

Reach-scale Denitrification and Nitrogen Concentrations in Six Streams Draining Grassland and Cropland Landscapes

By

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Abstract

Denitrification is a critical microbial process that removes bioavailable nitrogen (N) in natural and human-altered ecosystems. Methods for measuring denitrification in aquatic ecosystems are fairly studied. However, the understanding of ecosystem metabolism and denitrification factors in agriculturally influenced streams is limited. We wanted to evaluate the connection between nitrogen (N) cycling and ecosystem metabolism in headwater streams affected by land use due to the increase of agricultural N pollution and its negative impact on downstream water quality. Here, we quantify N concentrations and denitrification in six headwater streams draining contrasting land use watersheds using the open-channel single station approach. We compared two contrasting watersheds; (a) cropland land use, representing the excessive agricultural N pollution to streams, and (b) grassland land use, representing original land use type prior to agricultural N pollution. To understand in-stream N processes and ecosystem metabolism, we analyzed diel patterns of nitrate (NO_3^-) concentrations and dissolved gasses (O_2 , Ar, N_2 , N_2O) during the summer of 2019. We estimated open-channel reach-scale denitrification, gross primary production (GPP), and ecosystem respiration (ER). Streams draining primarily cropland had higher NO_3^- concentrations ($4.2 \text{ NO}_3^- \text{-N mg L}^{-1} \pm 0.11$) associated with higher metabolism rates. Higher N concentrations drove higher denitrification rates among cropland-dominated streams (mean denitrification rates [$0.31 \text{ g/m}^2 \text{ day} \pm 0.4$] in cropland-dominated streams vs. [$0.01 \text{ g/m}^2 \text{ day} \pm 0.01$] in grassland-dominated streams). Overall, the excess nutrients from the agricultural landscapes resulted in higher nitrate concentrations and rates of metabolism compared to the grassland landscapes. Estimates of denitrification and stream metabolism are needed from underrepresented systems to aid in the quantification of nitrogen fluxes, locally and globally.

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Dedication

This is dedicated to the many people who supported me throughout my education journey, from K-12 to Haskell to the hill.

I would also like to dedicate this to my brothers and other native youth—always believe in yourself and never give up.

Kepiichihii

Table of Contents

Abstract.....	iii
Acknowledgements.....	iv
Introduction.....	1
Methods.....	4
Site Description.....	4
Field Sampling.....	6
Laboratory Methods.....	9
Denitrification and Metabolism Modeling.....	9
Statistical Analysis.....	10
Results.....	11
Discussion.....	16
Conclusion.....	21
References.....	22

List of Tables

Table 1: Study site characteristics and parameters.	5
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List of Figures

Figure 1: <i>Map of Delaware River Watershed</i>	6
Figure 2: <i>Nitrate and Chloride concentrations</i>	12
Figure 3: <i>Nitrate and Dissolved oxygen concentrations</i>	13
Figure 4: <i>Denitrification and Metabolism rates</i>	14
Figure 5: <i>Ecosystem Respiration versus Gross Primary Production</i>	15
Figure 6: <i>Relationships between DEN and Nitrate, GPP, and ER</i>	15

Introduction

Agricultural nitrogen (N) pollution in the Mississippi River watershed has continued to create problems for downstream water quality as N loading to freshwater ecosystems continues to increase yearly (Green et al., 2004; Gruber & Galloway, 2008). Excessive N export consequently impacts the downstream water quality. For instance, the Gulf of Mexico's infamous "dead zone", an oxygen-depleted zone that ultimately makes water quality unsuitable for aquatic life and recreational use (Rabalais et al., 2002). Additionally, 58 percent of streams in the lower Mississippi River basin (Midwest) are N impaired, which is a management classification indicating the water may not meet quality standards for desired uses (Sobota et al., 2015). Land use change, primarily the intensification of agricultural land and management practices, is the driver of much of the excess nitrogen in the basin (Giri & Qiu, 2016). Synthetic N fertilizers are applied to improve crop yield for an owner's yearly production, conversely degrading the quality of surface water and groundwater supplies (Carpenter et al., 1998). Moreover, a vast amount of the synthetic N-based fertilizers are not fully absorbed into soils but eventually runoff into streams to either be removed through denitrification or integrated into biomass (Mulholland et al., 2008). Thus, we must continue to work toward a better understanding of how human-influenced streams remove excess N through reach-scale denitrification.

Denitrification is a focal microbial process for studying nitrogen processing in streams and rivers due to the relatively "permanent" removal from these impaired systems (Groffman et al., 2006). Denitrification produces di-nitrogen (N_2) gas by microbially converting nitrate and nitrite (Groffman et al., 2009). Thus, it is an important process as it is the only mechanism in aquatic ecosystems that transforms nitrate or nitrite back into N_2 , the dominant gas of our

planet's atmosphere (Boyer et al., 2006). Components from the landscape, such as nitrogen-rich fertilizers applied to croplands that flow into streams, function as hot spots for denitrification (Groffman et al., 2009). In the Midwest, agricultural sources of N input dominate the Mississippi River watershed because it drains the heartland of American agriculture. Fortunately, up to 75% of N exported through the Mississippi River watershed can be removed before reaching the infamous dead zone at the mouth of the Mississippi River (Burgin & Hamilton, 2007; Howarth et al., 1996). Thus, it is important to understand the factors that control the process of denitrification over space and time to help quantify the effects of anthropogenic activity on the N cycle (Boyer et al., 2006).

