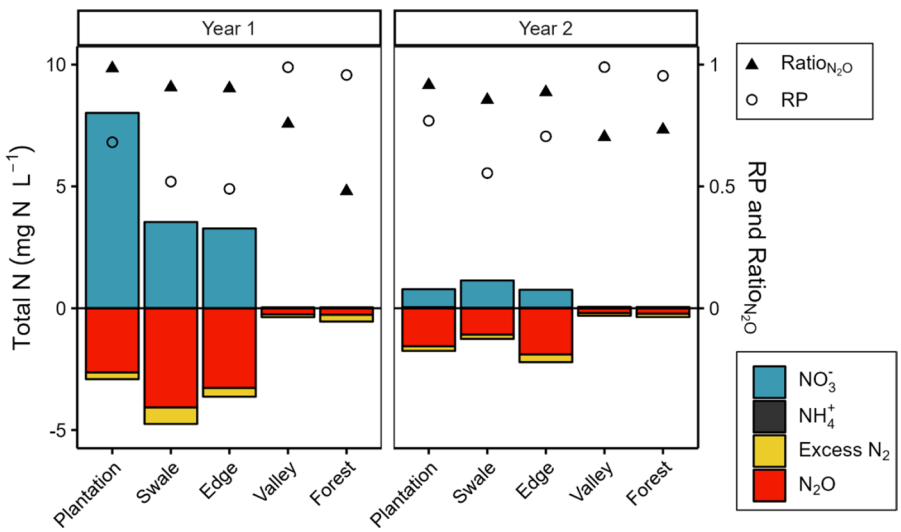


Fig. 5 Median concentrations of terminal ($Excess_{N_2}$) and intermediary (N_2O) denitrification end products, as well as NO_3^--N and NH_4^+-N, represented as stacked bars. Reaction progress (RP; Eq. 5) and the ratio of end products ($Ratio_{N_2O}$; Eq. 6) are represented by circles and filled triangles, respectively, on the secondary y-axis. Denitrification end products ($Excess_{N_2}$ and N_2O) are shown as negative values as they represent forms of N that are lost from the watershed

Dissolved gases and denitrification products

Valley and forested hillslope locations had significantly lower concentrations of N_2O than other landscape positions ($p < 0.001$, Table 3, Fig. 5). N_2O was the primary form of reduced N within Watershed C when compared to N_2 (Table 3). Median measures of the $Ratio_{N_2O}$ (Eq. 6) ranged from 0.56 and 0.64 in the forest and valley respectively, to 0.92 and 0.90 within the plantation and edge, respectively. Reaction progress (RP) varied by landscape position (Table 3). Median estimates of RP in the valley and forest were high, 0.99 and 0.97 respectively. RP values outside the SMZ ranged from 0.58 in the swale, to 0.69 and 0.75 in the edge and plantation, respectively. RP values outside of the SMZ were significantly lower than inside of the SMZ ($p < 0.001$, Table 3).

N_2O was the primary denitrification end product across all topographic positions (Fig. 5). Median N_2O production in upslope locations was much greater in Year 1 (2.9–4.9 mg N L^{-1}) than in Year 2 (1.5–2.5 mg N L^{-1}) (Fig. 5). Median $Ratio_{N_2O}$ increased in the forest from Year 1 (0.45) to Year 2 (0.73) (Fig. 5).

Correlations and principal components: gases, nutrients, hydrogeochemistry, and hydrology

N_2O concentrations were positively correlated with ORP ($\rho = 0.53$), DTG (0.45), and GW_Z (0.55) negatively correlated with DOC (-0.44) and had a strong, positive correlation with TN (0.7) and NO_3^--N (0.78). EP similarly was positively correlated with ORP (0.55), DTG (0.45), and GW_Z (0.56), and negatively correlated with DOC (-0.45), and had strong positive

