THE EFFECTS OF LOW-HEAD MILLDAMS ON STREAM NITROGEN PROCESSES

by
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TABLE OF CONTENTS

LIST OF TABLES vi
LIST OF FIGURES vii
ABSTRACT xi

Chapter

1 INTRODUCTION 1

REFERENCES 5

2 LITERATURE REVIEW 8

2.1 Milldam History, Recent Removal, and Management 8
2.2 Stream Hydrology and In-Stream Processes 11
2.3 Effects of Dam Removal on Stream Health and Management Implications 15
2.4 Natural River Obstructions Altering Stream Hydrology 17
2.5 N Pollution and Importance of Stream Sediment Denitrification 18
2.6 Controlling Factors of Denitrification and Rates from Literature 20
2.7 Methods for Measuring Denitrification 28
2.8 Alternative Nitrate-N Removal Pathways 29
2.9 Effects of Natural and Manmade Dams on N Processes and Stream Sediment Denitrification 31

REFERENCES 37

3 METHODS 47

3.1 Study Sites 47
3.2 Experimental Design 51
3.3 Hydrologic Monitoring 53
3.4 Stream sample collection for nutrients 56
3.5 In-Situ Water Quality Monitoring 57
3.6 In-stream Denitrification Potential Assessment 59
3.7 Particle Size Analysis 63
3.8 In-Situ Measurement Analysis 63
3.9 Stream Grab Sample and Sediment Core Analysis 64
3.10 Statistical Analysis 64

REFERENCES 66

4 RESULTS 68

REFERENCES 95

5 DISCUSSION 96

REFERENCES 115

CONCLUSION 121

LIST OF TABLES

Table 2.1: Biological, chemical, and physical controls on denitrification from literature.. 23

Table 2.2: Stream sediment denitrification enzyme assay (DEA) rates, methods used, and habitat studied from selected literature. = data not reported. ~ = test not performed. Mean (standard error) shown. 26

Table 2.3: Riparian zone and beaver pond denitrification rates from selected literature.. 33

Table 3.1. Chemical and biological sampling (A) and high frequency data (B). 53

Table 4.1: Velocities (m/s), residence times (h), and difference in residence time above versus below each dam. 73

Table 4.2 ANCOVA results of stream nutrient concentrations by sampling date, distance along the 500 m sampling reach, and interaction of date by distance for Christina (top) and Chiques (below). Factors marked with * are significant at α =0.05. 78

Table 4.3: Average and standard error values for DEA rates (by treatment), % organic matter, net nitrification, and net mineralization of streambed sediments by site and sampling location. At Christina, no significant differences were seen above the dam versus below in DEA rates, OM%, or sediment incubations (all p values > 0.13). Overall, amended DEA samples were significantly different than unamended DEA rates, $p < 0.0007$. 82

Table 5.1: Denitrification rates from this study compared to other literature values, converted to µgN kg^{-1} h^{-1} if applicable. 104

LIST OF FIGURES

Figure 1.1: Schematic showing side-view of a milldams and its physical, chemical, and biological effects upstream and downstream. Note difference in water depth and denitrification potential. 2

Figure 2.1: Depiction of mill density in the Eastern US by 1840 (Image from Walter and Merritts, 2008). 9

Figure 2.2: Schematic representing how dam presence alters factors that increase denitrification potential above the dam compared to a more free-flowing environment below. 36

Figure 3.1: Christina River watershed and Cooch's Mill dam (39.645556°, -75.742500°) (A) and Chiques Creek watershed and Roller Mill dam (40.108306°, -76.443111°) (B). Watershed map inserts (a) indicating entire watershed outlined in black, individual streams in dark grey, main river stem highlighted, and red dot representing dam sites. Photos of dam looking upstream (b). Study site maps (c) overlaid on LIDAR digital elevation model indicating monthly stream grab sample collection above and below dam (yellow squares), seasonal streambed sediment core collection (yellow circles), and water quality sensors installed in the stream (yellow triangles). 48

Figure 3.2: Images of water and large debris flowing over Cooch's Mill dam (left) and view of downstream of dam (right). 49

Figure 3.3: Roller Mill dam after a storm event above the dam (left) and below (right). 51

Figure 3.4: Timeline of site installation and sampling from August 2019-August 2021. 52

Figure 3.4: PVC pipe structures deployed at Cooch's Mill site to host water quality sondes (a) and conductivity and stream level loggers (b). 56

Figure 3.5: Discharge rating curve developed for downstream of the Roller Mill dam on Chiques Creek using SRBC high frequency stage height (ft) and discrete flow measurements (cfs). 56

Figure 3.6: Collection of stream sediment cores upstream of the milldam using a coring rod on Chiques Creek in Manheim, PA. 61

Figure 3.7: Streambed sediment samples being flushed with N_2 to produce an anaerobic environment for denitrification enzyme assays. 62

Figure 3.8: Images comparing streambed sediment from Chiques Creek (left) and Christina River (right) collected from various distances from dam and seasons. Note variation in sediment size and type. 63

Figure 4.1. Temporal trends in stream level (ft) and conductivity (μS/cm) above and below the dams from Nov. 2019-Sept. 2021 for Christina River (a) and Chiques Creek (b). Dilution events in the streams due to rain events are denoted by (*). Conductivity spikes likely due to road salt runoff from a nearby highway at Christina denoted by (**). Note difference in Y axis scales due to higher conductivity at Christina. 69

Figure 4.2. Average daily difference in temperature (°C) above the dam compared to below the dam plotted against streamflow (m^3) for Chiques Creek (A) and Christina River (B). Black line represents the moving average of daily difference in temperature. 70

Figure 4.3. Daily percent dissolved oxygen saturation versus streamflow ($m^3 s^{-1}$) for Christina (A) with values from July 1, 2020- July 20, 2020 (a) and July 19, 2021- September 6, 2021 (b), and Chiques (B) with values from September 9, 2020- October 12, 2020 (a) and July 20, 20921- August 31, 2021 (b) over selected time periods of best data available. Red points represent above the dam dissolved oxygen percent saturation; black dots represent below. 72

Figure 4.4: Stream nutrient concentrations upstream and downstream of the dam shown in boxplots for Christina (A) and Chiques (B). No significant differences were seen in nutrient concentrations upstream versus downstream of the dams at either site, $p > 0.5$. Matching letters indicate no significant differences at $\alpha = 0.05$. 74

Figure 4.5: Average stream nutrient concentrations (NO_3-N, NH_4-N, TN) for upstream versus downstream of dam at Christina (above) and above the dam at Chiques (below). Matching letters indicate no significant difference. No significant difference was seen in above the dam versus below at Christina ($p > 0.3$, $n=16$, Student's T-test, $\alpha = 0.05$). Bars represent standard error. 76

Figure 4.6: Boxplots of stream nutrients by distance from dam for Christina (top, n=16) and Chiques (bottom, n=17) over two-year study period. Positive distances indicate farther upstream, negative distances indicate farther downstream. Dam is represented by dashed line. Christina samples taken along a ~500 m reach upstream and downstream of the dam. Chiques samples taken along a ~500 m reach upstream of the dam. No significant differences were found among TN, NO_3-N, and NH_4-N by sampling distance at Chiques ($p > 0.5$, n=17, ANCOVA, α =0.05). A significant difference by distance was seen at Christina for downstream TN ($p < 0.01$, n=16, ANCOVA, α =0.05) but not for upstream TN or NO_3-N, and NH_4-N. 77

Figure 4.7 Temporal pattern of dissolved stream nutrients from grab samples collected above (open markers) and below the dam (closed markers) for Christina River (left, n=24) and Chiques Creek (right, n=22). No significant difference detected between upstream vs downstream samples at either site (α =0.05). 81

Figure 4.8: Average denitrification rate (ugN kg sediment h^{-1}) for each site by treatment (amended in black bars, unamended in gray bars) and sample location (upstream of the dam versus downstream). Bars represent standard error. A significant difference was found between upstream and downstream samples for both treatments at Chiques, $p < 0.0014$ as indicated by non-matching letters. 83

Figure 4.9: Boxplots of DEA rates by distance from dam for Christina (top) and Chiques (bottom). Dam marked by dashed line. N= 8 seasonal sediment core samplings from October 2019-Aug. 2021 for all Christina samples and all upstream Chiques samples, downstream Chiques samples n= 4. Boxplots with same letters are not significantly different at α =0.05. Values are amended and unamended samples combined. Note Y axes are different due to sampling distance differences among sites. 85

Figure 4.10: DEA rates (µg N kg sediment h^{-1}) by treatment (amended results in black bars, unamended in white) and sampling month for each site. Note Chiques (top) has a different Y axis than Christina (below) due to differences in overall DEA potential. 86