Denitrification, however, is a challenging process to measure because 1) it is difficult to quantify the output of N_2 given its high background concentration within the atmosphere and 2) denitrification is hindered by high spatial and temporal variation in the process, leading to an abundance of methods to assess realistic rates that can be scaled (Groffman et al., 2006). For instance, the ^{15}N tracer method relies on an incubating technique that adds a ^{15}N tracer to aquatic ecosystems to differentiate in-stream denitrification. However, this addition increases N availability and so may overestimate denitrification in N limiting streams, for example those in relatively unimpacted headwaters (Groffman et al., 2006). The method is also quite expensive. New techniques for measuring reach-scale denitrification have been developed in freshwater research but are rarely used in studies of headwater stream denitrification (Nifong et al., 2020). One key new technique is open-channel N_2 quantification which uses a $N_2:Ar$ ratio and membrane inlet mass spectrometry (MIMS) to quantify N_2 production, assuming Ar concentrations are at equilibrium with the atmosphere (Kana et al., 1994). This method is increasingly found in studies of reach-scale denitrification because open-channel + MIMS

measurements are rapid and precise and has minimal disturbance to streams allowing for natural conditions (Baulch et al., 2010; Hanrahan et al., 2018; McCutchan et al., 2003; Reisinger et al., 2016). Even with new effective techniques like open-channel N_2 and MIMS analysis, a majority of reach-scale denitrification estimates have been conducted in small streams with discharge less than $0.1 \text{ m}^3 \text{ s}^{-1}$ (Hall et al., 2016). Moreover, previous studies do not entirely represent whole-ecosystem denitrification rates because past techniques do not incorporate the diel (day to night; 24 hours) dynamics of headwater stream conditions of light and temperature (Nifong et al., 2020). A central goal of this work is to contrast reach-scale denitrification among sites using the open-channel N_2 flux method by sampling *in situ* over diel cycles (Kana et al., 1994; Reisinger et al., 2016).

Stream ecosystem metabolism integrates production and respiration and plays an important role in biogeochemical processes. Ecosystem metabolism can be determined by measuring gross primary production (GPP) and ecosystem respiration (ER) to help indicate the fixation and mineralization of organic carbon sources in aquatic ecosystems. Metabolism, in turn, controls biogeochemistry and the transport of nutrients within and through streams (Dodds et al., 2018; Ensign & Doyle, 2006). Reach-scale ecosystem metabolism measurements are useful for determining abiotic and biotic factors that control nutrient dynamics in streams that are anthropogenically impacted, including stream temperature, light availability, hydrology, nutrient concentration, and organic matter availability (Bernot et al., 2010; Dodds et al., 1999; Mulholland et al., 2001). However, ecosystem metabolism is not well-characterized in headwater streams draining agriculturally influenced landscapes (Griffiths et al., 2013). Agriculturally influenced streams are primarily eutrophic, but can be net sinks for CO_2 , due to high rates of in-stream primary production from the increased light availability from vegetation conversion

(Wiley et al., 1990), or in other cases are net sources of CO₂ due to respiration of allochthonous organic matter, such as riparian grasses and crop debris (Fuß et al., 2017; Griffiths et al., 2012). Studying diurnal variations can help establish which biogeochemical processes play an integral role in human impacted streams. Additionally, better quantifying stream GPP and ER variation among contrasting land uses and incorporating diurnal fluctuations is critical to better estimate nitrogen fluxes and the removal of nitrogen from streams by incorporation into biomass.

To better understand the effects of human-influenced landscapes on N cycling, we conducted a comparison between three watersheds that are predominantly row crop agriculture, to three watersheds that are predominantly grassland, representing pre-agricultural land cover. Our overall objective in this study is to quantify how land use affects in-stream denitrification and metabolism over a 24-hr period. We hypothesized that: 1) cropland-dominated streams would have higher nitrate concentrations due to greater N export from agricultural landscapes, 2) nitrate concentrations among cropland-dominated streams will demonstrate a diel (day-night) pattern while displaying shallow variation in grassland-dominated streams, and 3) rates of denitrification, GPP, and ER would be greater among the cropland sites compared to the grassland sites due in large part to the greater input of nitrogen. To measure N removal in our headwater streams via the process of denitrification, we estimate reach-scale denitrification using the open-channel N₂ approach based on reach-scale metabolism methods (Reisinger et al., 2016).

Methods

Site Description

Six study streams were selected in the Delaware River Watershed in northeast Kansas, USA (Table 1; Figure 1). Additionally, the Delaware River Watershed is home to three tribal

reservations; The Kickapoo Tribe in Kansas (who utilizes the Delaware River surface water for drinking water purposes), The Sac and Fox Nation of Missouri and Nebraska in Kansas, and The Prairie Band Potawatomi Nation. Stream sampling sites were selected based on land-use in their respective sub-watershed and catchment size (1st-3rd order streams). The selected streams were categorized as cropland or grassland after determining the dominant land cover within each sub-watershed found with the use of geographic information system (GIS) data layers. DEM data layers were downloaded by county from USDA Geospatial Data Gateway (<http://gdg.sc.egov.esda.gov>) at 30m resolution. This study was a part of a larger Kansas NSF EPSCoR Track-1 project that studies Microbiomes of Aquatic, Plant, and Soil Systems (MAPS) across Kansas' land-use and precipitation gradient. Land use data for MAPS is available on the Kansas Data Access and Support Center (DASC). The 2005 Kansas Land Cover Patterns(KLCP) Mapping initiative was created by the Kansas Applied Remote Sensing (KARS) Program within the Kansas Biological Survey (KBS).

Study site/code	Subwatershed size (ha)	% subwatershed in designated land use		Latitude, Longitude	Sampling date	Average depth (m)	Wetted width (m)	Discharge (L/s)	Stream temperature (°C)	
		Cropland	Grassland							
Cropland										
C1	746	77.9	20	39°53'6.87"N, 95°49'55.50"W	July 25-26 2019	0.10	3.5	0.11	19.99	
C2	938	73.1	24	39°46'27.5"N, 95°45'08.5"W	August 4-5 2019	0.21	3	1.41	21.89	
C3	930	82.7	14	39°45'37.04"N, 95°36'5.60"W	August 19-20 2019	0.1	0.9	0.75	26.21	
Grassland										
G1	751	3.7	81.7	39°27'36.91"N, 95°50'56.60"W	August 24-25 2019	0.34	7	3.24	19.75	
G2	879	5.3	78.6	39°26'3.66"N, 95°47'34.42"W	August 24-25 2019	0.08	2.5	5.12	20.56	
G3	675	22	71.4	39°21'41.04"N, 95°43'10.52"W	August 13-14 2019	0.07	4.9	1.00	25.92	

Table 1: Study site characteristics and parameters.