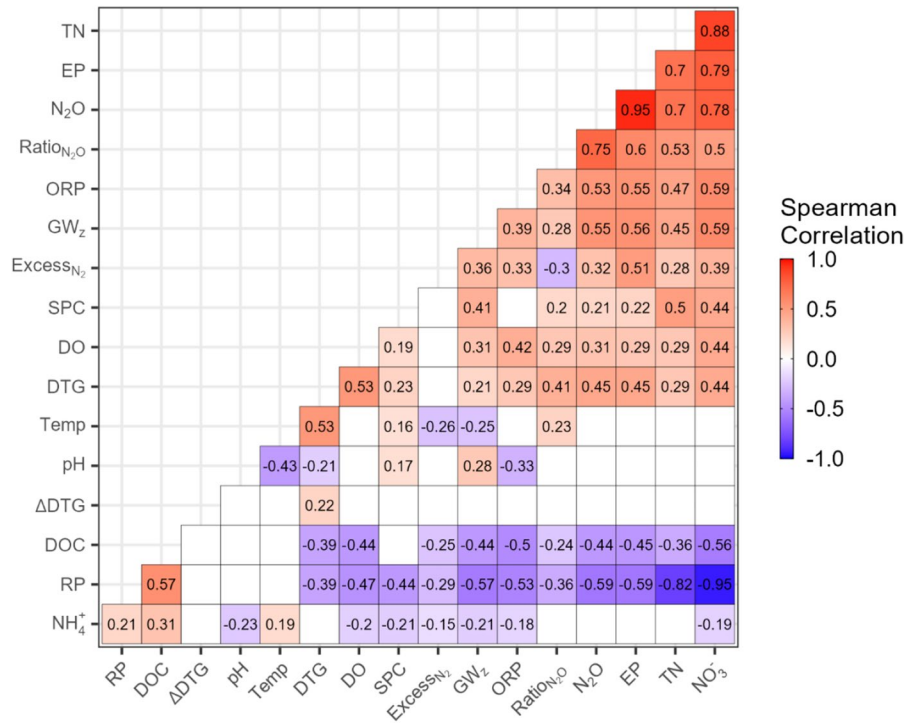


Fig. 6 Plots of **a** nitrous oxide (N_2O) vs. nitrate (NO_3^-) concentrations on a log–log scale; **b** nitrous oxide (N_2O) vs. ammonium (NH_4) concentrations on a log–log scale. All nutrient and dissolved gas concentrations in mg N L^{-1}. Marker shape and color identify landscape position

correlations with TN (0.70) and NO_3^--N (0.79). NO_3^--N was positively correlated with GW_Z (0.59) DO (0.44), SPC (0.44), ORP (0.59), DTG (0.44), and had a negative correlation with DOC (− 0.56). RP was positively correlated with DOC (0.57) and had a negative correlation with DO (− 0.47), SPC (− 0.44), GW_Z (− 0.57), and ORP (− 0.53). Looking at scatter plots of N_2O with NO_3^--N and NH_4^+-N (Fig. 6), as well as NO_3^--N with DTG (Fig. 3), we can see distinct groupings based on landscape position.

During Year 1, the two largest principal components together explained 54.5% of the variation in denitrification and associated parameters (Fig. 7a). RP, SPC, NO_3^-, and N_2O contributed significantly to PC1 (40.6% of variation), while DO, DOC, NH_4^+, and DTG contributed significantly to PC2 (13.9% of variation). There were distinct differences in landscape position, with the valley and forest separated from the areas outside the SMZ, with similar orientations. The patterns in Year 2 varied from Year 1 (Fig. 7b). The two largest principal components together explained 41% of the variation between water chemistry, hydrology, and denitrification parameters. RP, NO_3^-, $Ratio_{N_2O}$, and DTG contributed significantly to PC1 (25.9% of variation), while $Excess_{N_2}$, pH, and temperature contributed significantly to PC2 (15.1% of variation). There were again distinct differences by topographic position, with the valley and to a lesser extent the forest grouped away from the positions outside the SMZ. We see that in both years, DOC, NH_4^+, and RP differentiate the separation between the valley and forest (SMZ) and the rest of the landscape.

