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SPATIAL AND TEMPORAL CONTROLS ON DENITRIFICATION IN AN URBAN RIPARIAN ZONE

A Thesis in

Ecology

by

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ABSTRACT

Humans have drastically altered the nitrogen cycle at global and regional scales through anthropogenic activities and influences on land use. As urbanization increases globally, anthropogenic inputs of nitrogen from burning fossil fuels, lawn care practices, and sanitary wastewater are heightened in cities and their surrounding areas. Despite this, urban watersheds in the Baltimore area remain highly retentive of nitrogen likely due to the presence of control points for denitrification. Nearby forested and agricultural watersheds are consistently more retentive of nitrogen across wet and dry years; with denitrification largely relying on landscape features in riparian soils. Stream incision and erosion of urban streams, however, has degraded these landscape features in urban watersheds and therefore removed many control points for denitrification from the watershed. Here, we examine the spatial and temporal controls on denitrification in contrasting riparian locations to estimate high-frequency denitrification rates. Soil oxygen and soil moisture data were collected from four riparian soil pits in the Dead Run watershed in Baltimore, MD that span gradients of upslope accumulated area, total upslope impervious surface area, and downslope riparian incision. Soil cores were collected seasonally and analyzed to determine rates of denitrification at each riparian location. The objectives of this research are to (1) identify control points for denitrification across a heterogeneous urban riparian zone and (2) determine the characteristics of an urban riparian zone that are limiting denitrification.

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Chapter 1

Introduction to Riparian Soil Biogeochemistry

N pollution and retention

Upstream Nitrogen (N) loading from anthropogenic inputs of reactive N is increasingly related to downstream water quality. Eutrophication, or an excess of nutrients is responsible for algal blooms in the Chesapeake Bay and other coastal waters on the east coast of the US (Nixon, 1995). Streamflow N exports from urban watersheds are increasingly related to downstream eutrophication in the Chesapeake Bay causing algal blooms and anoxic conditions which are increasing in severity and frequency (D'Elia, 1987, Nixon, 1995; Shields et al., 2008). Algal blooms occur when nutrients limiting algae population growth (often N and Phosphorus) are added to a water supply by way of upstream nutrient loading. During a bloom, water O_2 concentrations often drop to hypoxic (near-zero) conditions, thus altering the biological communities and ecosystem functioning of aquatic environments. As a result of these events happening with more frequency and severity, increasing interest in upstream nutrient management has been a hot topic for research across many fields.

Anthropogenic influence on the nitrogen cycle has dramatically increased the abundance of reactive nitrogen (N) in the atmosphere (Galloway et al., 2004). Reactive forms of N are added to the atmosphere through anthropogenic practices such as burning fossil fuels, fertilizing soil for crop production or lawn care, and leaks in sewer infrastructure; therefore, urbanization amplifies the input of N into the watershed. Inputs of N in urban areas are increased by factors such as traffic, industrialization, and are heavily influenced by lawn fertilization practices (Kaushal et al., 2011; Law et al., 2004). While N is a critical element for supporting life, in excess it can have detrimental effects on downstream ecosystem function. Additionally, Increased N pollution also has implications on global climate change as agricultural and industrial practices have exacerbated the input of nitrous oxide (N_2O), a potent greenhouse gas.

Despite increased inputs of N to the region, urban watersheds in the Baltimore, Maryland area retain the majority of N added to the landscape (Bettez et al., 2015). Nitrogen retention of a landscape is a function of N inputs and N outputs of a defined spatial and temporal scale. Efforts to calculate retention in several watersheds in and around the Baltimore, MD area estimated inputs of N from atmospheric deposition, nitrification, and fertilizer applications. The outputs of N used in this study were estimated from the N supply exported in streams (Bettez et al., 2015). Using this method researchers calculated high retention estimates across an urban-rural gradient. One possible explanation for high N retention in these watersheds is that current estimates of retention on the landscape do not account for any additional removal of N from the landscape through denitrification. This is the microbial conversion of reactive N in the forms of nitrate (NO_3^-) , nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O) to dinitrogen (N_2) gas (Kulkarni et al., 2008).

Denitrification on the Landscape

Denitrification is dependent on soil NO_3^-, microbial presence, an energy source to support microbial activity—often in the form of organic matter, soil oxygen (O_2) concentration and temperature. The microbial conversion of NO_3^- is a critical process which removes excess NO_3^- from the landscape and therefore, aids to mitigate downstream effects of nitrogen pollution. Denitrification rates are very difficult to accurately capture with current field methods due especially to high background concentrations of N_2 gas in the atmosphere (Groffman et al., 2006). Additionally, there is great spatial and temporal variability in denitrification which poses a major challenge in understanding these dynamics on the landscape. The removal of NO_3^- through denitrification is found to happen disproportionately in small areas (hot spots) and short periods of time (hot moments) where conditions are more likely to support denitrification at a high rate (McClain et al., 2003). This concept is further understood as ecosystem control points which expands on the hot spots hot moments concept to further describe the mechanisms driving the disproportionate biogeochemical influence of patches on the landscape. These mechanisms are defined as permanent, activated, export, and transport control points (Bernhardt et al., 2017). Because of this behavior, determining where and when denitrification is occurring across the scale of an entire watershed becomes increasingly difficult.

Urban watersheds are also tremendously heterogeneous across space and time and often alter certain landscape features that act as control points for denitrification in other landscapes—such as functioning riparian zones. This necessitates a more detailed examination of the role of riparian zones in denitrification processes within urban settings. Therefore, this study specifically investigates the function of riparian zones as permanent and activated control points across multiple distinct riparian features.

Riparian Biogeochemistry

In many watersheds, riparian zones are critical for managing excess NO_3^- and functioning and intact riparian areas often act as permanent control points of denitrification (Hill, 1996; Pinay et al., 1993). Riparian areas are defined by their position in the landscape, as the land directly paralleling a stream channel which acts as the transition between aquatic and terrestrial ecosystems. Thus, these areas frequently have soils with high moisture levels and organic matter content making them optimal locations to sustain high denitrification rates. Intact, functioning riparian zones are also critical features for instream function as they manage nutrient distribution, reduce erosion, slow the velocity of in stream flow, and reduce flooding extent. Urbanization however, is often linked to lower water tables and dry more aerated soils in the riparian zone, this in turn alters the biogeochemical function of urban riparian zones thus limiting their ability to provide the same critical ecosystem functions for in stream processes (Groffman et al., 2002).

Urban watersheds have several factors that alter the function of riparian areas and influence denitrification rates in these soils. Mainly, flow paths in urban areas are greatly altered through stormwater infrastructure which deliver large volumes of water to streams (Walsh et al., 2005). Near stream water table levels in urban watersheds are often low due to reduced upland infiltration as a result of stormwater infrastructure (Groffman et al., 2003). Lower water tables in urban and suburban watersheds with more oxygenated soils promote higher rates of aerobic processes such as nitrification and decreasing rates of anaerobic processes such as denitrification (Groffman et al., 2002).

Mechanisms for NO_3^- removal from the landscape

This study specifically explores the dynamics of denitrification in urban riparian zones as a source of nitrate (NO_3^-) removal in one urban watershed. Denitrification describes the reduction of NO_3^- to inert N_2 gas via microbial transformation. This is an anaerobic process by which reactive forms of N are reduced to N_2O and N_2 gas. Denitrification is a function of NO_3^-, soil O_2, microbial activity, organic matter, and temperature. On the landscape, denitrification trends vary disproportionately through space and time (McClain et al., 2003). While this study looks at microbial denitrification as the primary mechanism for transforming NO_3^- from riparian soils, there are additional mechanisms which could also contribute to high retention of N in urban landscapes.

Dissimilatory nitrate reduction to ammonium (DNRA) is the transformation of NO_3^- to NH_4^+ under anaerobic conditions. DNRA can be heterotrophic by way of fermentative bacteria or chemolithoautotrophic. The latter is another mechanism of transforming NO_3^- in which chemolithoautotrophic bacteria oxidize inorganic sulfur compounds (and other inorganic compounds) to reduce NO_3^- under anaerobic conditions. Where denitrification reduces NO_3^- to N_2 gas thus removing the reactive N from the landscape, DNRA cycles reactive N in the ecosystem through transformation and N transport and thus, is more likely to be retained in the ecosystem (Burgin & Hamilton, 2007). While the extent of DNRA varies across ecosystems; DNRA seems to predominately happen in marine soils and has some significance on removing NO_3^- in freshwater ecosystems (Burgin & Hamilton, 2008; Giblin et al., 2013; Handler et al., 2022)

Anaerobic ammonium oxidation (anammox) is the conversion of NH_4^+ and NO_2^- to N_2. Unlike denitrification, no N_2O is produced in this conversion. Anammox is an anaerobic process and is carried out by autotrophic anammox bacteria. In marine environments, anammox is estimated to account for half the NO_3^- loss, however the terrestrial influences of this are far more variable and the extent of anammox driven NO_3^- removal is uncertain (Dalsgaard et al., 2005; Kuenen, 2008). While, anammox has the potential to be a significant driver of NO_3^- in wetlands and other environments, studies have found that while there is evidence of annamox bacteria in some riparian zones, this abundance does not result in significant NO_3^- removal from anammox compared to NO_3^- removal from denitrification (Wang et al., 2012; Zhu et al., 2013)

Methods for measuring denitrification

Denitrification is a notoriously difficult process to quantify in a landscape due to several considerations; and thus, the measurements that are available to capture this have unique and challenging limitations (Groffman et al., 2006). Fundamentally, denitrification is tricky to calculate due to the high atmospheric concentrations of N_2. As the product of denitrification is N_2 gas, it becomes extremely challenging to discern between N_2 produced via denitrification and that which is in the atmosphere. As a result, the common thread amongst all methods to measure denitrification is controlling for N_2 gas in a soil sample.