Figure 4.11: Average net nitrification and net mineralization for Christina (top) and Chiques (bottom). Bars represent standard error. Note different Y axis due to differences in over net N processing rates. 88

Figure 4.12: Unamended DEA rates (µg N/kg sediment/h^{-1}) versus net nitrification converted to the same units as DEA for comparison. Chiques is in blue, Christina in orange. Dashed line represents zero, or when net nitrification is equal in magnitude to unamended DEA. Winter is represented by a square, up triangle is spring, down triangle in summer, and circle is fall. 89

Figure 4.13. Scatter plots representing organic matter (%) per samples versus DEA rates (µg N kg sediment h^{-1}) by reach (a) with upstream in closed circles and downstream in open circles, and season (b) with Christina in blue closed circles and Chiques in open red circles. 90

Figure 4.14. Percent sediment particle size by volume and by distance from dam for Christina (a) and Chiques (b). Dam represented by black line. No sampling was done for Chiques downstream. 92

Figure 4.15. Percent sediment particle size by surface area and by distance from dam for Christina (a) and Chiques (b). Dam represented by black line. No sampling was done for Chiques downstream. 93

Figure 5.1: (A) Artist's rendering of Pennsylvania valley bottom pre-colonial settlement based on plant macrofossils from Elliott et at., 2016. (B) Creation of milldams on stream networks changed waterways from a more open and connected flowing system (bottom left) to a disconnected and fragmented ponded system (bottom right.) 111

Figure 5.2: Schematic indicating biogeochemical processing ability as it relates to denitrification potential on a dam that is actively trapping sediment (left) and one that is full above the impoundment and at dynamic equilibrium (right). 112

Figure A.1: Boxplots of SUVA values (L/mg*m) and DOC (mg/L) for Christina (top) and Chiques (below) by sampling locations. No significant differences were found across sites (Christina: $p > 0.3$, n=24, Students T-test, $\alpha = 0.05$; Chiques: $p > 0.7$, n=22, Students T-test, $\alpha = 0.05$). 123

ABSTRACT

Stream fragmentation is prevalent throughout the US mid-Atlantic due to historic low-head milldams altering stream hydrology and biogeochemical processes. While numerous dams have breached or been purposefully removed, many remain creating slower flows and enhancing organic matter (OM) and sediment deposition above the dam. These factors promote anaerobic nutrient processing including denitrification which may alter overall nitrogen (N) removal across the watershed. We studied two streams with existing low-head milldams (Christina River in Newark, DE; drainage area of 50.7 km^2 and Chiques Creek in Manheim, PA; drainage area of 127 km^2, dam heights 4.0 & 2.4 m, respectively) over two years. We expected streambed sediment denitrification rates to be higher upstream of the dam compared to below and to vary seasonally, with highest rates in summer. Denitrification enzyme assays (DEA), net mineralization and nitrification, sediment particle size, and % OM, were determined from streambed sediments collected seasonally. Monthly stream grab samples were analyzed for total nitrogen (TN), nitrate (NO_3-N), ammonium (NH_4-N), and dissolved organic carbon. Contrary to expectations, DEA rates and nutrient concentrations did not differ above and below the dams, and highest denitrification rates occurred during autumn at Christina and winter at Chiques. Streambed sediment was dominated by sand at both sites, which has less surface area for denitrification to occur compared to smaller silt or clay particle sizes. A multilinear regression model showed OM and sediment NH_4-N accounted for 33% of variability in denitrification rates. This study indicated the two milldams did not have a significant effect on streambed denitrification or stream water N concentrations, possibly due to full sediment capacity above the impoundments reducing denitrification potential. These

findings will help watershed managers make informed decisions on dam removals and potential consequences for N exports.

Chapter 1

INTRODUCTION

Across the world, human activities such as agriculture, mining, damming, and navigation have greatly impacted our surface waters and aquatic ecosystems, with 48% of global river volume moderately to severely impacted by dams (Tshantz 2014, Poff et al. 2007, Grill et al. 2015). In the US, starting in the 1700s, thousands of milldams were constructed on streams and river, especially in the Mid-Atlantic region (Walter and Merritts 2008). Early settlers harnessed the power of water by damming rivers and streams to fuel their grist and water mills, with 65,000 milldams built by 1840 (Walter and Merritts 2008). Many of these structures have been naturally breached and removed, however numerous original structures remain and have fallen into disrepair (Tschantz 2014). More than 1,700 dams have been deliberately removed since 1912 due to safety and environmental concerns, including 90 removed in 2019 alone across 26 US states (American Rivers 2020, Tschantz 2014, Bellmore et al. 2019). Dam removal has become a convenient way to address aging and obsolete infrastructure as an alternative to costly repairs to meet safety standards and liability concerns, which often fall on private landowners (Foley et al 2017, Bellmore et al, 2019, Wyrick et al., 2007). Understanding how the historic creation of dams altered or impaired regional hydrology and biogeochemistry is integral to monitoring and maintaining stream health today.

Stream health is impacted by both natural and humanmade dams that alter stream hydrology and in-stream biogeochemical processes. Beaver dams can alter the landscape by creating ponds and wetlands where there was once free-flowing water (Larsen et al., 2021). This channel alteration raises stream water levels above the dam increasing the interaction of ground and surface water (Lazar et al., 2015), along with altering oxygen (O_2) exchange in the hyporheic zone from the water column and streambed (Briggs et. al, 2013). Overall, dams enhance surface storage and groundwater recharge while lowering dissolved oxygen (DO) and redox potential due to the anoxic environment created by impoundments (Burchsted et al., 2010). This pool of slower and more stagnant water created by dams breaks up dynamic river flow and allows for sediment to accumulate, while increasing residence time and potentially decreasing DO above the dam (Figure 1.1).

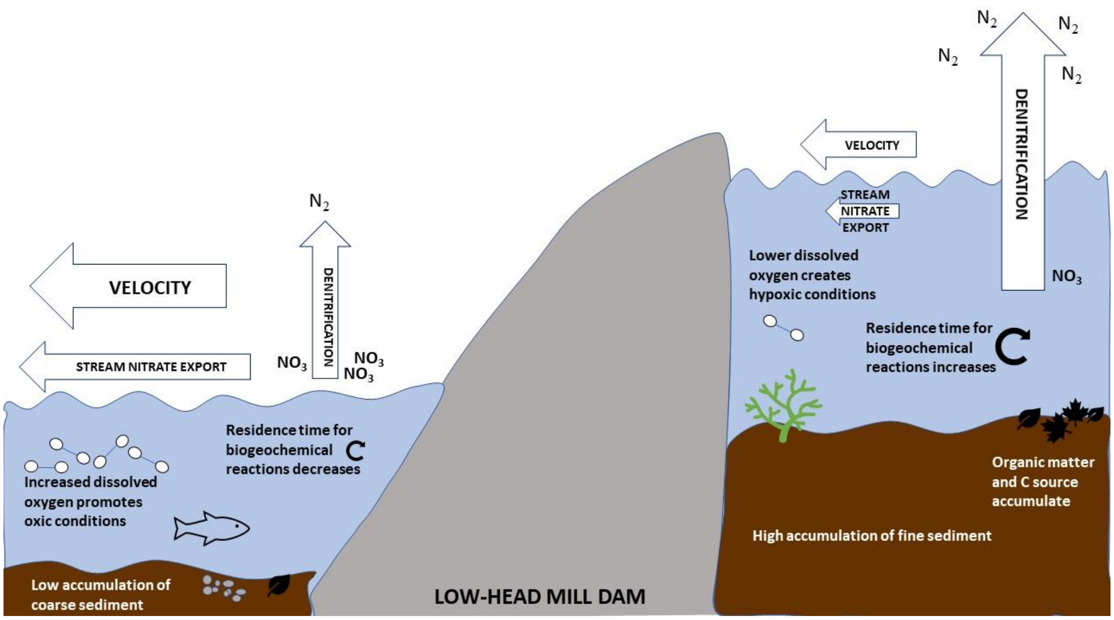

Figure 1.1: Schematic showing side-view of a milldams and its physical, chemical, and biological effects upstream and downstream. Note difference in water depth and denitrification potential.