Discussion

Nitrogen gradients along a hillslope

Nitrogen concentrations, speciation, and dynamics differ substantially across topographic positions reflecting differences in biogeochemical controls. In particular, the valley and forest locations are similar to one another and distinct from the fertilized and upslope locations (plantation, swale, and edge). Reductions in nitrogen concentrations from Year 1 to Year 2 could be due to a decrease in the overall fertilizer signature, though we do not see that in the long-term monitoring (Fig. 2). Differences in moisture conditions between the years is another possibility to explain the interannual

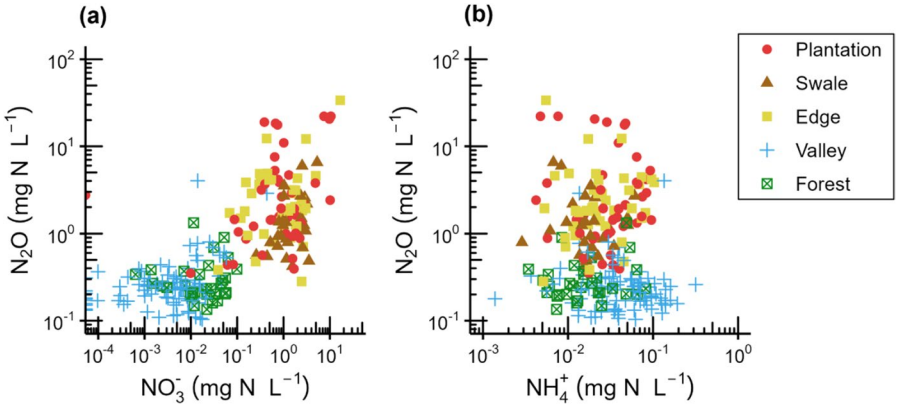


Fig. 7 Spearman correlation matrix showing significant correlation coefficients ($p < 0.05$). Non-significant correlations are blank ($p \geq 0.05$)

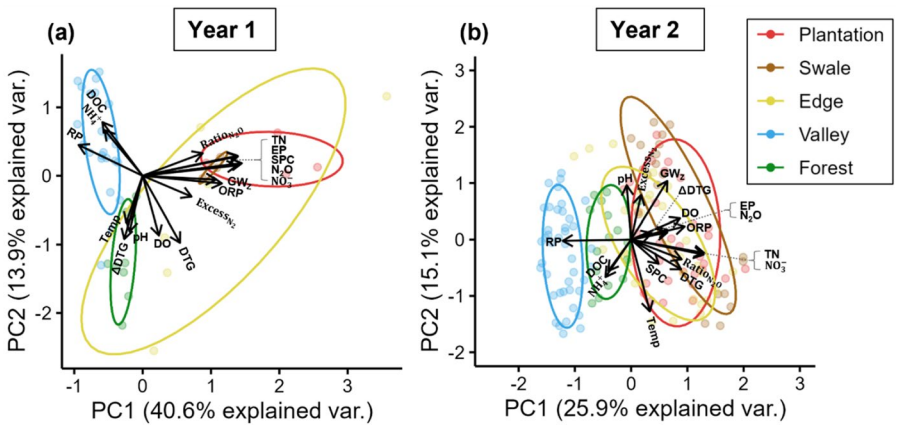


Fig. 8 Principal component analysis of all measured and calculated parameters (water chemistry, nutrient concentrations, dissolved gas concentrations, and denitrification parameters) grouped and colored by landscape position for Year 1 of monitoring (**a**) and Year 2 (**b**)

variability of nitrate, though here we have comparable precipitation year to year (947 vs 1043 mm). NO_3^--N concentrations in valley groundwater (median = 0.005 mg N L^{-1}) were three orders of magnitude less than in groundwater below the plantation (median = 0.807 mg N L^{-1}), and TN in the valley groundwater (median = 0.134 mg N L^{-1}) was one order of magnitude less than in the plantation (median = 0.964 mg N L^{-1}), indicating a substantial loss of N as groundwater moves through the toe slopes and valley. The decrease in NO_3^--N is likely greater than the decrease in TN because one component of TN is dissolved organic matter which is greater in the valley than upslope positions. We know that nitrate availability is one of the primary drivers in differentiating among landscape positions and is an important component in driving incomplete denitrification (Figs. 6, 7 and 8). Upland locations where fertilizer was applied had higher DO and lower DOC, with much higher ORP values when compared to the valley (Table 2). This further supports the observed gradient of nitrate and net denitrification processing across the hillslope and indicated that these parameters are strongly controlled by site-specific environmental factors (e.g., DO, DOC, temperature, pH, ORP).