Acetylene inhibition method

A breakthrough for this challenge was the discovery that the compound acetylene (C_2H_2) inhibits the production of N_2 in the reduction of NO_3^- (Balderston W L et al., 1976). When adding acetylene to soil samples, N_2O production can be read as denitrification instead. Because N_2O is much less abundant in the atmosphere, it is easier to measure small changes in concentration from a sample. Acetylene inhibition methods have been used for decades to understand denitrification trends across the landscape however, these methods do not come without limitations. The biggest limitation to these methods is that nitrification is also inhibited with the addition of acetylene thus, NO_3^- pools in soils deplete as samples are incubated (Hynes & Knowles, 1978). Because of this, often time methods using acetylene to prohibit N_2 production underestimate denitrification rates.

Today the most common use of these methods is to determine denitrification potential using denitrification enzyme activity (DEA). This method is used to measure the maximum rates of denitrification by removing the limiting factors to denitrification in the form of O_2 concentration, NO_3^-, and Carbon (C) availability. N_2 production is inhibited in this method, thus N_2O flux is measured as denitrification potential. Because this study measures denitrification under ideal soil conditions, it is limited in its use for understanding temporal variation of denitrification as soil conditions change. However, DEA remains a valid method for understanding denitrification trends across the landscape and has proven to be very useful in studies that compare soil ecosystems and treatments (Groffman et al., 1999). A huge benefit of DEA is the ability to run large batches of samples in a short amount of time. Other methods of measuring denitrification through N_2 flux are heavily limited by time consuming methods.

Measuring direct N2 flux from denitrification

Efforts to calculate denitrification from N_2 flux directly are limited by the background atmospheric concentration of N_2. Currently, methods directly measuring N_2 from denitrification use variations on a flow through core measurement system which isolates a soil sample from any atmospheric gas (Burgin et al., 2010; Burgin & Groffman, 2012; Butterbach-Bahl et al., 2002; Swerts et al., 1995). The method used in this study is the Nitrogen-Free Atmospheric Recirculation Method (N-FARM). In this method, the air in the soil sample is replaced with a blend of gases, making the samples free of any N_2. This allows for small changes in N_2 concentrations to be measured over time; these changes are used to calculate the flux of N_2 from denitrification. This method allows for the synthetic atmosphere in the sample jars to be set by the researcher, meaning denitrification rates can be observed at specific O_2 concentrations which is important for understanding spatial and temporal denitrification trends. One of the biggest limitations to the N-FARM is the length of time samples take to run. These limitations are discussed more in depth in Chapter 3.

Chapter 2

Soil Denitrification Fluxes in Urban Riparian Zone

Introduction

In this study, we used consistent soil gas and moisture sensors and collected soil core samples from four riparian soil plots in a Baltimore County, Maryland watershed to understand the temporal and spatial variability of denitrification across the top 1m of a heterogenous riparian zone. Given the factors controlling denitrification in a forested watershed, we would expect denitrification in this landscape to occur in shallow soils of low-lying positions in forested reaches of the riparian zone. With increasing alteration of the riparian zone however, it is unclear the extent to which these areas are acting as control points for denitrification as they are in forested and agricultural watersheds. The objectives of this study are to (1) identify control points for denitrification across an urban riparian zone and (2) determine the characteristics of an urban riparian zone that are limiting denitrification. Given the sample sites chosen for this study, we test the following hypotheses: (1) Denitrification rates are highest in a low-lying stormwater riparian wetland given the high organic matter, consistent soil moisture, and large drainage area to this site. (2) Riparian zones on highly incised segments of the stream channel will have the lowest rates of denitrification due to dry and oxygenated soils through the profile. (3) Denitrification will decrease with depth as NO_3^- supply, organic matter, and soil oxygen will decrease with depth across all sites.

Methods

Site description

Dead Run 5 (DR5) is a nested catchment in the upper reaches of the Dead Run watershed in Baltimore, MD (Figure 2-1). DR5 is a 1.6 km^2 urban watershed, with 44.6% impervious land cover. In this study, four sites were chosen in the riparian zone across the watershed to represent variations across the riparian zone of spatial features including upslope accumulated area, total upslope impervious surface area, and downslope riparian incision (Table 2-1, Figure 2-2).

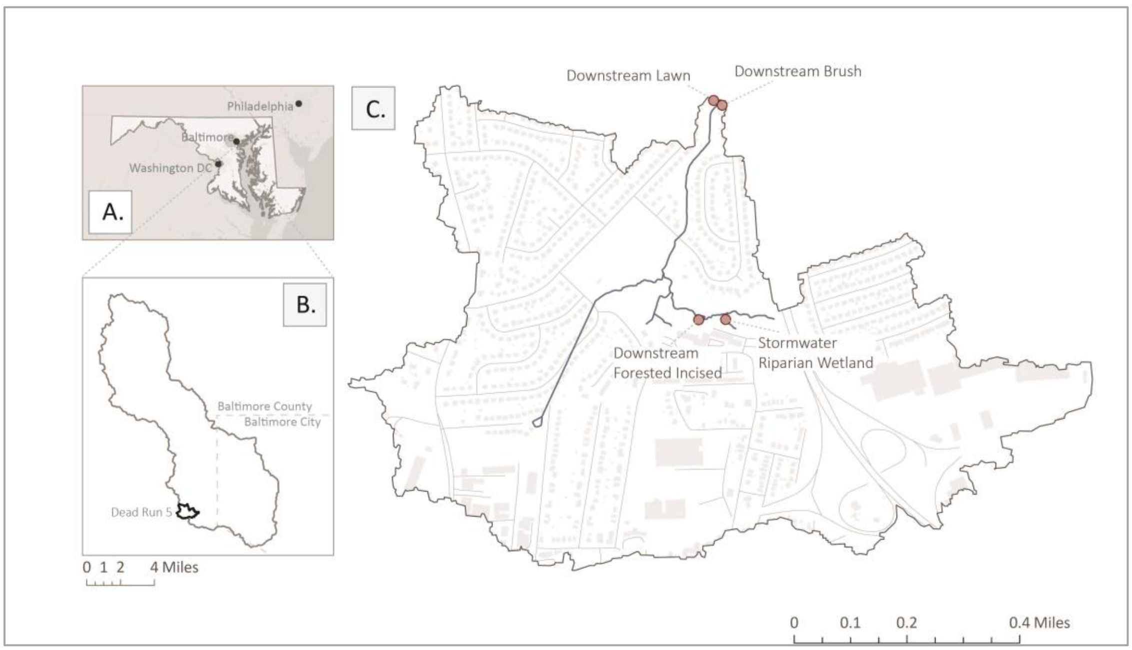

Figure **2-1**: (A) Position of the Dead Run 5 (DR5) watershed in the state of Maryland, USA. (B) Position of the study area in the Gwynns Falls watershed. (C) Watershed extent of DR5 and soil plot locations with their contributing area in the DR5 riparian zone.

Table **2-1**: Site descriptions for riparian soil plots. Upslope accumulated area (UAA), and Height Above Nearest Drainage (HAND).

Site	Downstream Brush	Downstream Lawn	Stormwater Riparian Wetland	Upstream Forest Incised
UAA (m^2)	0.0002	0.0003	0.0142	0.0011
HAND (m)	0.63	0.93	1.25	1.92

Two sites are positioned on opposing banks on either side of the stream, immediately upstream from the outlet of DR5. These sites have small upslope drainage areas with contrasting vegetation management. These are on opposite sides of the stream; downstream west is intensively maintained with mowing and lawn fertilization (Downstream Lawn). Typical lawns in the watershed are comprised of a mixture of *Festuca arundinacea* (tall fescue) and *Festuca* (fine fescue) (Raciti et al., 2011). The downstream east plot has unmanaged overgrown brush (Downstream Brush). These two sites are located on a segment of the stream channel that is ~1m incised. The Downstream Brush site is on a steeper slope (15.7%) compared to the Downstream Lawn side (9.4%)

The additional two sites are positioned further upstream in a forested reach of the watershed located behind Winters Lane. This reach is primarily dominated by *Quercus rubra* (red oak) and *Liriodendron tulipifera* (tulip poplar). The Upstream Forested Incised plot in this area is in the riparian zone adjacent to a highly incised section of the stream with 2m of incision between the top of the banks and the streambed (Table 2-1, Figure 2-2). The furthest upstream site is in a riparian stormwater swale eroded through remnant wetland vegetation and drainage from upslope stormwater infrastructure (Stormwater Riparian Wetland). This swale is surrounded by large patches of *Symplocarpus foetidus* (skunk cabbage). This site is located ~10m downslope from a storm water outlet pipe and ~20m downslope from an additional stormwater drainage system which drains the parking lot directly upslope from this site. This plot has the largest upslope accumulated area (UAA) out of the four chosen study sites.