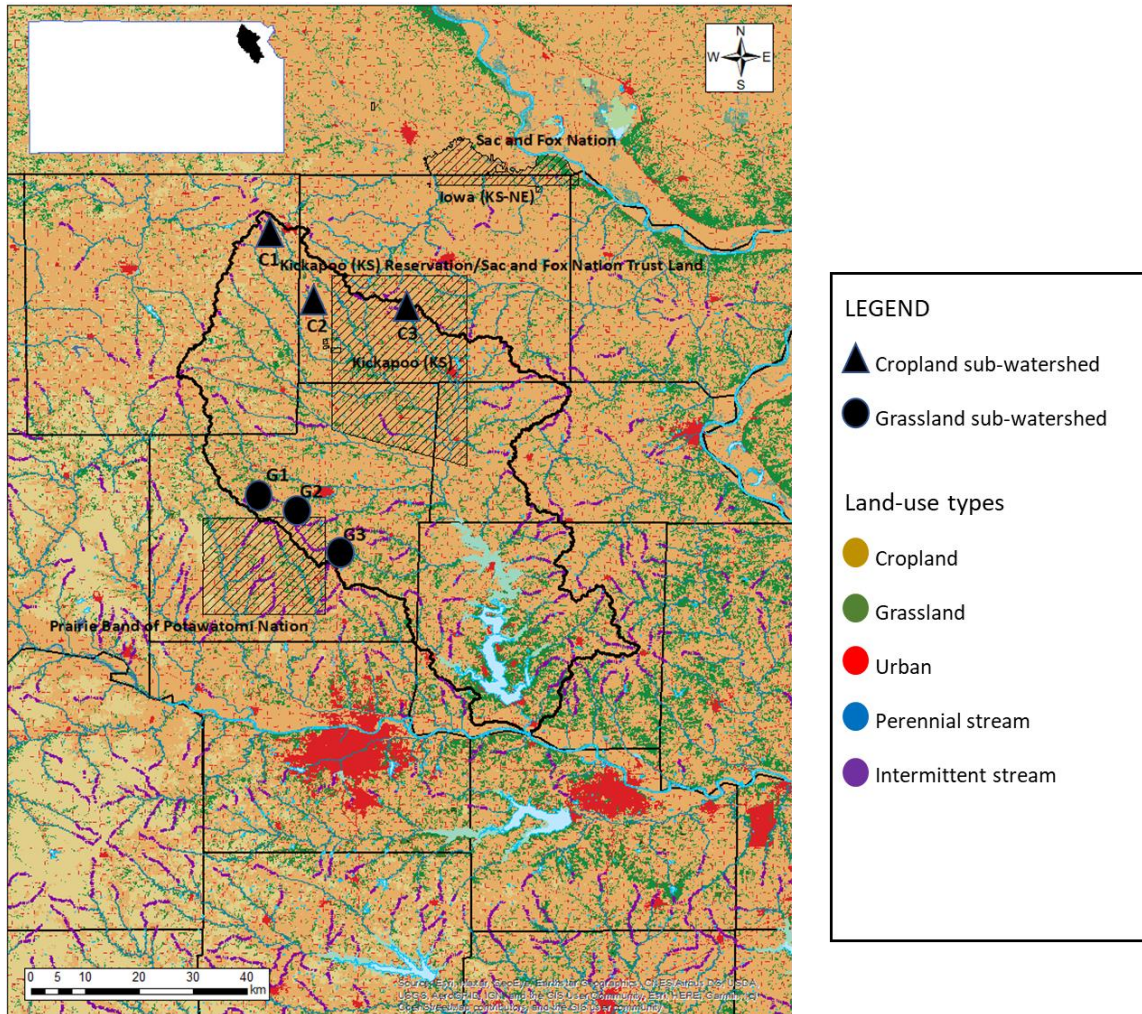


Figure 1: Study sites in the Delaware River Watershed, Kansas, USA.

Field Sampling

To measure nutrient supply, uptake, and stream metabolism, we deployed sensor arrays at each study site (Table 1). The sensor arrays were secured within 10 ft of nearby public road access from bridges at each site. Stream access was limited due to surrounding private properties at five of our sites except for site G3, we were granted access upstream from the public bridge to station our sensor array and complete the diel sampling. Dissolved O₂ (DO) and stream water temperature were measured every 30 minutes with a PME miniDO₂T® logger with an

antifouling copper plate attachment (Vista, CA) that was stationed upstream from each sampling site and calibrated before deployment. The sensor array also included a Photosynthetically Active Radiation (PAR) Odyssey logger, HOBO® conductivity logger, and HOBO® water level logger; all collecting in 10-minute intervals. Sensor arrays were secured throughout the entire field season (March-December). Measurements from the sensor arrays allow us to quantify spatiotemporal changes within the headwater streams. Stream discharge (Q , m^2/s) were estimated from measurements of depth, width, and velocity taken twice; within the first two hours and last two hours of diel sampling. Using a 10-point cross-section transect of the stream; depth and width were measured manually with a 36-m tape measure and velocity (m/s) was measured using a flow meter.

We conducted hourly sampling with 24 time points for the open-channel approach among each study site on separate days (Table 1). To measure reach-scale metabolism, we collected triplicate dissolved gas samples using a 24-inch PVC pipe with tubing attached to the bottom-side and a rubber stopper bottom. We submerged the whole PVC pipe into the water column, parallel to flow, allowing the stream to flow through for ~10 seconds before capping the PVC pipe. Next, we overfilled three 12mL Exetainer (Labco, UK) sample vials with the collected stream water, from the bottom-up to limit any atmospheric interaction and discarded the first 5 mL. We injected 0.2 mL of zinc chloride ($ZnCl_2$) to preserve the sample which was stored underwater in a 4°C cooler until analysis.

We retrieved barometric pressure data for each site using Weather Underground (www.weatherunderground.com) from the closest weather station nearby each site during their respective sampling dates. Real-time measurements of pH, DO, conductivity, and water

temperature were provided by the YSI ProDSS sensor and a HATCH Nitratex Plus SC UV sensor with an optical path length of 2mm measured real-time NO_3^- -N.

Water chemistry samples (e.g., Sulfate (SO_4), Nitrate (NO_3^-), and Chloride (Cl)) were collected in triplicates hourly from the stream water column and immediately filtered into clean 65 mL Nalgene bottles through 0.45 mm filters that were later stored at 4°C pending analysis. Triplicate unfiltered samples (e.g Soluble Reactive Phosphorus (SRP) and Ammonium (NH_4)) were collected in clean 125 mL Nalgene bottles and acidified with 2-3 drops of sulfuric acid, shaken well and when returned to the lab they were stored at 4°C pending analysis.