Inhibition of complete denitrification

Nitrous oxide (N_2O), the intermediate product of denitrification, was the dominant form of reduced nitrogen within our study area. High N_2O concentrations may reflect coupled nitrification–denitrification processes or environmental conditions (low DOC, high dissolved oxygen, low pH, low conductivity, short residence times) unsuitable for complete denitrification. We found higher concentrations of N_2O in upland locations. Shorter and intermediate groundwater residence times can be associated with incomplete denitrification reactions, and result in more N_2O

production (Jurado et al. 2017; Reeder et al. 2018; Nogueira et al. 2021; McEachran et al. 2023), but our upland locations are still within 150 m of the stream. Based on the close proximity of the denitrification wells to one another and the stream itself, we can speculate that groundwater residence times may not be the driving factor of N_2O production at our site, but it is rather the differences in environmental conditions driving the production of N_2O. In the plantation areas where N_2O production was high, there was higher DO and lower DOC than the valley and forest locations where N_2O production was low.

Environmental conditions ideal for preventing complete denitrification are likely driving high N_2O production. We found that RP was positively correlated with DOC and negatively correlated with DO, conductivity, GW_Z, and ORP. Acidic soil and groundwater are known to prevent complete denitrification, resulting in the production of more N_2O (Nagele and Conrad 1990; ŠImek and Cooper 2002; Jurado et al. 2017). The study catchment drains a blackwater stream with relatively low pH (4–5). There was not a significant relationship between pH and N_2O, but that is likely due to the narrow range in observed pH values throughout the study. Median pH was 4.5, with 83% of pH measurements falling between 4 and 5. Dissolved oxygen was much lower in the valley than any other landscape position, with median concentrations around 1 mg L^{-1}. Median concentrations in all other locations were between 2.2 and 3.4 mg L^{-1}. Conditions for complete denitrification typically require DO levels to be less than 2 mg L^{-1} (Jahangir et al. 2013b). Higher levels of DO upslope reflect a thicker vadose zone, and with that thicker vadose zone, a greater change in groundwater levels (ΔDTG) between sampling points introducing more air into groundwater. DO was positively correlated with depth to groundwater (DTG) and N_2O. Excess nitrate can limit complete denitrification, as microbes will use nitrate for respiration, instead of further reducing N_2O into N_2 (Rivett et al. 2008; Jurado et al. 2017; Zhou et al. 2018). Low pH in the presence of high levels of nitrate has been shown to inhibit the reduction of N_2O to N_2 (Rivett et al. 2008; Zhou et al. 2018).

When observations of N_2 fell below that of water in equilibrium with the atmosphere, calculated excess N_2 values were negative. This occurred in 11.3% of potential N_2 samples, even when we set our pumping rate at 90 mL/min to discount the effects of ebullition. These samples were excluded from analysis due to the concerns of degassing and ebullition. Negative concentrations of excess N_2 can reflect degassing or ebullition during pumping. Degassing can occur from oversaturation of dissolved gases from gas producing processes (i.e., denitrification), or by the stripping of N_2 from other gas production procedures like methane. Ebullition during peristaltic pump operation is a concern when using pumping rates over 100 mL/min (Blicher-Mathiesen et al. 1998; Weymann et al. 2008; Jahangir et al. 2013b; Fox et al. 2014; Hester and Fox 2020).

Separating nitrate-reducing processes

Because of the combination of multiple nitrate-reducing processes at play, it is difficult to separate which process is primarily contributing to N_2O production. The measurements of dissolved gases are estimates of net denitrification; we can only look at the driving or inhibiting factors of denitrification to define these different processes among landscape positions.

Transformation of nitrate to ammonium through dissimilatory nitrate reduction (DNRA) will produce N_2O, though typically only when nitrate levels are low (Rivett et al. 2008). Ammonium increased between the SMZ edge and the valley, where nitrate concentrations were lowest. The valley had much higher DOC and lower ORP values, which would benefit the DNRA process. However, ammonium concentrations were not significantly correlated with N_2O, and we did not find increasing N2O concentrations with ammonium reduction, which would indicate nitrification is occurring in the valley rather than DNRA. There is evidence at our site that N cycling processes (nitrification and denitrification) are coupled and driven by seasonal changes in water chemistry, especially with regard to water temperature (Griffiths et al. 2016, 2017). Changes to temperature can regulate the availability of microsites for denitrification or nitrification, due partly to changes in oxygen conditions (Griffiths et al. 2016).