The increase of residence time of water upstream of dams may also influence nutrient cycling, as biogeochemical interactions have a longer reaction time. In particular, changes in stream velocity and stream-sediment interface are known to alter nitrogen (N) and carbon (C) cycling and solute concentrations (Bardini et al, 2012). The effects of dam removal on dissolved nutrients, particularly N and phosphorus (P) may be highly site-specific and dependent upon watershed and impoundment characteristics such as hydraulic residence time (Velinsky et al., 2006). Sediment and nutrient export can increase significantly following dam removal, due to the export of nutrients stored upstream of the dam prior to removal (Ahearn and Dahlgren 2005, Gold et al., 2016, Riggsbee et al., 2007).

Of concern is excess N in aquatic ecosystems due to human activity including atmospheric deposition from fossil fuels; synthetic N production and fertilizer use; agricultural practices (e.g., land application and leaching of manure), and sewage contamination (Pennino et al., 2017). Nitrate-N, the most common form of inorganic N in the environment, does not adsorb well to soil particles as it is extremely water soluble. While some nitrate-N will be used for plant uptake or biological assimilation the remainder is transported to nearby streams and waterways through runoff and the movement of groundwater (Ranalli et al., 2010). Excess nitrate-N in freshwater systems poses risks to water quality and aquatic life and can cause contamination of shallow groundwater wells and drinking water sources in the US (Pennino et al., 2017). Better understanding of the impact of dams on stream nutrient processing, particularly N, is integral to monitoring stream nutrient loads and their watershed export consequences.

One natural pathway of N removal in streams, denitrification, is the microbial reduction of NO_3-N (nitrate-N) and NO_2-N (nitrite-N) to gaseous forms of nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) under anoxic conditions. Denitrification, the only pathway that truly removes NO_3-N from a system as a conversion to inert N_2 gas, occurs mostly in the top 2 to 5 cm of stream sediment (Seitzinger, 1988, Burgin and Hamilton, 2007). This process is enhanced in sediment containing high labile C availability, fine particle accumulation, and low oxygen saturation (Merill and Tonjes, 2014). Enhanced denitrification leads to an increased potential removal of NO_3-N from the watershed. These conditions are often created above the dam as water pools and collects, increasing residence time and lowering DO availability. It is possible, therefore, that removing these milldams could decrease denitrification potential, and these changes could enhance N exports from watersheds, potentially exacerbating the N pollution of our waterways.

This study aims to investigate how low-head milldams affect stream hydrology and biogeochemical processing of N, and if streams adjacent to these dams are “hotspots” for denitrification. We studied two sites with existing low-head milldams on Chiques Creek in Pennsylvania (2.4 m tall milldam) and Christina Rier in Delaware (4 m tall milldam) beginning November 2019 and concluding October 2021. Key questions that were addressed were:

1. How do milldams influence stream hydrologic and biogeochemical conditions upstream and downstream of milldams?
2. How do milldams affect stream water N concentrations? Are streams adjacent to milldams “hotspots” for denitrification, and what are the controlling factors?

We hypothesize that stream sediment upstream of milldams will have higher denitrification rates due to hypoxic conditions, elevated deposition of OM, and fine sediments increasing surface-groundwater interaction. We also hypothesize that instream denitrification rates will vary seasonally and be highest in summer due to increased temperatures promoting microbial activity.

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Chapter 2

LITERATURE REVIEW

This literature review discusses a brief history of milldams and offers a review of their current status and impact on stream hydrology and in-stream processes, specifically denitrification and controlling factors in streambed sediment. Previous dam removal studies and studies of natural dams such as beaver dams are also reviewed. Finally, the impact natural and manmade dams may have on stream sediment denitrification is discussed.

2.1 Milldam History, Recent Removal, and Management

Thousands of milldams were constructed across the eastern US in colonial times (Walter and Merritts 2008**,** Figure 2.1), while hundreds more have been constructed since for irrigation, mining, and manufacturing (Tschantz, 2014). As these dams naturally breach or are reviewed for removal, the process becomes complex due to socio-economic issues including jurisdictional regulation (or lack thereof) and often conflicting interest between landowners, local county government, and state conservation departments (Bowman 2002; Tonitto and Riha, 2016). For example, a survey of homeowners in New Jersey (USA) with lake front property due to the presence of a nearby dam showed homeowners were not in favor of dam removal (90.9%), citing fears of property value and recreation use loss with the draining of the lake, even with the dam classified as "high hazard" for risk of failing (Wyrick et al., 2007). In addition, the management, classification, and regulation of dams varies by the states in which they are located, creating confusion over who is responsible for infrastructure maintenance and repair.

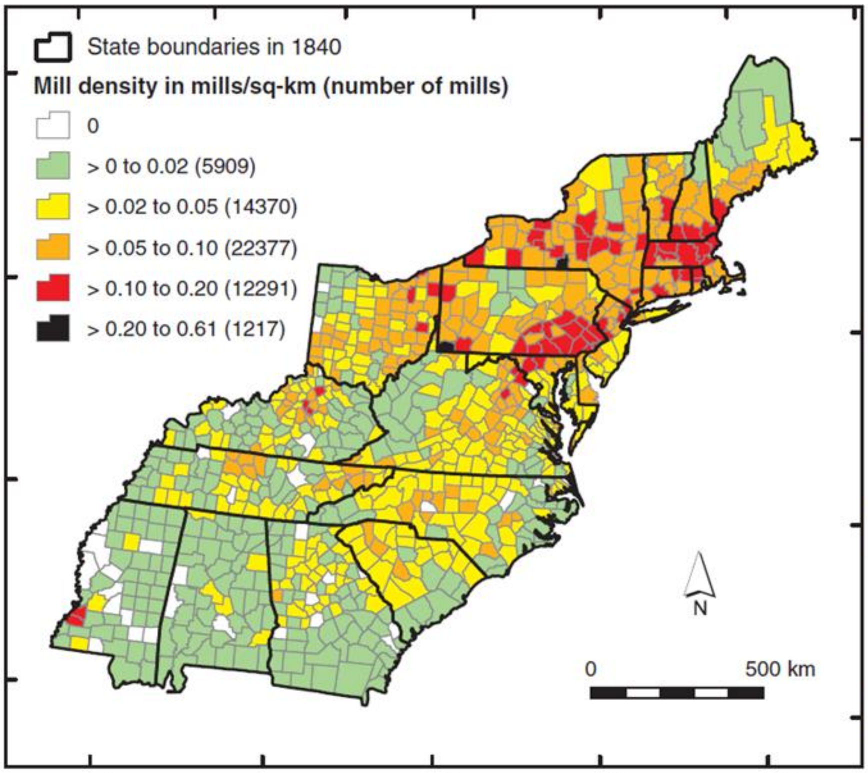

Figure 2.1: Depiction of mill density in the Eastern US by 1840 (Image from Walter and Merritts, 2008).

There are 2.5 million dams on American rivers, yet only 91,000 are considered large enough to be included on the National Inventory of Dams (NID) (US Army Corps of Engineers, 2019; Environmental Protection Agency, 2016). NID monitors dams more than 7.6 meters in height and 15 acre-feet in storage, or equal to or exceeding 50 acre-feet storage and higher than 1.8 meters (American Rivers). Smaller dams (also referred to as low-head dams) are broadly considered to be shorter than 4.6-9.1 meters in height, depending on the state or agency regulating them (Brewitt and Colwyn 2019).

Both large and small dams fall under state jurisdiction for regulation, inventory, and inspection. However, the above definitions fail to incorporate the smallest structures less than 1.8 meters in height, which make up the majority of American dams. In a 2014 survey of state dam safety managers, 37 states said they were aware of low-head dams in their state but only three states (IL, PA, and VA) had statutory authority for regulating public safety around these dams, including the creation of exclusion zones around the dams marked by buoys and signage (Tschantz, 2014). Pennsylvania had the most (253) self-reported low-head dams in states that conducted an inventory, followed by Iowa (246) and New Hampshire (244) (Tschantz, 2014). Estimates from states that do not keep dam inventories range from 1,814-3,660 unmonitored dams collectively across the US (Tschantz, 2014). These dams lie outside state jurisdiction and have fallen through regulatory cracks, with some states classifying them as “nondams,” These unregulated nonjurisdictional dams can pose a threat to public safety and environmental health (Brewitt and Colwyn 2019) as small dams not being inventoried or monitored for safety can pose a threat to residents using the area around the dam for recreation including fishermen, boaters, or swimmers. Recreators unaware of the potentially dangerous recirculating currents downstream of a low-head dam can become trapped. The number of low-head dam fatalities has increased since the 1960’s in the US, with 80 reported fatalities occurring between 2010-2014, while the number of fatalities from dam failures has decreased (Tschzntz, 2014).