Gas exchange samples (e.g., Nitrous Oxide (N_2O), Methane (CH_4), and Carbon Dioxide (CO_2)) were collected hourly in triplicate. We used 60 mL syringes to collect stream water from the water column, then pushed out excess air and water to reach 40 mL of stream water, avoiding any air bubbles left over. Next, we injected 20 mL of Helium (He) into the sample-filled syringe. The sample syringe was then manually shaken for 2 minutes to reach headspace equilibrium (Hamilton & Ostrom 2007). Carefully, we transferred the equilibrated gas into pre-evacuated 6 mL Exetainer (Labco, UK) vials, using stopcocks to transfer samples underwater to avoid outside air contamination. Samples were stored at room temperature pending analysis.

Due to an unforeseen lightning storm that occurred shortly during our G2 24-hr study at time point 20, we didn't sample for safety. To fill in the missing data point for analysis we averaged the bracket of concentrations from time points directly before and after time point 20. Additionally, our PME miniDO₂T® loggers lost measurement connection during diel sampling of sites G2 and G3. We used O₂ measurements quantified from the MIMS to replace missing data points.

Laboratory Methods

Using the MIMS (Bay Instruments, Easton, MD, USA) we quantified dissolved N₂, Ar and O₂ (Kana et al., 1994). Each water sample was pulled through a peristaltic pump and the dissolved gasses in the sample were diffused across a membrane vacuum to be measured by the mass spectrometer for abundance of ²⁸N₂, ³²O₂, and ⁴⁰Ar. Instrument drift was corrected with checks every 6 - 15 samples by analyzing a standard consisting of purified water (18 M Ω resistance; E-Pure, Barnstead International, Dubuque, IA) maintained at 18.2°C - 28.6°C, depending on site *in situ* temperature, using a circulating water bath (VWR International, Radnor, PA, USA) continuously stirring at a low speed to equilibrate with atmospheric gasses (Lab Egg RW11 Basic, IKA Works, Inc., Wilmington, NC, USA). The peristaltic pump was stopped while exchanging sample vials. To calculate concentrations of Ar, O₂, and N₂ for each sample, we multiplied the N₂:Ar and O₂:Ar ratios quantified by the MIMS by the Ar equilibrium concentration using equations derived by Hamme & Emerson, 2004 based on barometric pressure and room temperature within the laboratory. Ratios were used rather than concentrations due to the more precise output of measurements from ratios (Kana et al., 1994).

Analysis of NH₄⁺ was completed with the Fluorescence Spectrometer; NO₃⁻, Cl, and NO₂ were analyzed with Ion Chromatography (IC); SRP was analyzed with the SmartChem (Westco Instruments); and NO₂ was analyzed using Gas Chromatography.

Denitrification and Metabolism Modeling

Gas exchange and ecosystem metabolism modeling was completed using the open-channel diel N₂ model developed by Reisinger et al. (2016) and using the single station approach (Odum, 1956). The model included parameters of; stream temperature, barometric pressure,

discharge, DO diel time-series, PAR diel time-series, and concentrations of Ar, O₂, and N₂ sampled during our 24-hr studies. This model uses Bayesian parameter estimation to solve for gas exchange (K, d⁻¹), denitrification (DEN, g N m⁻² h⁻¹), gross primary production (GPP, g O₂ m⁻² d⁻¹), and ecosystem respiration (ER, g O₂ m⁻² d⁻¹). The model gives three outputs based on either real-time DO sensors, N₂:Ar ratio, and O₂:Ar ratio. The first output of ER, GPP, and K come from the “base model” using a single station, open-channel O₂ exchange metabolism method allowing K to be calculated from oxygen using data from our stationed sensor array (e.g., DO, temp, and PAR). The second output of DEN and K derives from the “N₂:Ar model” that uses estimates of K calculated in the first output series. A third output of ER, GPP, and K are given based on O₂:Ar ratio of dissolved gas collected from the stream, which were analyzed through MIMS. All model outputs were generated through R software (version 3.6.1) using the MIMSY package created by Michelle Kelly (<https://github.com/michelleckelly/mimsy>, 2018) and JAGS coupled with the *rjags* package (Reisinger et al. 2016).

Statistical Analysis

We used a two-sided, two-sample Student’s t-test to statistically assess the difference between means of GPP between the two contrasting land use types. The same statistical approach was used to test the difference between means of the absolute value of ER between grassland and cropland land uses. We used a one-way ANOVA to test the difference in variance between grassland and cropland land uses for GPP, ER, and DEN. We used a linear regression to calculate a relationship between DEN and its potential drivers (NO₃⁻-N, ER, and GPP) that assumes there is a direct correlation between variables. Additionally, we calculated the P:R ratio (GPP:ER), whereas streams with a P:R > 1 are considered autotrophic and streams with a P:R <

1 are considered heterotrophic. Statistical testing was completed using R software (version 3.6.1) with the default statistical testing package *stats* and the *data analysis* tool in Microsoft Excel.

Results

Northeast Kansas is majorly covered by agricultural related land use (Figure 1). All streams we sampled are in the Delaware River basin, which drains to Perry Reservoir (operated by the Army Corps of Engineers). Streams draining grassland sub-watershed are categorized as tributaries that lead to Banner Creek Reservoir. Cropland streams are perennial and have about 73 to 83 percent cropland land use type in their respective sub-watershed (Table 1). Watershed sizes were kept comparable among the six study streams (~675-938 ha) but were limited by access points given nearly all the surrounding land is privately owned.