Nitrification in the vadose zone could be occurring at the boundary between the unsaturated soil and water table. This process would produce N_2O when ammonium is oxidized to nitrate. In environments with high concentrations of organic matter,

high availability of ammonium, low pH, and high DO, the potential increases for this process to produce substantial amounts of N_2O (Wrage et al. 2001; Strauss et al. 2002; Marchant et al. 2016). The hydrology and environmental conditions of Watershed C fit those conditions where nitrification can play an outsized role in N_2O production. Further, coupled nitrification–denitrification can occur at the boundary between oxic and anoxic conditions, where there are persistent fluctuations in DO, and a ready supply of DOC and nitrogen.

Comparison to other studies of reaction progress

Higher reaction progress (RP) in the SMZ (valley and forest) corresponded with significant reductions in NO_3^--N and TN concentrations in these zones compared to upland locations. This is evidence of high rates of denitrification occurring within the SMZ, which is converting large loads of nitrate in hyporheic flowpaths of riparian groundwater into N_2O and N_2.

It is difficult to compare these estimates of RP with those of other studies due to differences in methods used to measure denitrification. This applies even to other studies which use the same N_2:Ar technique, as there are site and sample-specific factors to consider (e.g., sampling equipment and operation (pump rates), calculations of atmospheric contributions). These comparisons are also made difficult by differences in how $N_{initial}$ is formulated. Some studies are interested in nitrate reduction exclusively, keeping $N_{initial}$ and subsequent RP calculations as Böhlke et al. (2002) first presented, which combines NO_3^- concentrations and denitrification end product concentrations to calculate $N_{initial}$. We used the Böhlke et al. (2002) approach in our study. Others have incorporated TN, DON, NO_2, and other forms of nitrogen into the $N_{initial}$ calculation because of interests in gas production and separating individual N-reducing processes and intermediary denitrification components.

Keeping in mind the differences between studies, there are similar studies which have directly measured $Excess_{N_2}$ and calculated RP as an estimate of *in-situ* denitrification. Jahangir et al. (2013b) quantified denitrification and denitrification gas production in shallow groundwater at four agricultural sites, quantifying $N_{initial}$ based on N_2, N_2O, NO_2, NH_4^+, NO_3^-, and DON. Jahangir et al. (2013b) found mean RP of 46–77% at sites with low permeability, and 4–8% at sites with high permeability, while noting a maximum reaction progress between 97 and 99% in some of the low permeability sites. Weymann et al. (2008) calculated RP at four nitrate-contaminated watersheds in northern Germany using a conservative approach of Böhlke et al. (2002) by coupling it with additional measurements of intermediary denitrification (N_2O concentrations). Weymann et al. (2008) reports median RP values ranging from 33 to 68% (across sites), with ranges from 0 to 100%. McAleer et al. (2017) measured denitrification in deep and shallow groundwater of two agricultural catchments, one with slate bedrock and the other with a sandstone bedrock using the approach of Jahangir et al. (2013b). McAleer et al. (2017) found denitrification RP of 0–32% in the slate catchment, and 4–94% in the sandstone catchment. Zhou et al. (2018) investigated denitrification rates in three distinct agricultural settings in eastern China using the approach of Böhlke et al. (2002). They found that groundwater denitrification can consume 65–83% of leached nitrate. Zhang et al. (2022) present RP in terms of N removal efficiency and found that shallow groundwater rates ranged from 14 to 35%, noting the high variability among sites, and the importance of supplies of carbon and nitrate on the estimate. Zhang et al. (2022) modifies the approach of Böhlke et al. (2002) and substitutes TN for NO_3^-. For this study of a low-gradient, blackwater system, we found median RP values between 58 and 99% (among landscape positions), with maximums between 80 and 100%, which is within the ranges reported from the aforementioned studies. Median values of RP in the riparian areas important for nitrate removal were between 97 and 99%.

Conclusions

The maintenance of a forested SMZ encompassing the stream valley and toeslopes supported conditions for the rapid transformation and denitrification of excess nitrate being transported in groundwater from a fertilized pine plantation to the adjacent stream. We observed distinct gradients of N forms across hillslope positions from the pine plantation, downslope to the near-stream valley. Net denitrification was estimated to reduce 97–99% of nitrate in the shallow groundwater system within the near-stream valley, in contrast with upland locations which were

reducing between 58 and 75% of nitrate. Complete denitrification is not occurring at upslope locations due in part to nitrate excess, as well as several environmental factors (e.g., high DO, low pH, large changes in groundwater level) that are likely allowing for N_2O, rather than N_2, production. We see evidence of this at upslope locations where the intermediate product of denitrification, N_2O, contributes between 85 and 92% to the total dissolved gas pool, while in the forested valley N_2O only contributes between 56 and 64%.