In addition to safety concerns, it is also well documented that humanmade dams have widespread negative impacts on freshwater ecosystems and their downstream habitats (Gangloff 2013; Hart et al., 2002; Doyle et al. 2003c), and understanding how the creation of dams altered or impaired the streams they were built on is integral to monitoring and maintaining stream health today. States such as

Illinois, Pennsylvania, and Virginia have strong public dam safety programs, along with the Canadian Dam Association, that could be used as guidance for other states to develop similar programs to assess risk hazards, develop public policy, and create education and warning systems to better ensure public safety (Tshantz, 2014). Pennsylvania also found success in dam removals with nearly 200 removed between 1999 and 2015 by allowing permitting to be streamlined through one single agency (Tonitto and Riha, 2016; American Rivers, 2020). As dams continue to age and the need for coordinated regulation and repairs grow, these public risk concerns should be addressed and incorporated into discussion around dam safety, removal, and monitoring.

2.2 Stream Hydrology and In-Stream Processes

Rivers and streams are dynamic systems with complex hydrological processes, among the most diverse and complex of ecosystems on Earth (Humphries et al., 2014). Understanding how milldams affect stream health requires a holistic understanding of the dynamic nature and connectivity of stream ecology, hydrology, and biogeochemistry. A brief review of the hyporheic zone, stream biodiversity and metabolism, and various models of stream geomorphology and connectivity are discussed with attention to how dams alter key processing functions.

The hyporheic zone, an often overlooked but important function of stream hydrology, acts as a control of temperature and surface-water chemistry and provides a source of both dissolved C and terminal electron acceptors needed for biogeochemical reactions in the water column and streambed sediment (Merrill and Tonjes, 2014; Briggs et al., 2013). This zone occurs on streambed subsurface where groundwater mixes with stream water through vertical, lateral, and longitudinal flows. Water velocity also decreases as it enters the hyporheic zone, creating a calmer area of refuge for aquatic organisms such as benthic macroinvertebrates. This zone, however, is sensitive to shifts in stream and groundwater flow such as those due to damming, channeling, or change in discharge, causing negative impacts on chemical and biological functions (Merrill and Tonjes, 2014). In addition, stream flow and discharge vary among streams according to natural channel morphology, seasonality, and storm events. Native riverine biota are often influenced by these disturbances and flow conditions, which can then impact biota evolutionary adaptations and ecosystem functions (Poff et al., 2007), highlighting the complex interactions between biogeochemical cycling and the surrounding organisms in the hyporheic zone.

The biodiversity in rivers and adjacent riparian ecosystems is generated and maintained by geographic variation of stream processes, along with fluvial disturbance regimes. These are often controlled by regional differences in climate and geology, and therefore human-made dams can alter seasonal and interannual streamflow variability, further altering the natural dynamics of the system (Poff et al, 2007). Poff et al. (2007), argue that dams may have a continental scale effect on homogenizing environments, as there is an average of one dam every 48 km of third through seventh-orders river channels in the United States. These geographic areas are regionally different prior to damming, but dams allow for diverse nonindigenous species to spread at the expense of local biota that are adapted for their pre-dam environment and unable to compete with non-native species. Interestingly, the same dam can provide a benefit when invasive species are introduced to an area, acting as a natural block to minimize spread and contain the invasive species to one area, such as reducing spread of the sea lamprey (*Petromyzon marrinus*) in the Great Lakes region (Gangloff, 2013).

Streams, like living organisms, possess metabolic regimes with various controls and drivers. Autotrophic gross primary production (GPP) and heterotrophic ecosystem respiration (ER) are the underlying metabolic rates of ecosystems that control energy supply and dispersal in food chains, with net ecosystem production being the balance between GPP and ER. Stream ecosystem metabolism is calculated from the daily production and consumption of oxygen and is measured in g O_2 m^{-2} d^{-1}. Estimating river GPP and ER for at least a year is needed for accurate calculations due to seasonal patterns, frequent disturbance such as storms, and high light variability (Bernhardt et al., 2017). Although the first measurements of recorded whole ecosystem metabolism in a river was done over 60 years ago by H. T. Odum (Bernhardt et al., 2017; Odum,1956), there are no universal or regional predictors of river productivity known due to the highly dynamic natures of rivers. In addition, streams in deciduous forests are also under seasonal changes due to leaf emergence in spring. Shade from fully emerged leaves can reduce photosynthetically active radiation (PAR) reaching the streambed from >1000 to < 30 µmol m^{-2} s^{-1} (Hill et al., 2001). Extrinsic controls such as light, heat, nutrient input, and disturbance all alter metabolism and can be influenced by flow, climate change, land use, and eutrophication. Advancements in technology now allow for faster and more frequent measurement of dissolved gas and solutes fluxes along with new ecosystem modeling, bringing river metabolism calculations on par with the historical rates of streamflow measurements (Bernhardt et al., 2017).

Scientists have used various models and concepts to understand and describe stream processes by examining flow through flood plains and channels, sources of energy (C) input (autochthonous or allochthonous), and production ratios (autotrophic:heterotrophic/photosynthesis:respiration) and how they change longitudinally, laterally, and with discharge (Humphries et al., 2014). The first model was the river continuum concept (Vannote et al., 1980), which stressed a longitudinal link in rivers and a connection between organic matter inputs upstream and trophic levels downstream. The flood pulse concept (Junk et al.,1989) instead focused on the floodplain as a primary source of organic matter and the lateral connection of rivers between their floodplain and channels, while the riverine productivity model (Thorp and Delong, 1994), suggested energy production results from phytoplankton, benthic algae, and other aquatic plants derived from riparian zone organic matter, but also acknowledged the river continuum and flood pulse theories are applicable dependent on river type or section. Successive studies have shown that all three models are supported in different environmental conditions and climates (Humphries et al., 2014). Other models have also been proposed, including the river wave concept by Humphies et al. (2014) which aims to unify previous concepts. Here, waves are used to define river flow and energy input with the shape, amplitude, wavelength, and frequency combined with direction of travel (longitudinally and laterally) defining the type of energy input (local autochthonous and allochthonous, upstream allochthonous/ allochthonous and autochthonous floodplain production). These theories offer a robust starting point to begin to understand the complex processes that occur in streams, and each stream is of course particular to its physical, chemical, biological, and geological characteristics. The creation of dams has altered the landscape in such a way that rivers are no longer connected as the above models describe, and new studies and models must be created to understand stream flow and energy transfer while a dam is intact, immediately after removal, and throughout recovery post-removal.

2.3 Effects of Dam Removal on Stream Health and Management Implications

The historic creation of dams altered the landscape in ways that we are still coming to understand, and their subsequent removal invites the question of how, and at what rate, will the surrounding waterbodies respond. During the 1900s, more than 75,000 dams greater than 2 m were built in the US and occurred on average every 70 km of the ~5.2 million km of US river channels, ranging from small streams to 10th-order large rivers (Poff et al., 2007). Thus, dam removals act as "large scale experiments" that allow for an improved understanding of fluvial systems and how humans have impacted watersheds (Foley et al 2017). Previous studies have examined how dam removals influence river restoration, stream geomorphology, sediment flux and export, and aquatic habitat and biota health (Foley et al, 2017; Hart et. al, 2002; Poff and Hart, 2002; Riggsbee et al., 2007; Tullos et al., 2016). However, not many studies investigated how low-head milldams and their removal impact water quality and how these play a role in water quality compliances such as total maximum daily loads (TMDLs, EPA 2015) and the implications for larger watershed management such as the Chesapeake Bay.

Dam removal and subsequent total stream nutrient export may be affected by seasons, weather patterns, and the method used for removal. Riggsbee et al. (2007) found total suspended sediment export (TSS), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) loads to be greatest following dam removal and were dependent on size, quantity, and location of sediment stored prior in the above-dam reservoir. They concluded load reduction may increase if removals are done in multiple stages instead of all at once, and the influx of sediment after removal is best compared to previously recorded flood events in the river as opposed to regular base flow loads. On a second order tributary, Ahearn and Dahlgren (2005) calculated annual N export one year after dam removal. N export increased by an order of magnitude compared to the two-year prior average (223 kg^{-1} yr^{-1} TN to 1738 kg^{-1} yr^{-1} TN) with nitrate-N (73.7 kg^{-1} yr^{-1} to 689 kg^{-1} yr^{-1} NO_3-N) and TSS (1594.5 kg^{-1} yr^{-1} to 48,694 kg^{-1} yr^{-1}) also increasing. Nitrogen release was seasonally correlated with local flushing patterns, possibly affecting downstream habitats for years afterward. Gold et al., (2016) used algorithms to estimate N export following the removal of dams both with and without reservoirs. Over 2000 dams with reservoirs were found to potentially aid with N removal, with the majority of dams being on first order streams. However, site-specific studies are needed where dam removal may eliminate the N removal functions provided by the associated reservoir, and alternatives to removal that support other environmental restoration efforts should be explored (Gold et al., 2016), and long-term stream recovery including physical, chemical, and biological parameters after dam removal may takes years or decades to occur, sometimes with only partial recovery being reached (Tonitto and Riha, 2016).