Nitrate (NO_3^- -N) concentrations were highest among cropland sites compared to grassland sites (Figure 2). Nitrate was significantly higher in cropland streams compared to grassland streams ($t(2) = 4.3, p = 0.07$), with mean NO_3^- -N concentrations for cropland streams of 4.2 NO_3^- -N $\text{mg L}^{-1} \pm 0.11$ and 0.2 NO_3^- -N $\text{mg L}^{-1} \pm 0.01$ for grassland streams (Figure 2A). Mean Cl^- concentrations among streams of both land use types remained relatively consistent during their respective 24-hr study but averages varied among all streams and between land use types. Mean Cl^- averages among grassland streams (i.e., $14.7 \text{ Cl}^- \text{ mg L}^{-1} \pm 1.3$, $38.8 \text{ Cl}^- \text{ mg L}^{-1} \pm 3.0$, $30.5 \text{ Cl}^- \text{ mg L}^{-1} \pm 2.2$) were higher in concentration than cropland streams (i.e., $16.9 \text{ Cl}^- \text{ mg L}^{-1} \pm 0.3$, $6.5 \text{ Cl}^- \text{ mg L}^{-1} \pm 0.3$, $10.7 \text{ Cl}^- \text{ mg L}^{-1} \pm 1.0$) (Figure 2C, D).

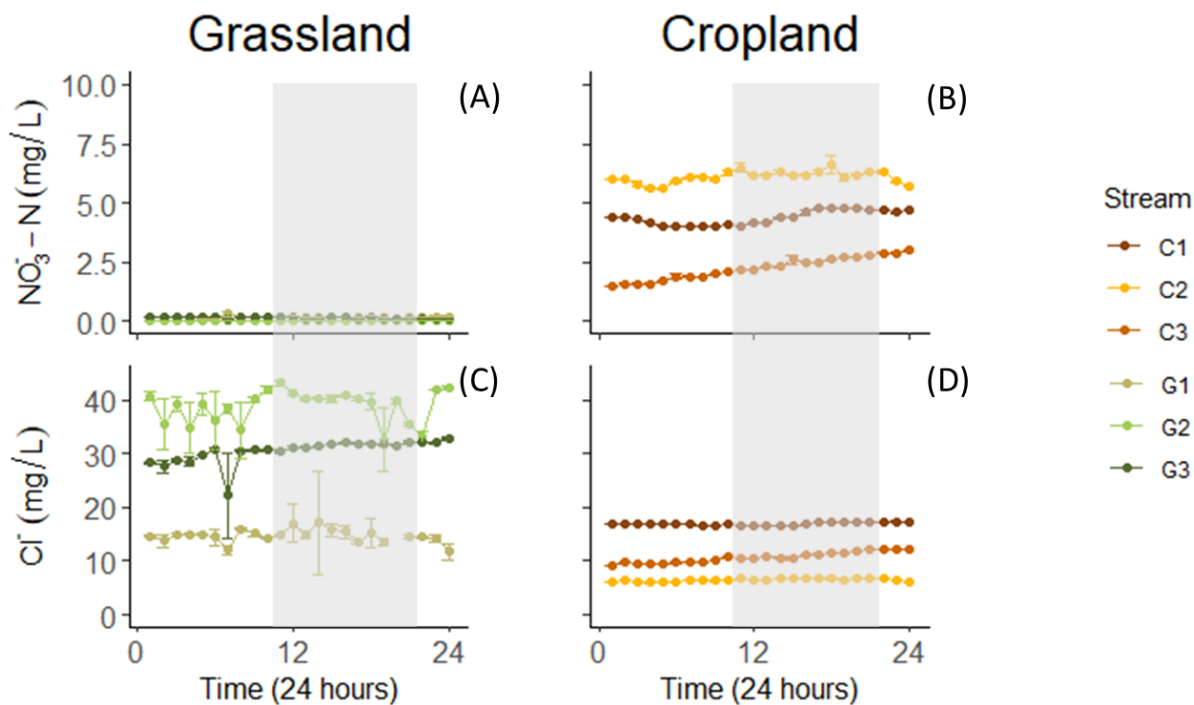


Figure 2: Mean (\pm SE) hourly NO_3^- -N concentrations among (A) grassland land use and (B) cropland land use. Mean (\pm SE) hourly Cl^- concentrations (C,D). Gray boxes indicate night hours.

We found distinctive diel swings in DO among two of the cropland subwatershed streams (Figure 3). The largest diel change in DO was in the cropland site C1 that ranged from 5.4 mg/L to 11.2 mg/L. Additionally, a similar swing was found in the cropland site C2 which ranged 6.4 mg/L to 8.2 mg/L. While the smallest variation was in the grassland site G2 with a change of 6.2 mg/L to 7.7 mg/L. However, DO presented dampened diel patterns in sites G1 and G3 of the grassland-dominated streams. And sites G1 and C3 presented distinct fluctuations in DO. Overall, oxygen consumption was generally higher during the night hours.