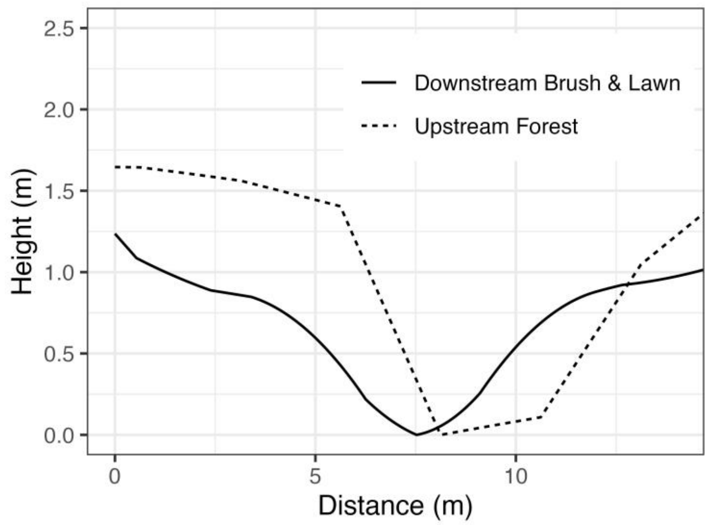

Figure **2-2**: Cross section of stream channel at the upstream forested site and the downstream brush/downstream lawn. The two downstream sites are on opposite banks of the stream channel. Height shown here was calculated as the height from lowest elevation in the channel.

Hypotheses

In the chosen riparian study sites, we expect several factors to control denitrification rates (Table **2-2**). Based on the controls identified, we test the following hypotheses.

(1) Denitrification rates will be highest in the stormwater riparian wetland given the high organic matter, large accumulated area and percentage of upslope impervious area, and the consistent soil moisture throughout the soil profile.

(2) The downstream lawn site will have higher rates of denitrification relative to the downstream brush site given the absence of a restrictive layer in the soil profile,

additional NO_3^- inputs from fertilizer application, and the gentler slope on the lawn side of the stream.

(3) The upstream forested incised site will have the lowest rates of denitrification due to the extensive stream incision nearby creating dry and oxygenated soils through the profile.

(4) Denitrification will decrease with depth at all four study sites as factors limiting denitrification such as root biomass, NO_3^- supply, and organic matter decrease with depth.

Table **2-2**: Additional site information informing denitrification at each riparian study site. Soil profile description, organic matter estimations, and plant uptake expectations are made from seasonal soil core extractions and site observations. NO_3^- supply and expectations are informed by upslope accumulated area (UAA) and additional known inputs. O_2 concentrations are informed by soil profile characteristics and stream incision.

Site	Downstream Brush	Downstream Lawn	Stormwater Riparian Wetland	Upstream Forest Incised
Soil Profile	Fragic layer (20-30cm)	No restrictive layer	No restrictive layer	Restrictive layer (~60cm)
Soil Organic Matter	Medium soil organic matter (0-15cm)	Medium soil organic matter (0-15cm)	High soil organic matter (0-15cm)	Lower soil organic matter
NO_3^- Supply	Low Small UAA, steeper slope	High Small UAA, Additional inputs from fertilizer	High Large UAA, High connected impervious percentage	Low Small UAA
Plant Uptake	High Dense brush vegetative cover	High Lawn cover	Low Dense wetland vegetation nearby	Low Minimal vegetative cover
O_2 Concentrations	Increases below fragic layer (20-30cm)	Decreases with depth	Decreases with depth	High at all depths

Soil sensors measuring soil oxygen and soil moisture

Soil pits were dug at each riparian site and instrumented to measure soil oxygen concentration, volumetric water content, and conductivity at three nested depths—10cm, 20cm, and 1m. Soil oxygen was measured with Apogee SO-421 SDI-12 Fast Response Thermistor Reference Oxygen Sensors; and soil moisture and conductivity were measured with Campbell Scientific CS655 Soil Moisture probes. Data was collected in five-minute intervals using Campbell Scientific CR1000x dataloggers.

Seasonal soil core denitrification rates

To determine denitrification rates, we collected multiple cores from each plot in every season of the year. The methods used were similar to those described by (Burgin et al., 2010; Burgin & Groffman, 2012; Weitzman et al., 2021). All soil core analysis took place at the Cary Institute of Ecosystem Studies in Millbrook, NY with seasonal samples beginning in early August 2022. Four rounds of sampling were completed in August 2022, October 2022, February 2023, and May 2023. For each of these seasonal sampling campaigns, three one-meter cores were taken from each site; after extraction, each core was cut into three 10cm segments based on sensor depth (0-10cm, 10-20cm, and 90-100cm).

In the Fall 2022, Winter 2023, and Spring 2023 seasons, two soil cores from each site and depth were used for the N-Free Atmospheric Recirculation Method (N-FARM) to determine N_2, N_2O, and CO_2 flux. Soil cores were held in gas tight chambers and attached to a flow injection system. The N-FARM begins with a ~16-hour period of vacuum flushes to replace any background N_2 in the headspace and soil pores with a constant gas blend of ultra-high purity He and O_2. After the vacuum flush cycle, soil cores are incubated over ~6 hours. Over this period, each sample was measured for concentrations of N_2, N_2O, and CO_2 production every ~2 hours. Flux was calculated from the production of each gas throughout the day.

Unlike previous approaches to quantify denitrification rates using the N-FARM method, we exposed the soil samples to O_2 concentrations that most closely reflected the soil conditions at each site. We analyzed soil samples under concentrations that reflected close to the average O_2 concentrations that were recorded from soil oxygen sensors in the week leading up to sample collection. Using these data, a soil core was either exposed to 0% and 5% O_2 or 10% and 20% O_2. One limitation of the N-FARM is, when running soil cores at multiple O_2 concentrations, denitrification rates tend to decrease over time as NO_3^- supply decreases in the sample. To limit this problem, two of the three soil cores collected from each site were used for gas flux analysis as duplicates. In this strategy, duplicate samples were analyzed only once and exposed to different O_2 concentrations. This allowed for a more accurate understanding of denitrification rates in each of these environments across multiple O_2 concentrations but assumes no spatial variability within a plot between the two cores for a given sample date therefore, heterogeneity is assumed to occur at a higher level.

In the Winter and Spring 2023 seasons, one additional core was taken from each site to be autoclaved. These “killed” cores were used to ensure the N_2 measured in this process was a product of denitrification instead of gas trapped in the pore space of the sample. One core from each site was killed and run at either 0% or 5% O_2 based on available chambers on the N-FARM.

Potential net N mineralization and potential net nitrification

The additional soil core was used to determine potential net N mineralization and potential net nitrification. Each core was separated by sensor depth location (0-10cm, 10-20cm, and 90-100cm depth) and homogenized. Subsamples from this were taken to measure gravimetric soil moisture, inorganic N (NO_3^- and NH_4^+), and potential net mineralization rates. One subsample was held in a forced air oven at 60°C for 48 hours and measured for gravimetric water content. Another subsample will be used to analyze initial NO_3^- and NH_4^+; to this sample, 2M Potassium Chloride (KCI) was added and samples were extracted after one hour. One additional subsample was held in glass jars for a 10-day incubation. After that period, KCI extractions were taken to measure NO_3^- and NH_4^+ concentrations. Potential net N mineralization was calculated from the change in ammonium and the change in NO_3^- after the 10-day incubation period. Potential net nitrification was calculated from the change in NO_3^- after incubation.

Statistical Analysis

Mean values and standard deviations of soil parameters were calculated for each depth position at all four sites across four seasons. To evaluate the differences in N_2, N_2O, and CO_2 flux across sites and depths, a two-way analysis of variance (ANOVA) was performed with site and depth as independent variables. A similar two-way ANOVA was performed to include seasons as a third independent variable to evaluate the differences in N_2 production across seasons for each site and depth. All statistical analysis was performed using R software (R Core Team, 2021).

Results

Soil oxygen and moisture

Continuous monitoring of soil oxygen and soil moisture uncovered patterns of the biogeochemistry of these four riparian sites over space and time. At the outlet of DR5, we positioned two soil pits on opposite sides of the stream with drastically different maintenance practices and vegetation. In the lawn, we found oxygen to decrease with depth generally however, between 10cm and 20cm below the surface, O_2 concentrations varied greatly. Beginning in Fall 2022, oxygen decreased to near 0% at the surface and remained there until Spring 2023 when O_2 concentrations returned to atmospheric levels. Meanwhile, O_2 20cm beneath the surface continued to fluctuate from near-zero to near-atmospheric concentrations with precipitation events. In May 2023, an additional soil O_2 and moisture probe was installed 10cm beneath the surface adjacent to the original soil pit to confirm these results nearby. While both O_2 sensors were reading similar values in the months following installation, the newer data show discrepancies beginning in late June 2023 when the new sensor began recording O_2 concentrations increasing. In the Downstream Lawn, soil moisture interestingly is shown to be decreasing with depth. Volumetric water content at all three depths ranged between 23.4% to 52.1% from August 2022 to June 2023.

On the opposite side of the stream in the Downstream Brush site, oxygen concentrations were highest in the shallow soils, however, concentration increased slightly 20cm beneath the surface at some points and decreased to near 0% O_2 concentrations at 90cm. Soil moisture was found to increase with depth here. Additionally, there is far less variability in volumetric water content 90cm beneath the surface while moisture in the shallow depths followed very similar trends over time. There was greater variation in soil moisture at 10cm and 20cm deep in the late summer to early fall 2022 as well as in the late spring to early summer 2023 months.