Bellmoore et al., (2019) found that river and riparian response to humanmade dam removal has many physical and biological components, but ecological recovery is possible when given the chance. This response may be different than the environment which was present prior to the removal due to many factors, but Bellmore offers three models (upstream to the previous reservoir, within the reservoir, and downstream of the previous dam) to help project managers predict ecological transition to help guide expectations of all parties involved in post dam removal restoration. In addition, Burchsted et al., (2010) state that natural river discontinuities (such as beaver dams) were part of precolonial rivers and as such play important roles in channel geomorphology, natural flow regimes, water quality and biota health. Because of this, it is argued that natural dams should be considered when creating a reference condition during river restoration projects.

2.4 Natural River Obstructions Altering Stream Hydrology

Natural river obstructions can have strong impacts on stream health and ecology, including the creation of beaver dams that redesign waterways and adjacent habitat. The North American beaver (*Castor canadensis)* was almost eradicated at the beginning of the twentieth century due to trapping and fur trade but rebounded due to stricter regulations in subsequent years (Naiman et al., 1988). Today there are an estimated 30 million beavers in North America which have created approximately 9,280-41,210 km^2 of beaver ponds and 204,000–908,000 km of new riparian interface due to beaver dam construction (Whitfield et al., 2015). Compared to free-flowing streams, beaver dams alter channel connection with the floodplain, longitudinal hydraulic patterns, and sediment transport (Burchsted et al., 2010), resulting in longer residence times and new patterns of hyporheic water chemistry which can alter biogeochemical reaction rates (Briggs et al., 2013).

Natural in-stream structures such as gravel bars and riffles/glides can also alter hyporheic chemistry but can be highly variable over short distances and are affected by other watershed characteristics such as physical location (urban versus forested stream) and high flow events (Briggs et al., 2013; Groffman et al., 2005). Briggs et al., (2013) also found a strong link between spatial and temporal hyporheic flux patterns in stream water with redox values, DO, and nitrate-N, all decreasing with increased residence time. Hyporheic water also varied from oxic conditions in the stream to anoxic (reduced) conditions, in relationship with hydrodynamic conditions and residence times.

2.5 N Pollution and Importance of Stream Sediment Denitrification

Nitrogen pollution in aquatic ecosystems is common due to human activity including atmospheric deposition from fossil fuels; synthetic N production and fertilizer use; agricultural practices (e.g.: application and leaching of manure); and sewage contamination (Pennino et al., 2017). The creation of synthetic N input has nearly doubled the amount of N available for terrestrial cycling, which has led to contamination of groundwater and surface water (Turner and Rabalais, 2003; Vitousik et al., 1997). Nitrate-N, the most common form of inorganic N, does not adsorb well to soil particles as it is extremely water soluble. Furthermore, nitrogen, as well as excess phosphorus and other contaminates, will eventually be moved downstream and enter a bay or ocean in coastal ecosystems, causing eutrophication and harmful algae blooms (HAB) (Kemp et al., 2005).

While high levels of nitrate-N reaching coastal zones remain a significant problem for human and environmental health, natural processes exist to support N load reduction before reaching coastal areas. Previous studies show much of anthropogenic nitrate-N is removed from waterways before reaching the ocean (Howarth et al., 1996; Alexander et al., 2000), and some of these mechanisms and controlling factors may be enhanced to increase nitrate-N removal efficiency. One pathway of nitrate-N removal, denitrification, is the microbial reduction of NO_3-N (nitrate-N) and NO_2-N (nitrite) to gas forms of nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2) under anoxic conditions. Aerobic and anaerobic forms of this heterotrophic respiration of organic matter can occur, meaning denitrification may be respiratory or chemoautotrophic (via sulfur or iron oxidation). It is known that DO levels decrease in aquatic sediment in anoxic conditions as available O_2 is consumed (Burgin and Hamilton, 2007). Microbes then turn to other available terminal electron acceptors beside O_2 in a predictable sequence related to energy yield; O_2 > NO_3-N>Mn IV > Fe III > $SO_4{}^{2-}$ (Briggs et al., 2013). In nitrate-N reduction involving iron oxidation, microbes can convert nitrate-N to nitrite by ferrous iron (Fe^2) or reduced manganese ($Mn^{2+)}$ then converted to N_2 (Burgin and Hamilton, 2007). In respiratory denitrification, an oxidation-reduction reaction, organic matter is oxidized under anaerobic conditions with nitrate-N acting as the terminal electron. This is the primary microbial process by which nitrate-N is completely removed from a watershed and released as N_2 back into the atmosphere, as opposed to being converted into other inorganic forms or temporary biological assimilation into algal and microbial biomass, where N is then released when organisms die (David et al., 2006, Burgin and Hamilton, 2007).

Through a mass-balance study of denitrification rates, David et al., (2006) found a 58% reduction in NO_3-N export in a reservoir along the Mississippi watershed due to reservoir retention time that maximized sediment denitrification rates. They showed increasing water residence time in reservoirs and wetlands may enhance the removal of nitrate-N, along with reconnecting rivers and flood plains to help mitigate nitrate-N export in the absence of reduction of high source inputs upstream. In addition, stream morphology and watershed characteristics can alter nitrate-N removal and retention pathways. Inwood et. al (2005) found stream channels of high width-to-depth ratios allow for increased water contact time with stream sediment, offering longer periods for biogeochemical processes to occur. A review of nitrate-N attenuation in streams and riparian zones by Ranelli et al., (2010) stated undisturbed headwater watersheds are efficient at retaining N, especially when compared to agricultural watersheds.

2.6 Controlling Factors of Denitrification and Rates from Literature

Denitrification occurs mostly in the top 2-5 cm of stream sediment (Seitzinger, 1988) containing high labile C availability and low oxygen saturation (Merill and Tonjes, 2014). Denitrification potential has many controlling factors including but not limited to redox conditions, temperature, pH, enzyme activity, residence time, substrate availability, nitrate-N availability, and microbial activity and functionality of denitrifying bacteria (Comer-Warner et al., 2020; Steinhart et al., 2000; Briggs et al., 2013; Kemp and Dodds, 2002; Bettez and Groffman, 2012; Wu et al., 2021). It is estimated that nitrification rates and nitrate-N availability (< 0.88 mg NO_3-N/L (Wall et al., 2005) or <0.4 mg NO_3-N/L (Inwood et al., 2005) can be a limiting factor (Steinhart et al, 2000). Kreiling et al., (2019) identified nitrate-N availability as the most important factor of sediment denitrification potential over an entire river network from headwaters to mouth. In a study by Wu et al., (2021), denitrification potential was higher in stream than pond sediment due to the steady organic carbon content and nitrate-N supplied by the stream discharge, as opposed these same microbial “fuels” being consumed quicker and being used up in the ponded system. Residence time also plays a role in biogeochemical reaction rates, acting as a control on hyporheic water chemistry and contact time among solutes and microbes, with a longer residence time correlated with higher denitrification rates (Briggs et al., 2013). Additional physical, chemical, and geological characteristics affecting denitrification rates are discussed below.

Sediment type and size also affect stream denitrification rates and are highly variable and localized (Steinhart et al., 2000, Inwood et al., 2005), with sand sediment having almost 10x higher denitrification rates compared to larger gravel sediment across all treatments (Comer-Warner et al., 2020), with finer sediments (sand particles) having highest NO_3-N reduction and highest N_2O concentrations compared to coarser gravel sediments. This is due to fine sediment having larger surface area and promoting longer residence times, higher nutrient attenuation (C and N concentrations) and lower DO than coarse sediments, all which can increase denitrification rates (Findlay et al. 2011; Comer-Warner et al., 2020). Porewater NH_4-N was highest in sand in all seasons and peaked in autumn, and porewater NO_3-N was overall equal in both sediment types. Additionally, drying and wetting of sediment can alter denitrification rates by causing N leaching in organic-rich sediments that experience seasonal drying out (Ahearn and Dahlgren, 2005). Sediment type may also play a role in microbial assemblies and their functional abilities. See Table 2.1 for a summary of factors affecting denitrification rates in stream sediment.