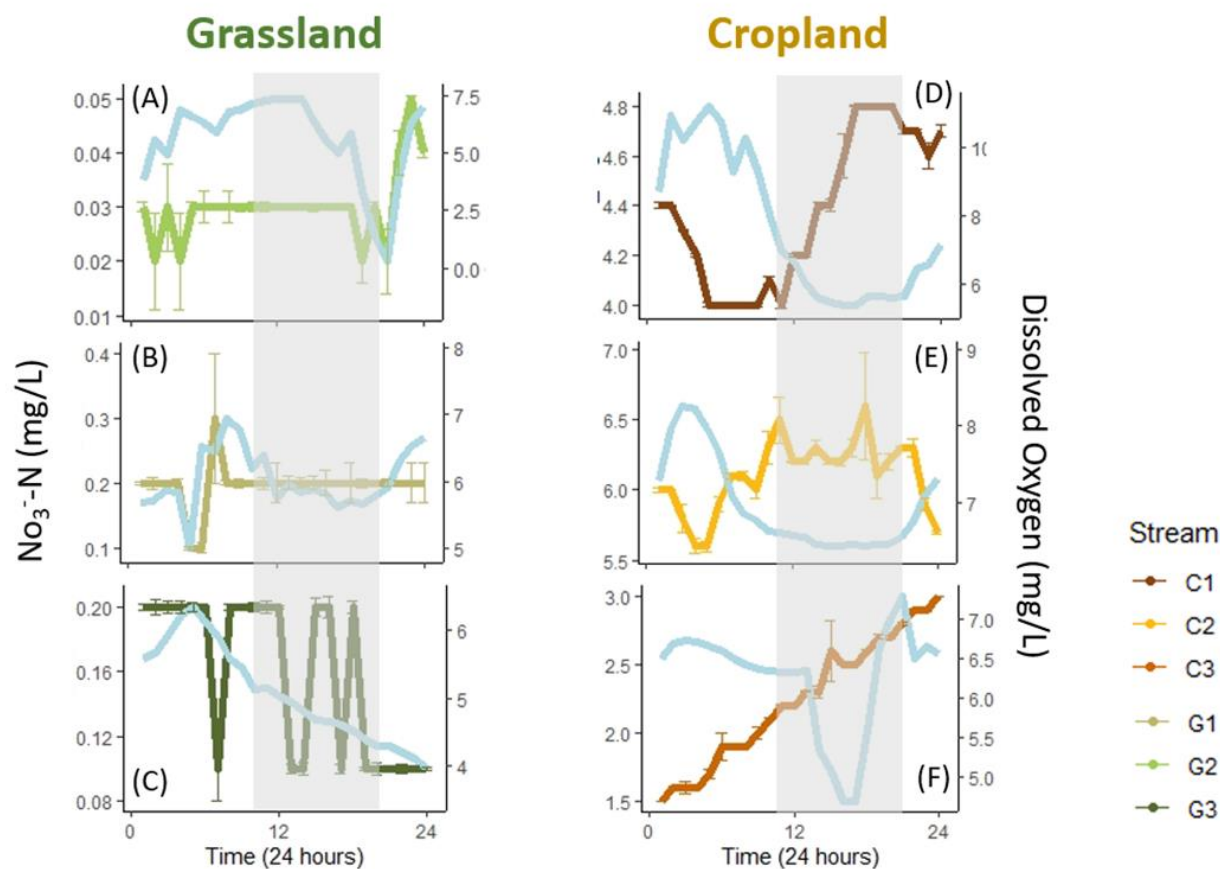


Figure 3: Mean hourly $\text{NO}_3\text{-N}$ concentrations (left y-axis) with Dissolved oxygen concentrations (right y-axis) indicated by light blue lines among grassland land use (A,B,C) and cropland land use (D,E,F).

Both GPP and ER varied several-fold between site but was not consistently different based on grassland and cropland land uses (Figure 4). For individual sites, mean GPP values ranged from 0.039 to $0.59 \text{ g O}_2 \text{ m}^{-1} \text{ d}^{-2}$ and mean ER values ranged from 1.69 to $9.70 \text{ g O}_2 \text{ m}^{-1} \text{ d}^{-2}$ (Figure 4 A, B). We found a strong correlation between ER and GPP, however, there was no significant relationship (Figure 5; $R^2 = 0.51$). ER rates were highest among cropland streams than grassland streams (Figure 5). Between the comparing land use types, there was no statistical difference among GPP rates, but GPP was consistently higher than ER in all study streams

(Figure 5). The calculated P:R ratio was less than 1 for all grassland-dominated and cropland-dominated streams.

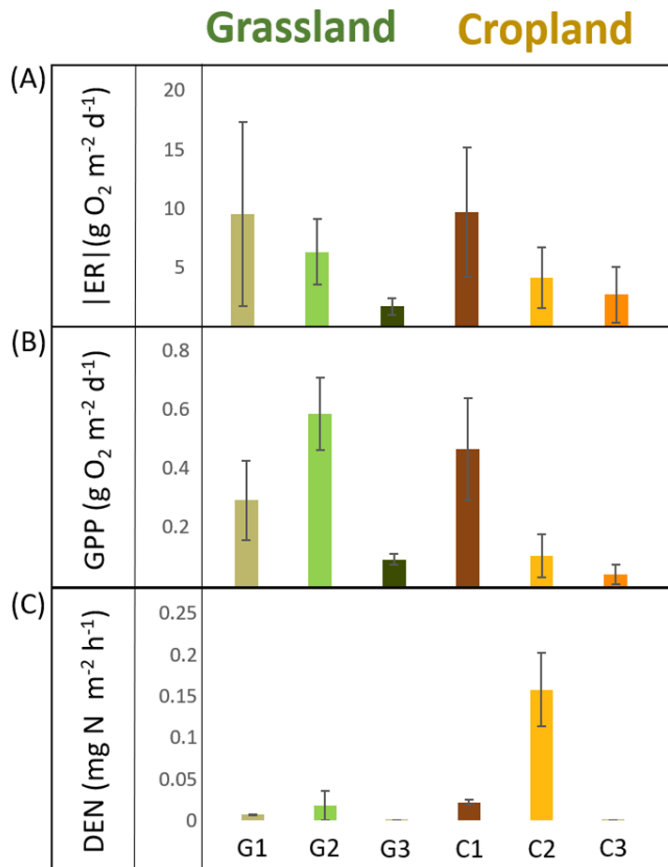


Figure 4: Denitrification (*DEN*) (A), gross primary production (*GPP*) (B), and absolute value of ecosystem respiration (*|ER|*) (C) among our 6 study sites.

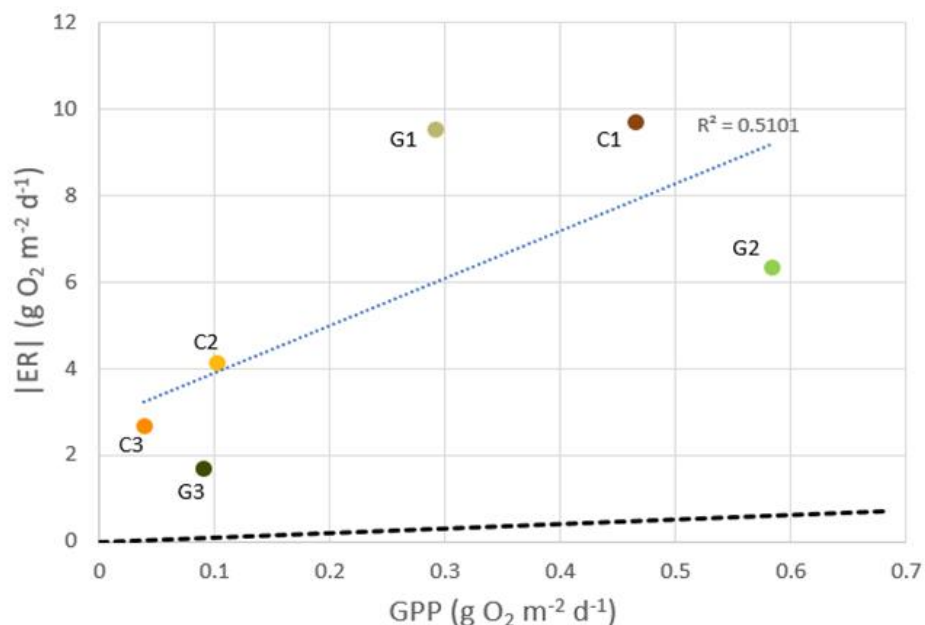


Figure 5: Ecosystem respiration (ER) versus gross primary production (GPP) for our 6 study streams. Dashed line indicates $GPP=ER$.