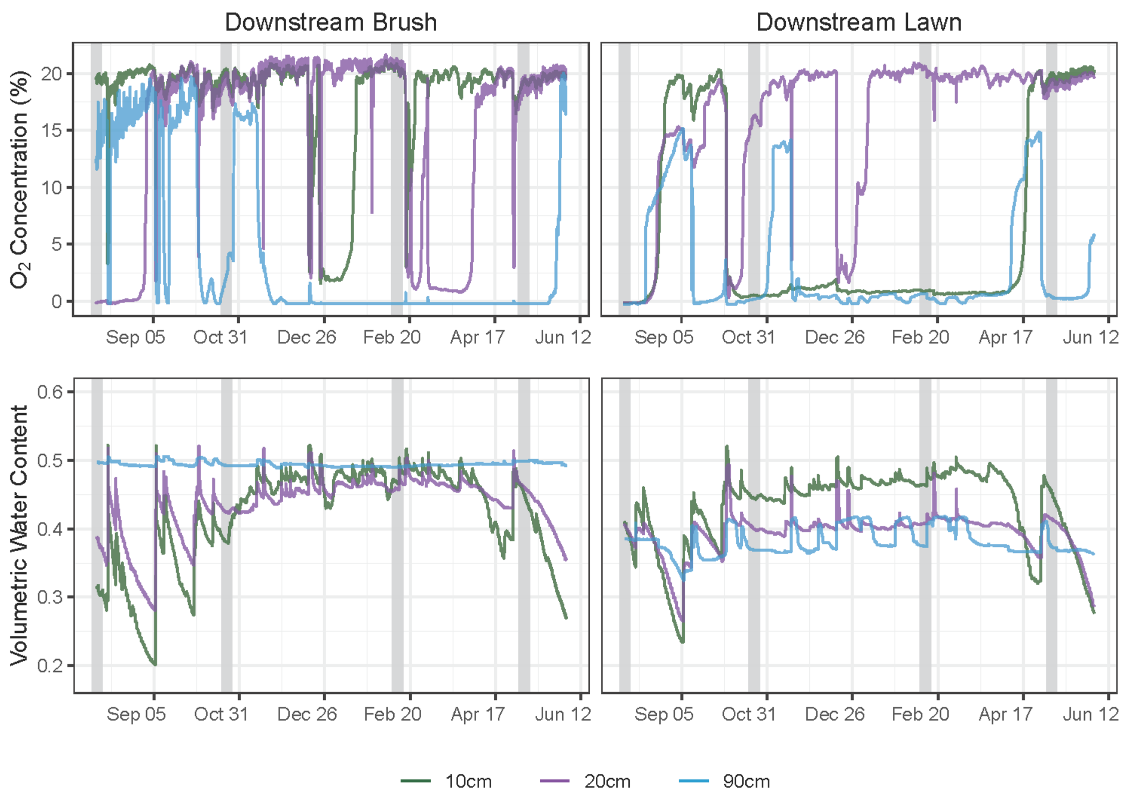

Figure **2-3**: Soil moisture and oxygen concentrations from two riparian sites at the outlet of Dead Run 5. At each site, soil O2 and moisture is measured at three depths (10cm, 20cm, and 90cm). Time series for each variable are plotted here at each depth. Data shown was collected from August 2022 – June 2023 Shaded bars are showing sampling days when soil cores for soil gas flux analysis were extracted.

Further upstream, soil O_2 and moisture conditions varied drastically in unique riparian features. On the most incised segment of the stream, these riparian soils were variable in moisture and O_2 over time however, soil O_2 remained over 5% most of the time at each depth. Here, oxygen did decrease with depth however, there were only a handful of times these concentrations reached anoxic conditions over this study period. Soil moisture did increase at 90cm below the surface and there was little variation in moisture over time in the deepest soils. At 10cm and 20cm deep, we found very similar temporal variations in both O_2 and moisture. Volumetric water content at this site ranged 11.5 – 54.1%.

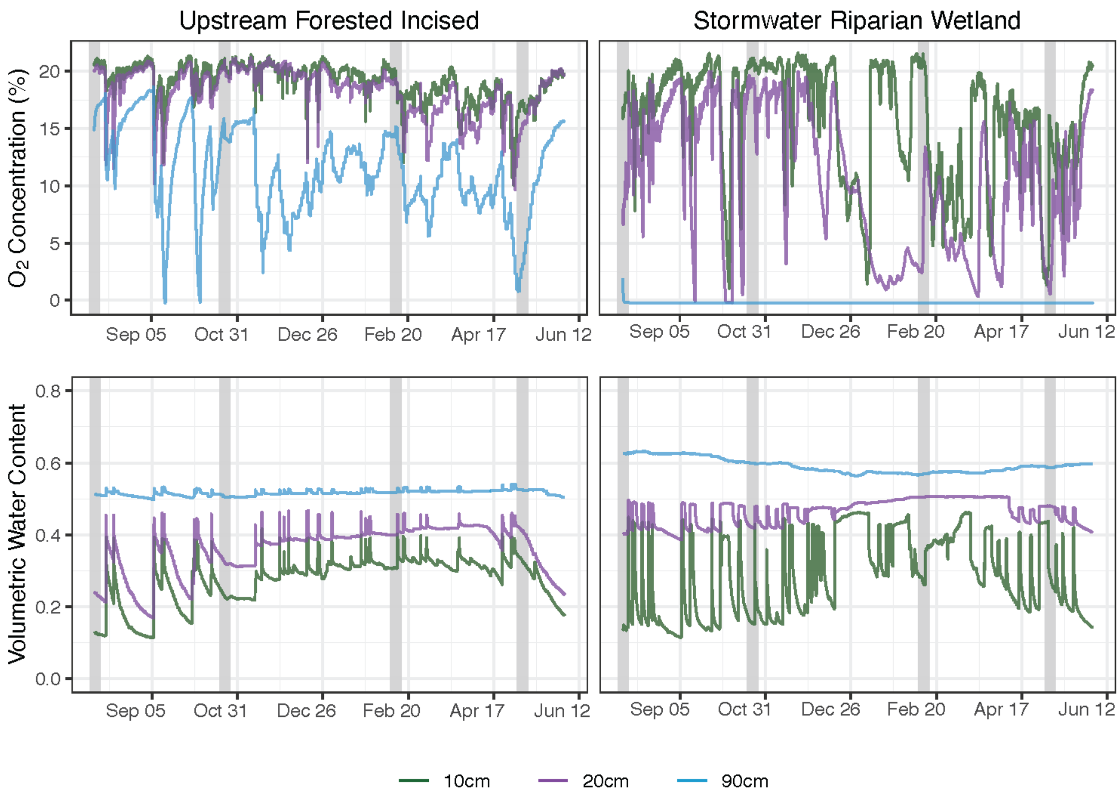

Figure **2-4**: Soil moisture and oxygen concentrations from two upstream riparian sites in Dead Run 5 (DR5). At each site, soil O_2 and moisture is measured at three depths (10cm, 20cm, and 90cm). Time series for each variable are plotted here at each depth. Data shown was collected from August 2022 – June 2023. Shaded bars are showing sampling days when soil cores for soil gas flux analysis were extracted.

In the stormwater riparian wetland, just upslope an incised stream channel, soil O_2 and moisture look drastically different 90cm beneath the surface. Here, soil O_2 decreased with depth and at 90cm deep, concentrations remained near 0% for the duration of this study. At depth, soils also remained consistently saturated and volumetric water content ranged 56.3 – 63.3%. In the two shallow positions, we observed far greater temporal variation in soil O_2 and moisture.

Riparian soil gas fluxes

Denitrification flux as calculated from N_2 production across sites and depth ranged from 0 – 1959 μg N/m2/hr and we found no statistically significant differences in N_2 flux among sites ($p > 0.05$). However, the highest rates were found in an upstream lawn located near the outlet of DR5. Here, N_2 production was highest at 90cm deep with the highest observed rate calculated here across all sites and depths. The range of N_2 production rates in the lawn is 0 – 1959μg N/m^2/hr. We did however find statistically significant differences in N_2 flux across depths. At both outlet riparian locations, denitrification was found to increase with depth and higher rates overall were found in the lawn. In the stormwater riparian wetland, the 20cm position had the overall highest rates of N_2 production across depths here and had an average denitrification rate of 587 μg N/m2/hr. The observed range of denitrification rates at the site was calculated to be 0 – 1597 μg N/m2/hr. In the downstream forested incised riparian zone, we found consistently low rates of N_2 production with the N_2 flux rates ranging 0 – 379 μg N/m2/hr. There were no clear trends of seasonal variability of N_2 flux from denitrification across all landscape positions (Table 2-3).

N_2O production across all four sites was significantly lower than N_2 production and, there were no significant differences in N_2O flux across sites and depth. Although N_2O flux rates were overall low, the highest flux came from the two downstream sites in the lawn and brush. In the downstream lawn, N_2O flux ranged 0-15 μg N/m2/hr; and average rates increased slightly with depth. The highest N_2O flux was found in fall 2022 at 10cm deep. In the downstream brush site, production rates ranged from 0-11 μg N/m2/hr; and average rates decreased with depth. Here, the highest N_2O flux was also observed to be at 10cm deep in fall 2022. The upstream forested incised site had rates measured in the range of 0-8.3μg N/m2/hr; and the highest observed rate was found in summer 2022 at 20cm. However, the highest average rate of N_2O production was found 90cm beneath the surface. The lowest N_2O flux was found in the stormwater riparian wetland with rates ranging 0-1.4 μg N/m2/hr here and the highest flux rate was observed 10cm below the surface in winter 2023.

Across all four riparian soil pits, CO_2 production decreased with depth. The downstream brush site had the highest rates of CO_2 production across all sites with a max recorded rate of 27,031 μg C/m2/hr. The downstream lawn site ranged 675-9874 μg C/m2/hr. The lowest CO_2 flux was found at the upstream forested incised site where CO_2 rates ranged 0-9334 μg C/m2/hr. In the stormwater riparian wetland, CO_2 flux ranged 119-3876 μg C/m2/hr.