Seasonal changes also affect stream nitrate-N levels and sediment denitrification rates. In forested stream reaches, nitrate-N levels are lower in spring before canopy leaf emergence as primary production is high with light penetrating surface water for algae growth, resulting in an increase of autotrophic uptake of N while also providing a food source for denitrifying microbes that consume nitrate-N. With high canopy cover during summer, algae growth is kept low after leaves emerge, reducing primary production and thus food availability for heterotrophic microbes responsible for denitrification, resulting in a slight increase in stream levels of nitrate-N. Nitrate-N levels drop in autumn as allochthonous C input from leaf litter provides organic matter for denitrifying microbes, resulting in a short increase in denitrification rates before microbial activity and autotrophic uptake decreases in winter as temperatures decrease, allowing nitrate-N levels to accumulate (Ledford et al., 2016). It should be noted that urban streams may follow a different pattern due to lack of canopy cover. Despite high anthropogenic nitrate-N inputs, spring through fall typically has steady and low nitrate-N levels, as autotrophic uptake of N is high throughout due to optimal algal growth and warmer subsurface temperatures. This constant biological assimilation is thought to outpace denitrification as a means of N retention in urban streams (Ledford et al., 2016; Arango et al., 2008). Denitrifying bacteria can also use either particulate or dissolved C sources and may use C from within sediments instead

Table 2.1: Biological, chemical, and physical controls on denitrification from literature

Control	Effect on Denitrification	References
Organic Carbon Content	Denitrification increases with labile dissolved organic carbon (DOC) availability	Inwood et al., 2005, Merill and Tonjes 2014, Wall et al., 2005, Findlay et al., 2011, Wu et al., 2021
Organic Matter Availability	Food source for denitrifying microbes	Korol et al., 2019, Inwood et al., 2005, Bettez and Groffman, 2012
Residence Time	Increased contact time among sediment, biogeochemical solutes, and microbes increases denitrification	Briggs et al., 2013, Inwood et al., 2005
Dissolved Oxygen and Redox Conditions	Denitrification increases under anoxic conditions as O_2 becomes less available	Comer-Warner et al., 2020, Inwood et al., 2005, Merill and Tonjes 2014, Wall et al., 2005
Nitrate-N Availability/Seasonal Variability	<0.88 mg NO_3-N/L can be limiting (Wall et al., 2005) or less than <0.4 mg NO_3-N/L (Inwood et al., 2005)	Inwood et al., 2005, Kemp et al., 2005, Kreiling et al., 2018, Steinhart et al., 2000, Wall et al., 2005, Wu e al., 2021
Sediment Type/Substrate Availability	Fine (sand) and FPOM dominated sediment have higher denitrification rates than coarse (gravel) or CPOM dominated sediment due to higher surface area	Comer-Warner et al., 2020, Findlay et al., 2011
Drying and Wetting of Sediment	N may leach from organic-rich sediments during drying periods	Ahearn and Dahlgren 2005
Temperature	Warmer temperatures encourage microbial activity	Steinhart et al., 2000, Wall et al., 2005
Microbial Activity	Microbial respiration and presence of denitrifying bacteria promotes high denitrification rates	Comer-Warner et al., 2020, Steinhart et al., 2000, Bettez and Groffman, 2012
Habitat, land cover, and use	Undisturbed watersheds correlated with higher denitrification rates, land use affect NO_3-N availability	Korol et al., (2019), Kreiling et al., (2019), Findlay et al., 2011
Natural in-stream structures	Organic debris dams, riffles, pools, gravel bars may alter hyporheic chemistry	Groffman et al., 2005, Inwood et al., 2005
Hyporheic Zone	Controls biogeochemical reactions by altering, temperature, acting as a source of dissolved C, and providing terminal electron acceptors	Merrill and Tonjes 2014, Briggs et al., 2013
pH	Control of microbial activity	Briggs et al., 2013, Comer-Warner et al., 2020

of the water column, as shown by an inconsistency of DOC and denitrification potential by Inwood et al., (2005).

Korol et al., (2019) found catchment forested land cover, catchment urban land cover, and flood plain sedimentation correlated with denitrification potential in nontidal Chesapeake Bay watershed floodplains, and large-scale predictors could explain 43-57% of denitrification variation. Kreiling et al., (2019) did not find a positive correlation between stream sediment denitrification potential and land use (watershed land cover) except for the most undisturbed study site across the watershed with denitrification enzyme assay (DEA) rates ranging from 0.2-418 µgN cm^{-2} h^{-1}. Denitrification rates can also vary depending on location in stream geomorphic structures and the accumulation of organic matter content, with denitrification potential highest in organic debris dams compared to pools and runs (Groffman et al., 2005; Steinhart et al., 2000; Jacinthe et al., 1998).

Agricultural runoff can also impact denitrification rates by adding excess nitrate-N to the system (Kemp and Dodds, 2002, Inwood et al. 2005). Inwood et al. (2005) found higher denitrification rates in agriculturally influenced headwater and urban streams due to higher NO_3-N input (23-75 mgN m^2 d^{-1}) compared to forested streams (15 mgN m^2 d^{-1}), but both stream types denitrified a smaller portion of stream NO_3-N load compared to the forested stream. Agricultural streams also had higher levels of DOC, soluble and reactive P, and NH_4-N. Primary influence on denitrification potential was availability of nitrate-N (<0.4 mg/L was limiting), followed by DOC, DO, temperature, and OM. Denitrification rates measured through DEA ranged from 5.22-12 µgN-N_2O g $AFDM^{-1}$ h^{-1}. See Table 2.2 for a comparison of stream sediment denitrification rates from literature.

Reservoirs also serve as areas of high sediment denitrification rates due to longer residence time and higher organic matter deposition. Understanding reservoir denitrification is critical to better predict watershed ability to remove excess N before it reaches coastal areas creating hypoxic zones (David et al., 2006). Water residence time ranged from 2.5 to 10 months from 1981-2003 on a reservoir situated on a tributary to the Mississippi River, which empties into the Gulf of Mexico (Wall et al, 2005). Highest denitrification potential occurred at the upper reservoir out of the river-reservoir continuum due to high organic matter, N percentage, and high nitrate-N concentration in the water. NO_3-N varied seasonally and was low in fall and winter, with concentrations ranging between undetectable to 15 mg/L NO_3-N (Wall et al., 2005). Denitrifying bioreactors have also been proposed to remove large amounts of NO_3-N from agricultural watersheds and can be enhanced through the presence of certain denitrification functional genes (Zhang et al., 2017).

It is important to note that some studies choose to express denitrification potential in both dry mass (DM) and ash free dry mass (AFDM) due to differences in sediment quality and texture. For example, Wall et al., (2005) did not find a significant difference in denitrification rates and sediment characteristics when expressed in g/DM, but did find a significant difference when expressed as g/AFDM (positive correlation to both organic matter and N content).

Table 2.2: Stream sediment denitrification enzyme assay (DEA) rates, methods used, and habitat studied from selected literature. = data not reported. ~ = test not performed. Mean (standard error) shown.

Reference	Method	Unamended Denitrification	Carbon Source and Nitrate-N Amended	NO_3-N Only	Units	Stream NO_3-N - N (mg/L)	Habitat
David et al. 2006	Chloramphenicol, C_2H_2 inhibition	62-225	~	~	g N m $^{-2}$ · yr $^{-1}$	10-14	Lake Shelbyville, IL US Agricultural reservoir
Comer-Warner et al. 2019	C_2H_2 inhibition, combined and individual NO_3-N and glucose amended	~	0.134 ± 0.092 (sand) 0.0134 ± 0.003 (gravel)	0.042 ± 0.025 (sand) 0.003 ± 0.001 (gravel)	µg N_20-N g^{-1}/h^{-1}	--	Agricultural stream, UK
Wall et al. 2005	Chloramphenicol, C_2H_2 inhibition, nutrient-amended denitrification assays, NO_3-N $^-$ and glucose	0-63 (reservoir) 0-12 (riverine) OR 0-2.7 (reservoir) 0-0.27 (riverine)	--	~	µg N_2O gAFDM $^{-1}$ h $^{-1}$ µg N_2O gDM h^{-1}	< 10	Nine Midwestern US headwater streams
Steinhart et al. 2000	Chloramphenicol, C_2H_2 inhibition	~	0.7 (0.3) - 11 (2.0) 0 (0.0)-180.0 (39)	0 (0.0)-5.3 (1.4) (*sand and gravel)* 0(0.0)-340 (55) *(organic debris)*	N_2O production (ng N g^{-1} h^{-1})	0.002-0.160	Northeastern USA streams (NY, NH, ME)
Findlay et al. 2011	Chloramphenicol, C_2H_2 inhibition, combined NO_3-N and		~	~	(ngN g AFDM-1 h-1)	--	Samples from 65 streams across the US