DEN rates among all six sites were relatively low (0.16 to 0.00023 $g\ m^{-2}\ d^{-1}$) despite having higher rates among the cropland land use sites (Figure 4C). Factors controlling DEN represented a strong correlation between NO_3^- -N and DEN among both grassland and cropland land uses but the relationship was not significant (Figure 6A). DEN and GPP had a positive correlation among the cropland sites, but the relationship was not significant. However, among the grassland sites, we found a significant relationship between GPP and DEN ($R^2 = 0.99$, $p = 0.03$; Figure 6B). DEN and ER were positively related among both comparing land use types but not significant.

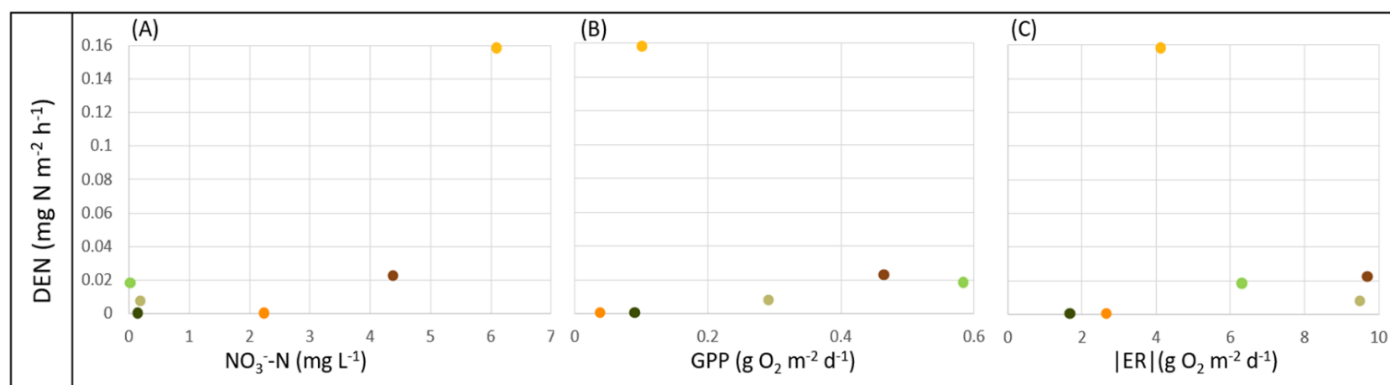


Figure 6: Relationships between denitrification and nitrate (NO_3^- -N) (A), gross primary production (GPP) (B), and ecosystem respiration ($|ER|$) (C) for all 6 study streams.

Discussion

For this study, we asked how contrasting land uses may affect denitrification and nitrogen cycling in small streams. We hypothesized that: 1) higher NO_3^- -N concentrations would be found in cropland-dominated streams due to nitrogen rich fields, 2) a diel pattern would be visible in NO_3^- -N concentrations among cropland-dominated streams while displaying shallow variation in grassland-dominated streams, and 3) cropland-dominated streams would have greater rates of DEN, GPP, and ER due to high nitrogen supply from the agriculturally impacted landscape. As expected, streams draining nitrogen rich croplands had higher NO_3^- -N concentrations compared to the grassland watershed sites (Figure 2A, B). The Cl^- concentrations were relatively consistent through each study sampling, supporting the integrity of our NO_3^- -N data (Figure 2C, D). ER and GPP were coupled among the cropland-dominated streams but not the grassland-dominated streams (Figure 5). We found higher ER rates associated with low rates of GPP (Figure 4) and positive relationships between metabolism (GPP, ER) and both land use types. Lastly, we found that NO_3^- -N, GPP, and ER had a positive correlation with DEN, except for GPP in grassland sites (Figure 6), however this was not significant. This suggests that NO_3^- -N concentrations, GPP and ER rates are all driving factors that influence DEN outcomes in our headwater streams that are agriculturally impacted.

Agriculture is a major source and supplier of excess N pollution, and this reflects in our cropland-dominated streams with higher NO_3^- -N concentrations compared to concentrations in grassland-dominated streams (Figure 2B). The NO_3^- -N concentrations that we observed in our cropland-dominated streams were similar to those observed in other agriculture watersheds (Bernot et al., 2006; Inwood et al., 2005; Mulholland et al., 2008). We expected nutrient rich, agriculturally influenced catchments to have measurable concentrations of nitrate compared to

less impacted streams such as our predominantly grassland catchments. However, the observed low nutrient concentrations and minimal DEN in grassland streams can be attributed to low discharge measured among our sites (Table 1) (Vanni et al., 2001). Overall, our observed cropland streams showed greater activity for nitrate and stream metabolism (Figure 6). It is important to continue studying agriculture ecosystems to bring diverse viewpoints of inputs and exports from agriculturally impacted ecosystems (Loucks, 1977).