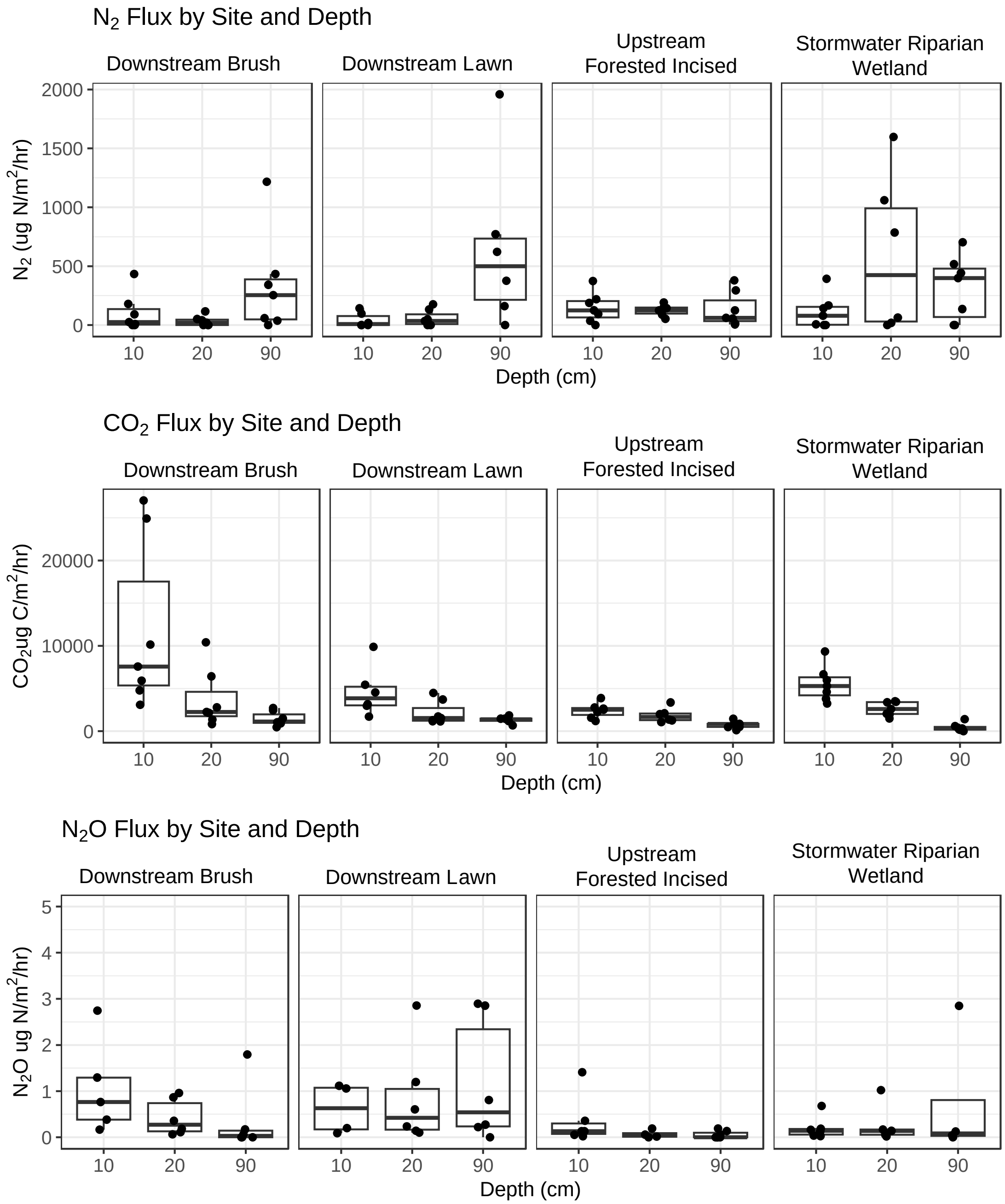

Figure **2-5**: N_2, CO_2, and N_2O rates calculated with N-FARM. Data shown was collected across four seasons (August 2022, October 2022, February 2023, and May 2023). Cores were either held at 0% O_2 and 5% O_2 or 10% and 20% O_2 depending on the oxygen concentration in the soil at the time of sampling.

Soil NO_3^- consistently decreased with depth across all sites, and NO_3^- concentrations ranged from 0.25-14.0 µg N/g soil. The outlet riparian sites had the highest recorded concentrations of NO_3^-. There was little variation of soil NO_3^- between sites despite varying sizes of contributing areas; and similar trends were present with depth across every riparian site. Soil ammonium also decreased with depth across sites and there was little variation in ammonium concentrations across all study sites. The highest average concentrations of ammonium were found in the stormwater riparian wetland 10cm beneath the surface (2.81 µg N/g soil) (Table 2-2).

Table **2-3**: Mean soil and gas flux data across four riparian sites in the Dead Run 5 (DR5) watershed, Mean values were calculated from soil cores taken in August 2022, October 2022, February 2023, and May 2023.

Mean Soil and Gas Flux Data from Riparian Soils in Baltimore County, Maryland

		Downstream Brush			Downstream Lawn			Riparian Incised			Riparian Wetland		
Depth (cm)		10	20	90	10	20	90	10	20	90	10	20	90
Potential net nitrification (µg N/g soil/d)													
	Mean	0.43	1.29	-0.03	0.55	0.13	0.07	0.30	0.31	-0.02	0.53	0.37	-0.04
	SD	0.28	1.77	0.05	0.55	0.34	0.07	0.40	0.67	0.03	0.46	0.12	0.07
Potential net N mineralization (µg N/g soil/d)													
	Mean	0.36	1.26	-0.16	0.53	0.10	0.05	0.15	0.20	-0.05	0.29	0.31	-0.10
	SD	0.26	1.79	0.21	0.59	0.38	0.03	0.24	0.52	0.05	0.05	0.15	0.05
Soil nitrate (µg N/g soil)													
	Mean	6.98	4.45	0.94	3.82	3.42	1.35	3.30	2.40	1.22	3.26	1.42	0.95
	SD	6.13	3.28	1.19	4.32	2.43	1.58	2.35	1.95	1.18	1.73	0.99	1.14
Soil ammonium (µg N/g soil)													
	Mean	1.71	0.51	1.47	1.17	0.74	0.47	2.65	1.46	0.56	2.81	0.87	0.80
	SD	1.00	0.37	2.10	0.91	0.51	0.38	1.52	1.55	0.50	3.95	0.78	0.56
N_2 flux (µg N/m2/hr)													
	Mean	93.0	31.3	334.7	51.6	38.8	648.3	110.4	122.9	133.2	103.6	527.3	313.9
	SD	170.3	46.0	422.5	64.8	49.8	702.3	85.0	49.9	146.7	152.8	629.3	272.4
N_2O flux (µg N/m2/hr)													
	Mean	2.73	0.42	0.29	3.52	0.86	1.17	0.20	1.62	0.37	0.35	-0.32	0.06
	SD	4.17	0.39	0.67	6.51	1.06	1.34	0.24	3.31	1.10	0.53	0.67	0.08
CO_2 flux (µg N/m2/hr)													
	Mean	12920	4012	1456	4451	1890	1327	4926	2488	444	2543	1814	732
	SD	10413	3701	830	3194	1288	391	1300	795	463	867	780	419

Data collected over four seasons were found to have no statistically significant differences in N_2 flux seasonally. While average rates were similar in all seasons, N_2 flux was found to be highest in February 2023 and lowest in October 2022 (Table 2-3). While average N_2O flux was low across all sites and seasons, rates were found to be the highest in October 2022 and lowest in May 2023. CO_2 flux measured in October 2022 was higher than all other seasons; and rates were lowest in August 2022.

Table **2-4**: Seasonal averages of N_2, N_2O, and CO_2 flux across four riparian soil plots in DR5. Mean flux is averaged across four sites measuring rates at three depths (10cm, 20cm, 90cm).

		Summer 2022	Fall 2022	Winter 2023	Spring 2023
N2 flux (µg N/m2/hr)					
	Mean	249.1	139.3	245.5	233.4
	SD	540.6	179.8	433.8	264.3
N2O flux (µg N/m2/hr)					
	Mean	1.2	1.5	0.5	0.3
	SD	2.6	3.8	0.7	0.8
CO2 flux (µg N/m2/hr)					
	Mean	2838.5	3776.0	3334.2	2890.3
	SD	2610.5	5201.3	5446.5	2754.6
Sample size		12	23	23	23

When averaging soil O_2 concentrations by depth from August 2022 – June 2023, O_2 decreased with depth at every site. In the downstream lawn, concentrations were highest at 20cm however, across all four sites, O_2 was lowest 90cm below the surface. The average O_2 concentrations at the forested incised site were higher than any other site at each depth. Volumetric water content, NO_3^-, and N_2 flux were all averaged the same way. Moisture increased with depth at the upstream forested incised, stormwater riparian wetland, and downstream brush sites. However, in the downstream lawn site, moisture decreased with depth being the only site where this trend was observed. Average soil NO_3^- consistently decreased with depth at every site and, average N_2 flux trends with depth varied by site. In the Downstream Brush and Lawn sites, average N_2 flux increased with depth. In the Forested Incised site average rates were consistently low at each depth and, there was little variation between depths. In the Stormwater Riparian Wetland, average N_2 production was highest at 20cm beneath the surface.