	glucose amended						
Inwood et al. 2005	Chloramph-enicol, C_2H_2 inhibition	15 (Forested) 75 (Agricultural) 23 (Urban)	~	~	mg N/m^{-2} d^{-1}	0.02-0.24	9 midwestern headwater streams MI, US
Opdyke et al. 2006	Chloramph-enicol, C_2H_2 inhibition	0.6-76.4 (separation zones) 0-36.5 (riffles, point bars, pools, run)	185 (31) - 4955 (2460) (organic matter debris dam) 7.9 (5)- 219 (216) *(pool)* 7.6- (7) - 73 (69) (*riffles)* 2.6 (1)- 21 (15) *(Gravel bar)*	~	mg N m^{-2} h^{-1}	<15	Channelized and meandering headwaters IL, US
Groffman and Dorsey 2005	Chloramph-enicol, C_2H_2 inhibition, combined NO_3-N and dextrose amended	~	0.7 (0.3) - 11 (2.0) 0 (0.0)- 180.0 (39	~	µgN kg^{-1} h^{-1}	--	Urban streams MD, US
Steinhart et al. 2000	Chloramph-enicol, C_2H_2 inhibition	~	0.2-418	0 (0.0)- 5.3 (1.4) (*sand and gravel)* 0(0.0)- 340 (55) *(organic debris)*	N_2O production (ng N g^{-1} h^{-1})	0.002-0.160	Northeast US streams (NY, NH, ME)
Kreilling et al. 2019	Chloramph-enicol, C_2H_2 inhibition, Combined glucose and	0-58	~	~	µgN cm^2 h $^{-1}$	--	108 sites, mixed land use watershed, WI, US

	NO_3-N addition						
Bettez and Groffman, 2012	Chloramphe-nicol, C_2H_2 inhibition	.01-10.25	.218 (.328)	~	mgN kg^{-1} h^{-1}		Stormwater control structures MD, US
Wu et al., 2021	C_2H_2 inhibition CH_3COONa and KNO_3-N additions	0.218 (.328)	~	~	µgN g dry sediment $^{-1}$ h^{-1}	41.08	Heavy agriculture catchment in south-west France

2.7 Methods for Measuring Denitrification

Measuring denitrification is a difficult and often challenging process due to naturally high levels of background N_2 present in the atmosphere and in water. Many methods exist for measuring denitrification in both terrestrial and aquatic environments, with methods used to measure aquatic sediments being more accurate that those used for upland terrestrial systems. Common methods include acetylene inhibition techniques, mass balance calculations, ^{15}N isotope tracers, direct N_2 flux measurement, N_2:Ar ratios, stoichiometric approaches, and molecular approaches (Groffman et al., 2006). Denitrification enzyme activity (DEA) is frequently used to measure denitrification potential through the acetylene inhibition technique as outlined by Tiedje et al. (1989) due to its simplicity and quick measurement ability by blocking the reduction of N_2O to N_2 which allows N_2O to accumulate for measurement with gas chromatography (Merill and Tonjes 2014; Seitzinger et al., 1993). Chloramphenicol is often added to stop production of *de novo* nitrate-N reductase enzymes to create measurements close to *in situ* measurements (Murray and Knowles, 1999) and samples may be amended with spikes of nitrate-N and/or a C source (commonly glucose or dextrose) to represent optimal conditions (Comer-Warner et al., 2020, Steinhart et al., 2000, Wall et al., 2005, Groffman et al. 2005, Kreiling et al., 2019).

However, this method is known to underestimate denitrification rates as acetylene inhibits nitrification (Groffman et al., 2006) and may not be best for systems with low levels of nitrate-N or when nitrification is the main source of nitrate-N for denitrifying bacteria (Seitzinger et al, 1993, Steinhart et al., 2000).

2.8 Alternative Nitrate-N Removal Pathways

Denitrification has been accredited as a primary pathway of nitrate-N removed from aquatic systems (rivers, lakes, and wetlands), but new methods in stable isotopes and tracer techniques are providing evidence for other microbially mediated processes (Burgin and Hamilton, 2007, Saunders and Kalff, 2001). The removal of nitrate-N "is no longer synonymous with denitrification…" (Burgin and Hamilton, 2007) and new techniques may help explain discrepancies in N mass budget studies by offering alternative processes of N removal aside from denitrification or biological assimilation. Nitrate-N is easily transported in soil pore water (Duff and Triska 2000) and may undergo various transformations and pathways of either retention or removal once in an aquatic system (Ahearn and Dahlgren, 2005; Burgin and Hamilton, 2007). Retention mechanisms include biological assimilation (aquatic organisms/vegetation), abiotic sedimentation, annamox (anaerobic ammonium-N oxidation) and dissimilatory nitrate-N reduction to ammonium-N (DNRA) in which nitrate-N is converted to ammonium-N.

DNRA occurs in systems high in NO_3-N and labile C, and as the name implies NH_4-N is produced and not incorporated into microbial cells. Ammonium-N is less mobile and a more biologically available form of N, and although somewhat more adhesive to soil particles than NO_3-N, may still leach into groundwater. Not much is known about the fate of ammonium-N produced through microbially mediated DNRA, but it may be converted back to nitrate-N via nitrification or assimilated in plant or microbial biomass. Two pathways exist for DNRA to occur, fermentative DNRA and sulfur oxidation DNRA. Fermentative DNRA occurs in high C:N ratios under non-sulfuric and nitrate-N-limited environments, while sulfur (S) oxidation occurs where S oxidizing microbes are present (Burgin and Hamilton, 2007).

Annamox is a chemolithoautotrophic process more commonly found in marine systems but can occur in freshwater sediments. Not much is known about the bacteria that perform annamox as no pure cultures exists but is thought to be a slow-growing organism with internal compartmentalization and structures called anammoxosomes (Burgin and Hamilton, 2007). Annamox occurs in the presence of low labile C and in areas nitrate-N and ammonium-N coexist (Comer-Warner et al., 2020; Burgin and Hamilton, 2007).

Additionally, incomplete denitrification can occur naturally where NO_2 and N_2O are not reduced to N_2 and accumulate in sediment. Comer-Warner et al., (2020) found highest levels of N_2O in gravel sediments compared to sand when studying ^{15}N and ^{18}O isotopes from stream surface water and porewater samples 10 and 20 cm below the surface. Wu et al. (2021) also observed high levels of N_2O present when denitrification potential was highest and called for further research to understand the seasonal trends of incomplete denitrification and its potential implication in better understanding N budgets in watersheds. In both these instances, nitrate-N is still removed from the water but N_2O, a potent greenhouse gas, may eventually be diffused into the atmosphere. N_2O emissions from rivers may be up to 0.68 Tg N_2O-N y^{-1} (Anderson et al., 2010), and highest estimates of river N_2O emissions account for 10% of global anthropogenic N_2O emissions (Beaulieu et al., 2011).

2.9 Effects of Natural and Manmade Dams on N Processes and Stream Sediment Denitrification

Similar to how human-created dams can alter hyporheic zone chemistry and in-stream processes as discussed above, beaver dams can also alter denitrification in stream sediments. Beaver dams reduce flow velocity and alter landscapes by shaping free-flowing streams into ponds and wetlands (Naiman et al., 1986) causing the water table to rise, ground and surface water interaction to increase, enhance residence time and organic matter accumulation, while decreasing oxygen levels (Naiman et al., 1986, Klotz et al., 2010, Lazar et al., 2015). These factors can enhance the natural denitrification process, and denitrification rates from beaver ponds can vary based on dam size. Lazar et al. (2015) estimated that a beaver pond 0.26 ha in size could remove 49-118 kg NO_3-N km^{-2} yr^{-1} and a beaver pond 1.0 ha ranges from 187-454 kg NO_3-N km^{-2} yr^{-1}. Over a larger area, Lazar et al., estimated beaver ponds across southern New England could remove 5-45% of water nitrate-N resulting from high N loadings (1000 kg km^{-2}). In mesocosm experiments, denitrification rates ranged from 97-236 mg N m^2, with spring having lower denitrification rates than fall. Seasonality influence was also seen by Klotz (2010) with warmer months (April-September) having on average higher denitrification rates (45.5% daily nitrate-N reduction) than colder months (12.4%) streams, implying biological processes acted as control on rates.