The presence of an SMZ in this southeastern US Coastal Plain watershed provides an effective buffer in efforts to help mitigate the impacts of excess N fertilizer application, as the C-rich, low DO, warm conditions in the buffer are conducive to biogeochemical processes which aid in losses of nitrate from groundwater before it reaches surface waters. Runoff reduction from vegetation, vegetative and microbial uptake of N, dilution and dispersion of N to the vadose and groundwater flow paths, and denitrification are all processes which benefit from the presence of a riparian SMZ (Ice et al. 2010; Merill and Tonjes 2014; Griffiths et al. 2019). We found that inclusion of an SMZ in the management practices of a short-rotation pine plantation with low N fertilizer-use efficiency (Ferreira et al. 2021) effectively helped protect stream water from N pollution, despite evidence of nitrate concentrations in the surficial aquifer that were much higher than in the stream (Griffiths et al. 2019, Fig. 2).

Atmospheric contamination, sample degassing, site-specific environmental conditions, and multiple biological processes and reactions producing gaseous N all add difficulty in measuring denitrification *in-situ*, as well as comparing denitrification rates between studies. These factors can make it difficult to understand and estimate the extent and magnitude of denitrification occurring from groundwater. Regardless, there is a need to understand and estimate the amount of N removal occurring in groundwater systems with consideration to the amount of N-heavy fertilizer that is applied in agricultural settings. Forested riparian buffers were effective at mitigating excess nitrate leaching impacts on surface water, but incomplete reaction progress along the flow path may lead to large N_2O production. Increased N_2O production can be hazardous for the environment, as N_2O is a potent greenhouse gas with a warming effect more than 300 times that of carbon dioxide (CO_2) and a residence time in the atmosphere of over 100 years (Ravishankara et al. 2009; Wang et al. 2017).

Acknowledgements Peter Groffman and Lisa Martel of the Cary Institute provided advice and guidance throughout the project and conducted the N_2 and N_2O analysis. Andy Horcher and John Blake (retired) provided logistical and in-kind organizational support. Kevin Fouts, Nathan Melear, and Katie Lamp'l helped in sampling the 2nd year of the project and in well installation and sample processing. Christopher Lewis, Nathan Melear, Wesley Gerrin, Tyler McIntosh, Gabriel William Dias Ferreira, and Fabio Henrique Silva Floriano de Toledo assisted in drill rig operation and in augering plantation soil samples. Seth Younger, Katie Lamp'l, and Jordan Horvieth assisted in the cryogenic extraction process. Dan Buhr assisted in the processing of N_2 and N_2O data. Lori Sutter at UGA, and Allison Fortner, Jana Phillips, and Sarah Ottinger at ORNL conducted the water chemistry analysis.

Author contributions All co-authors contributed to the study. SR wrote the initial draft. JJ wrote an initial manuscript outline. SR, NAG, BMR, and CRJ analyzed data. SR, JJ, NAG, BMR, CM, and CRJ NAG contributed to the study design. SR, JJ, CM, and CRJ conducted or facilitated field work. SR, NAG, BMR, and CRJ facilitated manuscript editing and review.

Funding This work was supported by the United States Department of Energy Bioenergy Technologies Office and the United States Forest Service—Savannah River Site Interagency Agreement DE-AI09-00SR22188. S. Raulerson was supported by the United States Department of Agricultural through a National Institute of Food and Agriculture AFRI Predoctoral Fellowship (2022-67011-36463/1028181). This material is based on work supported by the U.S. Department of Energy, Office of Energy Efficiency and Renewable Energy, Bioenergy Technologies Office. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. Department of Energy under contract DE-AC05-00OR22725.

Data availability The data from this manuscript will be freely available upon request, as well as through the Bioenergy Knowledge Discovery Framework (https://www.bioenergykdf.net/).

Declarations

Conflict of interests The authors have no relevant financial or non-financial interests to disclose.

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