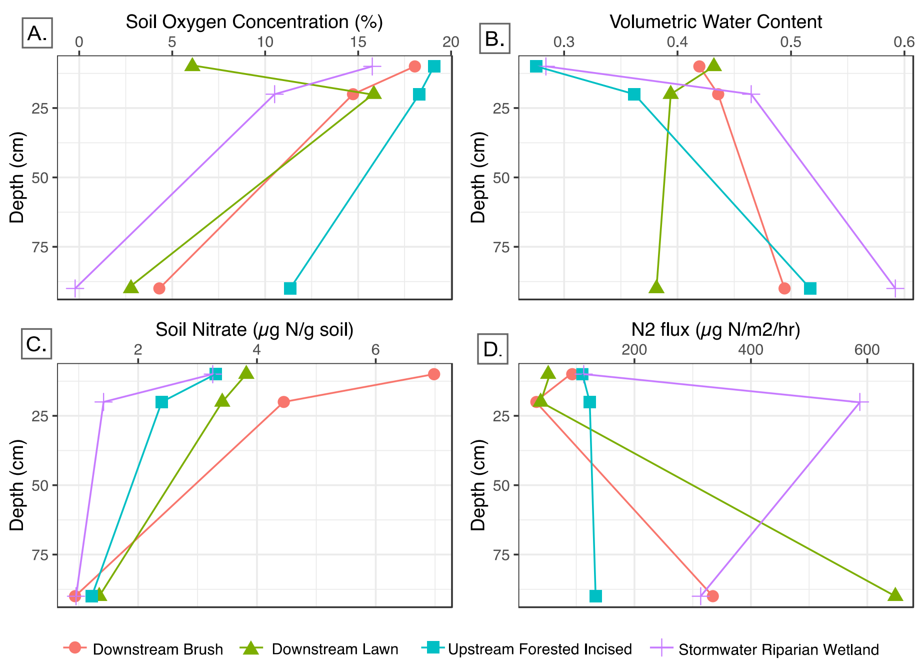

Figure **2-6**: Mean soil oxygen concentration, volumetric water content, soil NO_3^- and N_2 flux from denitrification at three depths (10cm, 20cm, and 90cm) from four riparian soil pits in DR5. Mean soil oxygen and moisture was calculated from data collected from August 2022-June 2023. Average N_2 flux and soil NO_3^- was calculated from soil cores collected in August 2022, October 2022, February 2023, and May 2023. (A) Mean soil oxygen concentration from soil O_2 probes. (B) Mean volumetric water content from soil moisture sensors. (C) Mean soil NO_3^-. (D) Mean denitrification N_2 flux from N-FARM

Discussion

Stormwater riparian wetland as a control point for denitrification

We hypothesized the stormwater riparian wetland study site to be a permanent control point because of the combination of high soil organic matter, a large contributing area increasing NO_3^-, and remnant wetland vegetation. Riparian wetlands in other watersheds are key features for NO_3^- removal from denitrification and often act as control points (Burns et al., 2005; Duncan et al., 2013). There is some evidence that in urban watersheds, these remnant wetland features provide a similar function for biogeochemical processes (Burns et al., 2005). However, these features are less commonly observed in urban areas as they are often eroded away or altered as channel incision intensifies.

In the stormwater riparian wetland, we found N_2 flux to be considerably higher at 20cm when compared to the denitrification rates found at 10cm and 90cm. There were marked differences in average N_2 rates between soils at 10 and 20cm (10cm: 104±153, 20cm: 587±629) (Table 2-2). While soil O_2 concentrations followed similar temporal patterns at both depths, in the shallowest position, O_2 was found to be higher during sampling times for soil gas flux analysis, therefore these samples were run at higher O_2 concentrations to capture denitrification flux under similar conditions. It is likely, given the higher NO_3^- supply in the shallow soils that these rates do not reflect N_2 flux during periods of low soil O_2.

This site had the largest contributing area out of the four and drained a considerable amount of upslope impervious cover. Additionally, drainage from stormwater infrastructure increases the runoff from impervious surfaces to this site. Here, we see consistently high soil moisture throughout the soil profile due to the contributing area to this site and stormwater infrastructure which created optimal conditions for dentrification in these soils. Upslope of

Stormwater riparian wetland site, there is a constructed swale draining stormwater from the upslope parking lot located as well as the road leading into the two upstream locations. This is a unique feature in the watershed that is a result of engineered stormwater infrastructure. Currently, these features are not mapped in the watershed, this one was found on foot while choosing sites for this study. Therefore, it is unclear how much of the watershed has similar conditions as found in the stormwater riparian wetland.

Controls driving unexpected control point beneath the lawn

Riparian soils at the outlet of DR5 had higher denitrification rates at 1m beneath the surface with higher rates found on the lawn side of the stream than on the side with unmanaged brush. Additionally, in this landscape (as well as all study sites) soil NO_3^- concentrations were found to decrease at 1m. However, NO_3^- concentrations at 1m deep were also higher on the lawn side which could be attributed to the frequent lawn care and multi-year fertilizer treatment in this lawn. Denitrification is limited across all sites by available NO_3^- supply, organic carbon, microbial activity, and soil O_2 concentration however, soil sensors show consistent redox conditions at depth that create an ideal environment for microbial denitrification (Figure 2-4). Despite more limited NO_3^- supply at this site, these results clearly show that riparian soils at this depth have an ability to support denitrification.

Our results point to a need for more data of soil biogeochemistry in deep riparian soils to better understand denitrification in urban watersheds**.** N_2 fluxes from deeper cores show that riparian lawns could be control points for NO_3^- removal in soils as deep as 1m where soil oxygen and moisture are ideal for denitrification and there is enough connection with the water table for a continuous NO_3^- supply. Additionally, these results indicate groundwater interactions are also variable across the riparian zone with some locations having more watertable interactions which can create more optimal redox conditions and deposit NO_3^-. This finding is further supported by research that has found lawns to be important features for denitrification in urban and suburban landscapes (McPhillips et al., 2016; Newcomer et al., 2012; Raciti et al., 2011).

Stream incision as a limiting factor to denitrification

At the forested incised location, we found the lowest denitrification rates from this study throughout the soil profile. Here, soil oxygen concentrations are rarely low enough at any depth to produce anoxic conditions for denitrification to occur (Figure 2-4). This site also exhibits the steepest stream channel incision than any other site, which limits the water table interactions at the 1m depth. Because of this, this site is also consistently dry, even at the deepest sensor depth. Despite the smaller upslope contributing area to this site, the NO_3^- supply here is comparable to all other sites. Because of the limited water table interactions, low soil moisture, and high O_2 concentrations, these elevated NO_3^- concentrations are likely due to nitrification (Groffman et al., 2002). Additionally, these soils are shown to support low levels of nitrification from potential net nitrification rates measured at this site (Table 2-2).

While N_2 flux increased with depth at the two outlet sites of DR5, the same trend was not found in a more incised riparian plot. The steeper incision at this site creates a lower water table here which alteres the redox conditions as soils become dryer and more oxic. In this area, where the riparian zone tends to exhibit the effects of the urban stream syndrome, the potential for denitrification hot spots may lie further upslope.

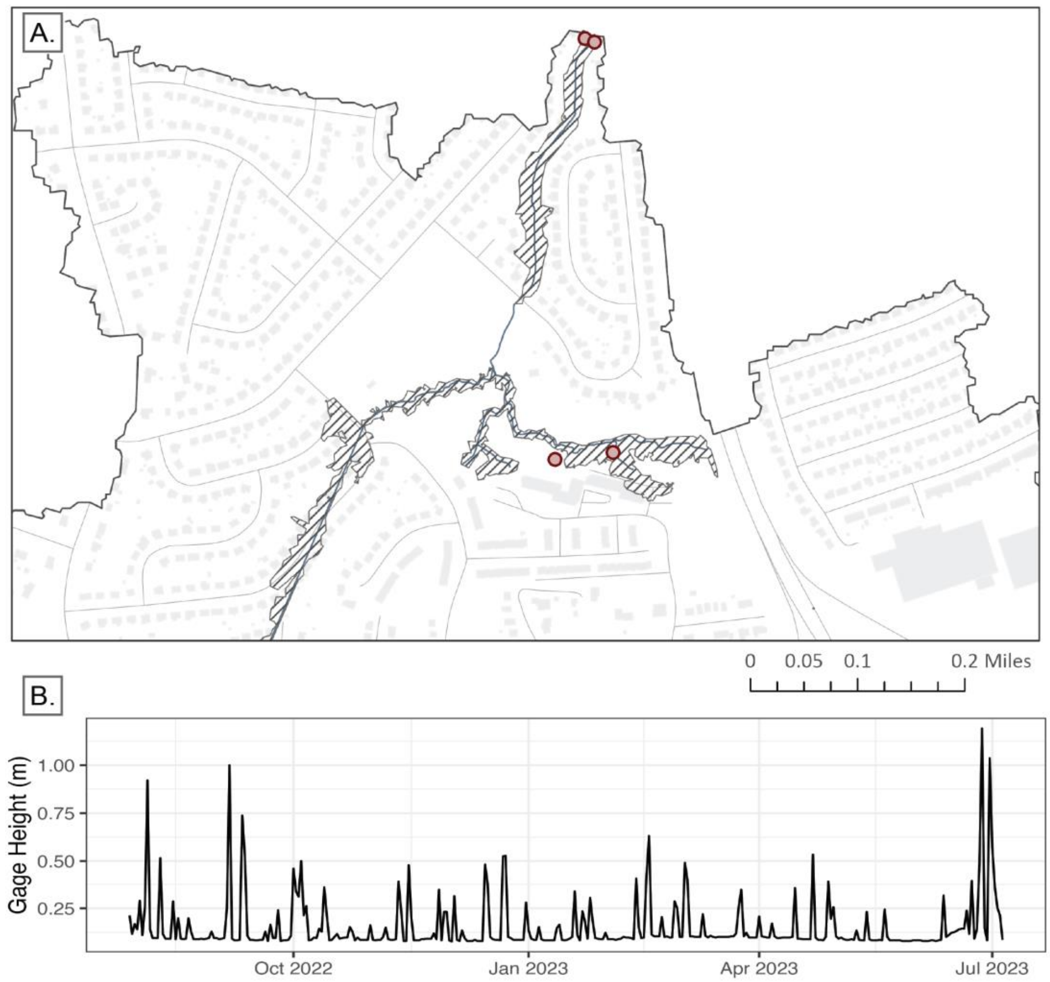

Figure 2-**7**: (A) Stream overbank flooding extent at 1.5m elevation calculated by vertical flow distance. (B) Max daily stream gage height at DR5 outlet from July 2022 to July 2023. Max daily stream gage height is calculated from stage height calculated at USGS station #01589312.