Despite our hypothesis of a prominent diel pattern among cropland sites for NO_3^- -N, only one site out of three presented the diel pattern (Figure 3D). This could be due to potential storage pools of N in our streams near our study sites, although we were careful to avoid pools upstream of our sampling site. Additionally, our nitrate observations among all study sites show little diel variation, this could be attributed to low and varying stream velocity among our streams (Flewelling et al., 2014). The diel NO_3^- -N response we observed in site C1 reflects the daily biological activity typical of a diel sampling day when flow is stable (Nimick et al., 2011). Observed dissolved oxygen dynamics, such as a diel pattern, reflect biogeochemical processes that drive ecosystem metabolism (Schindler et al., 2017). The diurnal swings of dissolved oxygen we observed were consistent among other studies. During our diel study, we saw a decrease in DO due to photosynthesis in the evening, especially in the cropland-dominated site C1 (Figure 3). The oxygen depletion creates ideal conditions for denitrification which coincides with the high rates of DEN we observed in site C1 (Figure 4), linking O_2 consumption to N_2 production (Harrison et al., 2005; Laursen & Seitzinger, 2004). Similarly, the low DEN rates we measured among the other five sites (C2, C3, G1, G2, and G3) were consistent with minimum diel change in DO, reflecting minimum oxygen consumption (Harrison et al., 2005). However, we observed lags in O_2 peaks which may have come from undefined processes that need further

attention (Dodds et al., 2018). In addition, we found distinct diurnal swings in dissolved oxygen for site C1 associated with the highest rate of GPP and ER compared to the other sites of both land use types, suggesting high respiration within the nutrient-rich cropland-dominated watershed. Observing diel biogeochemical cycles over a 24-hr period contributes to more than the collection styles and interpretation but also to the greater understanding of interrelated chemical processes and ecological studies of streams affected by excess N and their implications of nutrient transport downstream (Harrison et al., 2005; Nimick et al., 2011).

Mean gross primary production rates aligned with previous small stream metabolism meta-analysis data (Dodds et al., 2018; Kelly et al., 2021). We observed highest rates of GPP among streams with widespread riparian canopy coverage, suggesting that riparian zones have a considerable effect on small stream production (Koenig et al., 2019). Our streams had considerable canopy cover which could have increased allochthonous organic material from upstream, known to affect stream metabolism in grassland-dominated streams (Mulholland et al., 2001; Riley & Dodds, 2012). Contrary to the data presented in Hall et al. (2016), GPP and ER did not fall close to the 1:1 line (Figure 5) but aligns with estimates regularly found in small streams (Griffiths et al., 2013; Hall et al., 2016). In addition, GPP has been positively related to discharge in previous studies, and our study reflects this scenario as our relatively small GPP rates correspond with low measurements of discharge among all our study sites (Lamberti & Steinman, 1997). Overall, GPP tended to be higher in streams draining less disturbed landscapes, the grasslands sites, which is generally found in closed canopy headwater streams (Bernot et al., 2010; Mulholland et al., 2001).

We observed greater ER relative to GPP in our small streams due to limited light penetration in the water column in addition to the import of allochthonous particles from

upstream agricultural activities (Hall et al., 2016). Among all the study sites, the calculated P:R ratio was less than 1, suggesting that our small streams had high rates of heterotrophic respiration relative to GPP (Griffiths et al., 2013). Additionally, the P:R ratio averaged 0.04, relatively similar to other studies in headwater streams with P:R < 1 (Acuña et al., 2004). We found no significant correlation between ER and GPP among our sites, suggesting that the metabolism estimates are independent of each other (Dodds et al., 2018). Potential factors that we did not account for, such as turbidity and dissolved organic matter, could have been assessed for their impacts on metabolism rates, especially among our cropland streams where we observed lots of sediment loading (Dodds et al., 2018; Fuß et al., 2017). Specifically, organic-rich sediments could have been hotspots for heterotrophic respiration among our headwater streams (Hoellein et al., 2009).

Denitrification was generally higher in streams associated with higher NO_3^- -N availability, common in freshwater habitats (Piña-Ochoa & Álvarez-Cobelas, 2006). Nitrate is a known factor that can directly control DEN and in cases where NO_3^- -N concentrations were close to below the detection limit, DEN rates were lowest (Figure 6A) (Inwood et al., 2005). Streams with highest NO_3^- -N concentrations were associated with highest DEN, presumably as a result of the N loading from intense land use from the cropland-dominated watershed (Harrison et al., 2005; Kreiling et al., 2019). However, DEN rates were generally low across all our streams which could have been a result of our method of measuring N_2 with MIMS and not accounting for acetylene inhibition of the microbial conversion of N_2O to N_2 (Hanrahan et al., 2018). Additional challenges can arise when using the open-channel N_2 method that requires accurate and precise estimates of *in situ* N_2 (Reisinger et al., 2016). Using MIMS allows for those precise estimates, but then complex equations are used to convert MIMS data back into *in situ* N_2 ,

introducing potential error which may be the case in our metabolism estimates with high variability. Nevertheless, our estimations of reach-scale denitrification are similar to results from other estimations of small stream denitrification (Mulholland et al., 2008; Reisinger et al., 2016).

In summary, agriculturally impacted landscapes alter the biogeochemistry of headwater streams as we saw a difference in concentrations between the contrasting watersheds. Denitrification was measurable in headwater streams in the Delaware River Watershed but was limited by NO_3^- -N availability. The cropland-dominated watershed had the highest concentrations of NO_3^- -N associated with high rates of denitrification, providing evidence that our headwater streams cycle N when available. These results from our contrasting watersheds have implications for management problems associated with streams affected by agriculturally impacted landscapes. Our research may help facilitate further denitrification modeling efforts in headwater streams among varying land use types. Especially regarding biogeochemical N transformations in headwater streams affected by increased agricultural N loading. Our study provides supporting data on denitrification estimates using the open-channel N_2 method in small streams among contrasting land use categories (Hanrahan et al., 2018; Nifong et al., 2020; Reisinger et al., 2016). Any N that is not removed via denitrification will potentially transport downstream, such as to the Kickapoo Tribe in Kansas' drinking water treatment plant or further downstream to the coastal zones (Green et al., 2004; Gruber & Galloway, 2008). Our results suggest that effective management solutions should address excess N application from agriculturally impacted watersheds at the landscape scale to aid in reducing N loading (Inwood et al., 2005; Mulholland et al., 2008; Vanni et al., 2001; Vitousek et al., 1997).

Conclusion

The six streams and two contrasting land uses we studied can be used to broadly address factors that may drive reach-scale denitrification in headwater streams. Our highest denitrification estimates were associated with the highest nitrate concentrations, specifically among the cropland-dominated streams. Gross primary production increased with diurnal swings of DO and N availability, land use inputs may drive this relationship. Overall, our results of reach-scale denitrification and stream metabolism suggest that small streams affected by nitrogen rich inputs can remove N via denitrification, however, our data showed very low denitrification estimates and highlights the need for additional study on reach-scale denitrification regarding the effects of land use change on headwater streams. Understanding how nutrients transform throughout streams impacted by agriculture can help future generations address water quality issues.

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