Stream overbank flooding at a 1.5m extent varies across sites with incision. In more incised areas of DR5, this amount of flooding does not extend into the riparian zone. In fact, the most incised riparian site, rarely experiences over bank flooding at this extent and never within the duration of this study. Meanwhile, the sties at the outlet of DR5, experience more frequent innundation from floods, which adds additional water to drive anoxic conditions and possibly organic carbon and NO_3^-. While these 1.5m flooding extent events are infrequent, it gives an idea of moments where denitrification may be more supported further into the riparian zone. In the more incised areas, riparian soils are less likely to act as a hot spot of denitrification even in these high flooding moments as they are not experiencing them the same way as riparian zones on less incised segments of stream.

The potential for activated control points across the riparian zone

Across all sites in this study we observed temporal variability in soil O_2 and moisture to varying extents. Generally, we found that most sites had the more consistent O_2 and moisture conditions in the deepest soils however, there was much greater variability in the surficial soils across all sites. For example, in the stormwater riparian wetland, at the deepest position O_2 conditions are low and remain low in these saturated soils as a response to the shallow water table at this site. However, in the two shallow positions, we observe more variation in soil O_2 and moisture conditions as soils at these depths respond to the intermittent wetting and drying as storm events occur (Figure 2-8).

A similar trend exists in the downstream brush site where the average O_2 concentration at 20cm is ~15% O_2 however the same site is experiencing anoxic conditions at 20cm deep ~25% of the time (Figure 2-6, Figure 2-8). This indicates that at this site, there are times when denitrification is supported in these periods of low O_2 concentrations. Additionally, the highest NO_3^- concentrations in this study were found at the surface the downstream brush. Given the greater incline in this riparian zone and the lack of evidence of fertilizer application on that side of the stream, this could be due to nitrification in shallow soils here. This is further supported by measured rates of potential net nitrification which show these soils support low levels of nitrification (Table 2-2).

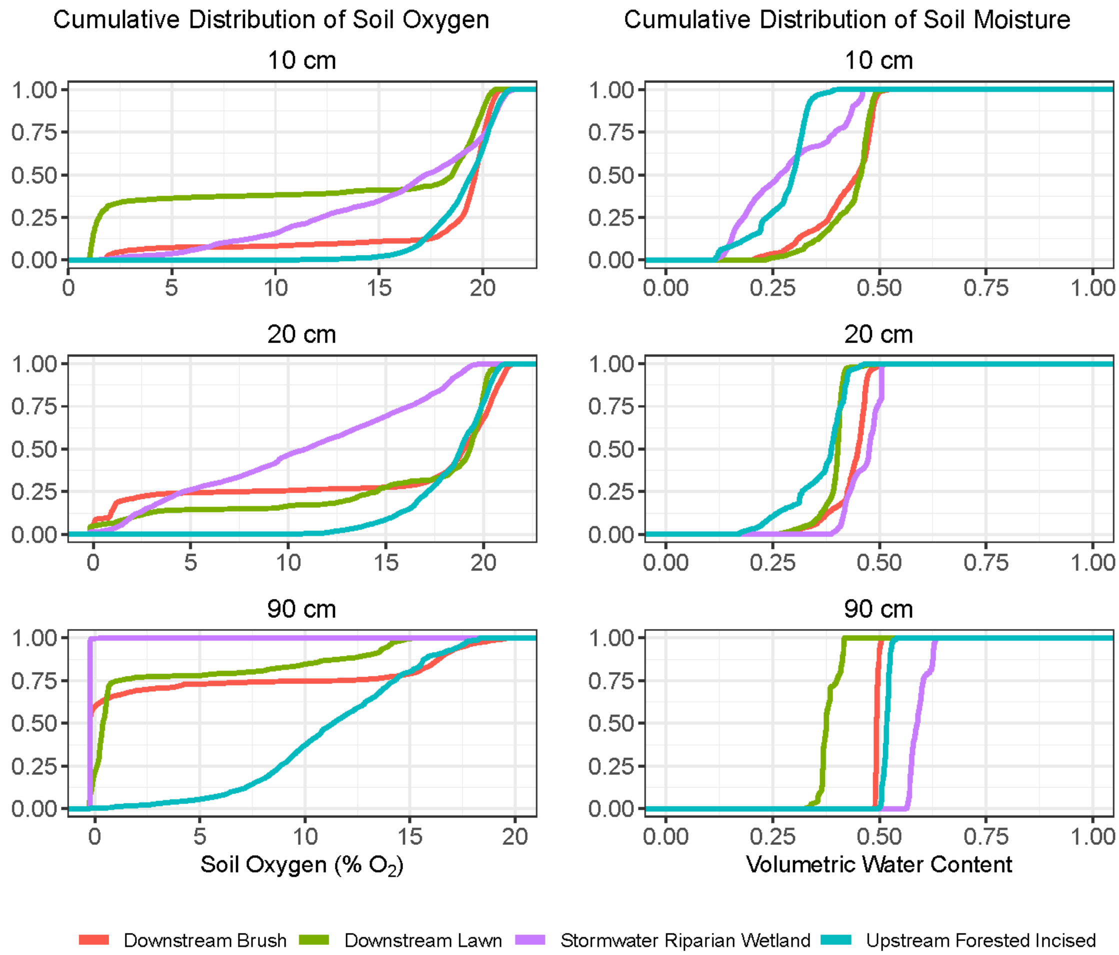

Figure **2-8**: Cumulative distribution of soil oxygen and volumetric water content data from soil sensors installed at 10cm, 20cm, and 90cm depths across four riparian soil pit locations.

Implications

Five studies used in a brief analysis of previously published N_2 fluxes using N-FARM revealed that mean N_2 flux rates across sites and depths in this study fall lower than most published N_2 rates (Burgin & Groffman, 2012; Duncan et al., 2013; Morse et al., 2015; Raciti et al., 2011; Weitzman et al., 2021). Papers were chosen across several land uses including agriculture, forests, and suburban lawns. Of the papers cited, one sampled at depths 1m below the surface (Weitzman et al., 2021). The remaining publications sampled in the top 20cm of soil to calculate denitrification rates. While our rates fell on the lower end of the range of published N_2 fluxes, this study found measurable production of N_2 from denitrification in four riparian sites that furthers the understanding of denitrification in these soils.

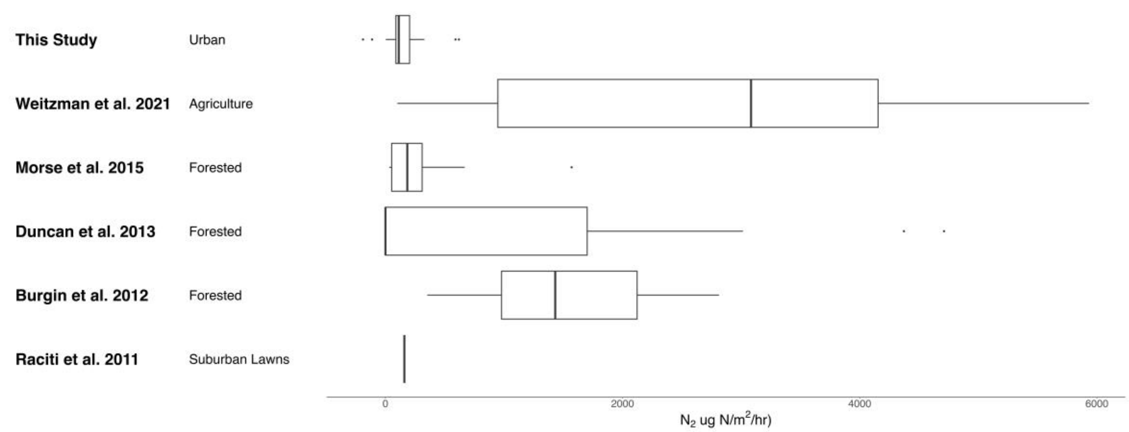

Figure **2-9**: Published denitrification rates from five papers using N-FARM to calculate denitrification flux from N2 production across land uses. Results from this study are presented here as an average flux by depth at four sites.

Our recorded rates were measurable and uncovered trends of urban riparian soils however, this landscape is highly retentive of N and there are likely hotspots unaccounted for. In Baltimore, MD, efforts to calculate retention of TN and NO_3^- have found urban watersheds to be less retentive of N across all study sites however, the most urban watersheds in this area remain highly retentive of N across wet and dry years (Bettez et al., 2015). These high levels of N retention expose gaps in understanding of the biogeochemistry of this landscape. It remains unclear to what extent NO_3^- removal from the landscape is contributing to these high retention rates and further work is important to gain a better understanding of this question.

Conclusion

This study used and built upon existing methods of capturing N_2 flux from denitrification in soil cores to observe the variability of denitrification rates across distinct riparian features. The objectives of this study were to (1) identify control points for denitrification across a heterogeneous urban riparian zone and (2) determine the characteristics of an urban riparian zone that are limiting denitrification. Unsurprisingly, we found high variability in soil gas fluxes across all four study sites and with depth however, we found the highest N_2 fluxes in the riparian stormwater wetland and at depth in the downstream lawn.

In residential neighborhoods, flow paths are often altered and interrupted by imperious road surface, roofs and gutters, and stormwater infrastructure. As a result, in these neighborhoods, often patches of lawn are key features of biogeochemical activity; and, lawns in suburban and urban areas are important for the retention, transformation, and transport of N (Raciti et al., 2008; Reisinger et al., 2016; Suchy et al., 2023). Here, we found the lawn site to have high N_2 flux 90cm below the surface. While there is high heterogeneity amongst lawns in terms of vegetation cover, maintenance, and fertilization practices, this finding is supported by previous studies which have demonstrated the potential of lawns to be key drivers of denitrification in suburban watersheds (Raciti et al., 2011). It also points to opportunities for future work to explore the biogeochemical function of deeper soil layers as it relates to N transformation. We found that at these deeper layers, despite decreasing NO_3^- supply, high N_2 rates were supported by optimal O_2 and soil moisture conditions.

We also measured high N_2 fluxes in the stormwater riparian wetland site. Initially, we hypothesized that this site would act as a permanent control point for denitrification due to the high drainage area and contribution from upslope impervious cover, high organic matter, and wetland vegetation. We found denitrification rates to be highest here at 20cm with high rates also found at 90cm deep. While this finding is somewhat unsurprising, it remains unknown to what extent similar features to this one exists in this urban watershed thus, it is unclear as to how much features like this one are contributing to high N retention here.

Additionally, we found that stream incision, limited denitrification rates. In a riparian zone on a highly incised segment of stream, soil O_2 remained high with dry soils throughout the soil profile. We found that while NO_3^- was comparable to other sites at the upstream incised site, the soil O_2 and moisture conditions limited high N_2 flux at this site. Because of the stream channel incision here, this site was far more disconnected to the floodplain than any other site in this study thus, creating more barriers for denitrification here. While this level of stream incision was only represented in one site in this study, stream channel incision to this extent extends throughout DR5 (Figure 2-7).

In this study we observed denitrification trends across a heterogeneous urban riparian zone and found great variability in soil biogeochemistry. These results confirm previous understanding of denitrification controls in urban riparian zones. However, they further demonstrate how variable biogeochemical processes are across the riparian zone. These results also point to a need for greater spatial analysis to put these rates into the context of a larger landscape. While we identified key features that are producing high N_2 flux rates, it remains unclear how widespread these features are across the watershed. To better predict the impact of these denitrification rates on the overall N budget, more spatial analysis is needed to identify similar patches across the landscape.

Chapter 3

Considerations for Future Work on Denitrification in Urban Riparian Zone

Avenues for future study

The results of the study laid out in Chapter 2 uncovered trends of denitrification across a heterogeneous urban riparian zone. We found great variability across sites with unique riparian features and found interesting and unexpected trends in deep soils at some sites. These results suggest the potential for ecosystem control points in riparian lawns and remnant riparian wetland features. However, we did not attempt to scale these findings to estimate the impacts of these features on NO_3^- removal from the landscape. To define these landscape features as control points in the watershed, efforts need to be made for further spatial analysis of DR5. Spatial scaling of denitrification—a highly variable process in both space and time—is made more challenging in a heterogeneous urban watershed. In this study we looked specifically at soil O_2, soil moisture, stream incision, drainage area, and upslope impervious area as some of the controls to denitrification. Mapping each of those variables individually as well as determining how closely they relate to one other is increasingly difficult.

One possible method for making broad estimates of denitrification across the riparian zone in DR5 is using topographic wetness index (TWI) as an indicator of soil moisture and O_2 conditions. TWI is a dimensionless index which considers both slope and local upslope contributing area (Beven & Kirkby, 1979). High values of TWI would indicate patches on the landscape that are frequently inundated and often collect runoff. TWI has been used previously to scale denitrification rates to the watershed scale and estimate the spatial distribution of denitrification on the landscape (Duncan et al., 2013; Whelan & Gandolfi, 2002). In the context of the results of this study, a TWI threshold could be assigned to each of the sites sampled based on their soil moisture and O_2 conditions. These thresholds could then be used to identify other patches in the riparian zone with similar soil conditions to the sites where N_2 flux was calculated. Then these rates could be scaled to the corresponding patches in the riparian zone. While this method would not explicitly scale denitrification rates with depth, these steps could produce compelling new results that give insight on the impact of NO_3^- removal in urban riparian zones at the watershed scale.

Estimating the impact of NO_3^- removal in urban riparian features is an important next step in unpacking high rates of N retention in urban watersheds. While there is great evidence to support that riparian zones in other watersheds are control points for denitrification, there is additional evidence to support the existence of control points in upslope positions of urban watersheds. One of the most surprising results from this study was that N_2 flux from denitrification were amongst the highest in a residential lawn at 90cm beneath the surface. This was surprising because we found that all sites had decreasing supplies of $NO3^-$ at 90cm deep. Additionally, factors such as root biomass and organic matter are known to decrease with depth (Gift et al., 2010). However, despite these limitations to denitrification the soil O_2 and moisture conditions were optimal at this depth to support high rates of N_2 flux from denitrification.

These results are supported by research on denitrification trends in more upslope lawns which have also found that these features are important patches for NO_3^- removal in urban and suburban watersheds (McPhillips et al., 2016; Newcomer et al., 2012; Raciti et al., 2011). Additionally, efforts to calculate denitrification potential within stormwater infrastructure have found these structures to be important features for denitrification (Bettez & Groffman, 2012; McPhillips & Walter, 2015). Evidence for control points of NO_3^- removal in urban watersheds exists beyond the riparian zone and, are important features to consider in any attempts to close the N budget gap.

Limitations to methodology

One major limitation to the N-FARM that we came up against is how time prohibitive the method is. As a result, there were some limitations to how many samples can be taken at a time and how often sampling can happen. The N-FARM mechanism allows for eight samples (in addition to one blank) to be analyzed at a time. Depending on the number of samples in a single run, analysis took between 9-12 hours a day. Each sample is exposed to the same set of flow through structures which only allows for one O_2 concentration to be used at a time. We exposed soil cores to four concentrations therefore, all analysis took at least four days to complete. These time constraints limited the number of cores we could sample in a single set because additional soil cores would remain chilled until they were able to be run thus, extending the time that samples were spending in the refrigerator. To reduce any effects on the soil samples due to these longer wait times, we limited the number of cores taken each season to only what could be analyzed over a four-day period.

In this study, we collected multiple 1m-deep soil cores four times over the period of one year. To minimize the effects of spatial variability, we tried taking soil cores from the same 1m by 1m plot at every site. As a result, it became increasingly challenging to find spaces in these plots to extract additional soil cores. This limited the frequency of sampling at each site as frequent core extraction would alter the conditions of the soil an ultimately influence the results of our analysis.

As a result of these limitations to frequency and quantity of soil core extraction, the methods in this study did not look for denitrification trends in post storm conditions and, in general conducted little temporal analysis of N_2 flux. We found that on the days where soil cores were extracted, soil O_2 and moisture most often reflect the average conditions at the sites rather than their response to wetting events (Figure 2-3, 2-4). Thus, the denitrification rates calculated and presented here are most closely reflecting the average condition of these soils. While the time series of soil O_2 and moisture give insight to soil conditions during storm events, we can only speculate that at some sites, wetting events are likely triggers for periods of high N_2 flux. We found in many surface soils, O_2 and moisture would react to storm events however, in some sites, these conditions would linger for days or weeks in some cases. Sampling during these post storm periods in sites that are retaining soil moisture after a precipitation event could give glimpses into denitrification rates in these activated sites that are experiencing more temporal variation.

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Appendix

Additional results from soil gas flux analysis

Table **A-1**: Site averages of N_2, N_2O, and CO_2 flux across four riparian soil plots in DR5. Gas flux data from autoclaved soil samples taken at the corresponding riparian soil plots. Only one sample at each site was autoclaved and analyzed. Samples for autoclaving were taken during the Winter and Spring 2023 seasons and cores were run at either 0% or 5% O_2 concentrations.

Mean Gas Flux Data from Riparian Soils in Baltimore County, Maryland

	Downstream Brush			Downstream Lawn			Riparian Incised			Riparian Wetland		
Depth (cm)	10	20	90	10	20	90	10	20	90	10	20	90
N_2 flux (µg N/m2/hr)												
Mean	93.0	31.3	334.7	51.6	38.8	648.3	110.4	122.9	133.2	103.6	527.3	313.9
SD	170.3	46.0	422.5	64.8	49.8	702.3	85.0	49.9	146.7	152.8	629.3	272.4
N_2O flux (µg N/m2/hr)												
Mean	2.73	0.42	0.29	3.52	0.86	1.17	0.20	1.62	0.37	0.35	-0.32	0.06
SD	4.17	0.39	0.67	6.51	1.06	1.34	0.24	3.31	1.10	0.53	0.67	0.08
CO_2 flux (µg N/m2/hr)												
Mean	12920	4012	1456	4451	1890	1327	4926	2488	444	2543	1814	732
SD	10413	3701	830	3194	1288	391	1300	795	463	867	780	419

Gas Flux Data from Autoclaved Soil Samples

	Downstream Brush			Downstream Lawn			Riparian Incised			Riparian Wetland		
Depth (cm)	10	20	90	10	20	90	10	20	90	10	20	90
N_2 flux (µg N/m2/hr)	0.00	0.00	696.71	507.54	0.00	0.00	0.00	238.15	0.00	114.26	15.29	129.71
N_2O flux (µg N/m2/hr)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CO_2 flux (µg N/m2/hr)	1095.63	0.00	47.98	246.02	172.00	60.94	203.07	0.00	65.91	250.00	212.86	72.00