Younger beaver dams also had higher rates of denitrification compared to older dam sites due to emergent macrophytes (Lazar et al., 2015). Table 2.3 shows stream sediment denitrification rates from beaver ponds and riparian zones for comparison. Briggs et al., (2013) examined the upstream impact of beaver dams on biogeochemical processes around two beaver dams in Wyoming, USA. They found a heterogeneous system with hyporheic flow linked to redox conditions and similar temporal variations in both stream water chemistry and hyporheic chemistry. However, riparian and groundwater had a different spatiotemporal mixing pattern and almost all streambed profiles intersected downwelling hyporheic flow paths that originated in the stream and did not mix with groundwater. A gradient also became apparent of aerobic to anaerobic transition.

Humanmade in-stream restoration structures, such as weirs, buried structures, and log dams, can also enhance denitrification and help remove N from the system. Hester et al., (2018) found in-stream structures enhanced hyporheic exchange and nutrient removal in areas where groundwater conditions neither induce strong gaining or losing conditions, and that these methods work best when paired with other restoration methods such as reducing overall nitrate-N input. However, while remaining effective methods to enhance denitrification on their own, natural streambed topography appeared to play a larger role in nitrate-N removal through denitrification compared to installed in-stream restoration structures. Lautz and Fanelli (2006) also found in-stream restoration structures created anoxic, biogeochemical hotspots upstream of the structures due to the increased interaction of ground and stream water and the settling of fine sediment in the reduced flow area. Restoration structures that span the full stream channel and are permanent in nature performed best, and the mixing of stream water and groundwater was unique in the hotspots, indicating a more complex mixing of the two sources. Lazar et al. (2104) also found the addition of large wood structures in low

Table 2.3: Riparian zone and beaver pond denitrification rates from selected literature.

Reference	Method	Habitat	Denitrification Rate	Units	NO_3-N (mg/L)
Lazar et al. 2015	^{15}N and ^{15}N-N_2O mass balance mesocosms	3 active beaver ponds in RI (US)	97-236	mg N m^2 d^{-1}	0.21-0.90
Welsh et al. 2017	DEA, chloramphenicol-amended acetylene-inhibition	Stream-Riparian Continuum in Restored and Unrestored Agricultural Streams, NC, USA	928 ± 116 (dormant season, riparian zone) 108 ± 149 (dormant season, stream) 355 ± 55 (growing season, riparian zone) 45 ± 40 (growing season, stream)	Ng N g^{-1} DM h^{-1}	0.47-1.19

Vecherskiy et al. 2011	DEA, acetylene-inhibition	Beaver landscape soils of the Borovna river valley, Kaluga, Russia	88 ± 4 (summer) 112 ± 8 (autumn)	Nmoles N_2O g^{-1} d^{-1}	Not reported
Kellogg et al. 2005	^{15}N-enriched nitrate-N "push-pull"	In situ groundwater denitrification rates at multiple depths (65, 150, and 300 cm) at 4 riparian wetland sites (RI, USA)	30-120 (within 10m of stream) <1-40 (>30 m from stream)	µg N kg^{-1} d^{-1}	<0.1-0.6
Kaushal et al. 2008	^{15}N-enriched nitrate-N "push-pull"	Geomorphic restoration of riparian-zone-stream interface of an urban stream (MD, USA)	77.4 ± 12.6 (restored reach) 34.8 ± 8.0 (unrestored reach)	µg N kg soil $^{-1}$ d^{-1}	1.47 ± 0.05 (unrestored reach) 1.15 ± 0.04 (restored reach)
Bettez and Groffman, 2012	DEA, chloramphenicol-amended acetylene-inhibition	Forested and herbaceous riparian zones (MD, USA)	0.07-.070	mg N kg^{-1} h^{-1}	Not reported

order streams may enhance nitrate-N removal (partially through denitrification), while producing lower N_2O emissions.

Manmade dams and levees also alter floodplain denitrification, with floodplains acting as critical areas of denitrification and N retention due to frequent inundation and wetland-like characteristics (Gergel et al., 2004). Dams lower the water height during floods and decrease floodplain inundation, while levees decrease lateral flooding events. Holding back water from the floodplain also holds back nutrients, an important source of organic matter and fuel for denitrifying microbes. Floodplain denitrification rates are highly variable due to spatial variability in terrain and soil type, along with hydrologic variability from dramatic water level changes from flooding. Through models, river floodplains that have been impacted by levees and dams showed a decrease in ability to process nutrients, including denitrification.

These impacts may be greater from cumulative small flood events as opposed to large, infrequent floods (Gergel et al, 2004). In addition, the reconnection of the stream channel to stream bank floodplains can increase denitrification rates (Kaushal et al., 2008).

Since methods of N retention are not well understood in freshwater systems, being able to better predict N processing is of increasing importance as anthropogenic inputs continue to increase (Saunder and Kalff, 2001). Dams can alter hydraulic residence time (HRT), resulting in associated upstream reservoirs acting as a sink for nutrients when HRT is high (Maavara et al., 2020). Low-head milldams reduce waterflow and create pools above the dam where water becomes more stagnant, lowering DO levels, enhancing residence time for biogeochemical reactions, and collecting fine sediments and nutrients from upstream. These combined conditions are ideal for denitrification to occur (Figure 2.2). Previous studies have called for the holistic and interdisciplinary approach to understanding the science and applied management of these low-head milldams including their cumulative spatial effects, nutrient cycling influence, and ecosystems alteration (Fencl et al., 2015; Van Cappellen and Maavara, 2016; Graham et al, 2019), and understanding how low-head milldams impact stream sediment denitrification is critical to managing and understanding stream health.

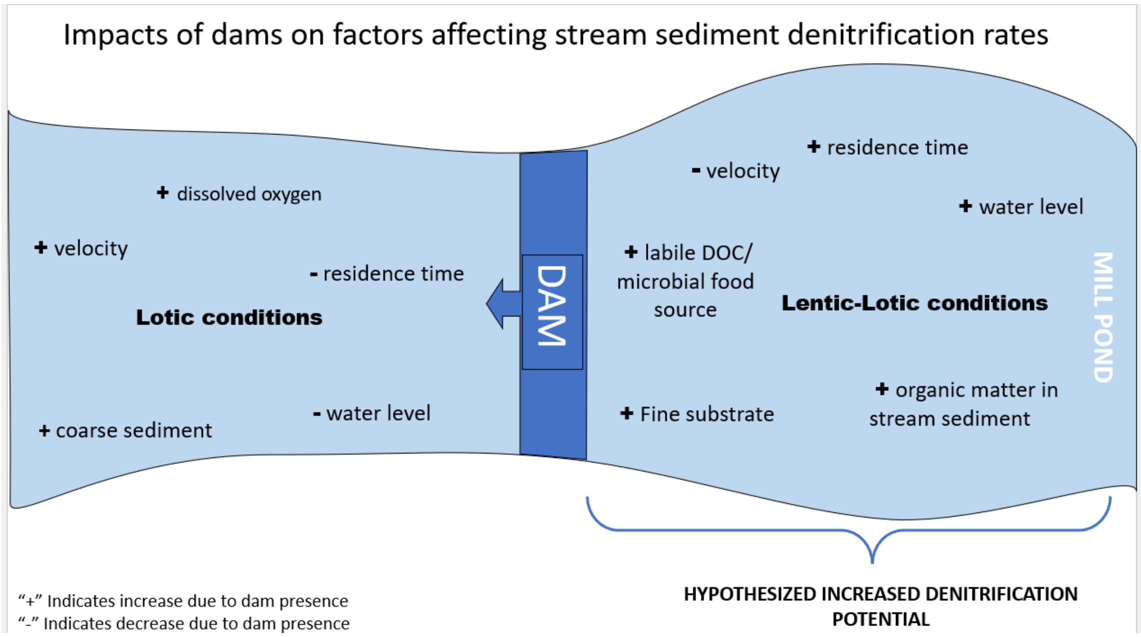

Figure 2.2: Schematic representing how dam presence alters factors that increase denitrification potential above the dam compared to a more free-flowing environment below.

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Chapter 3

METHODS

3.1 Study Sites

Two sites with existing low-head milldams were chosen for this study, Cooch's Mill dam (coordinates 39.645556°, -75.742500°) on the Christina River in Newark, Delaware (DE) and Roller Mill dam (coordinates 40.108306°, -76.443111°) on Chiques Creek in Manheim, Pennsylvania (PA). Cooch's Mill dam sits on the historic Cooch's Mill battleground from the Revolutionary War and the site has been largely preserved. Roller Mill dam sits on private property with many homes along the adjoining banks, with the dam slated to be removed in 2022 due to bank and abutment erosion.

Cooch's Mill dam sits south of Newark in New Castle County, DE on the Christina River, a third-order or higher tributary to the Delaware River and encompasses a drainage area of 50.7 km^2 (Figure 3.1A). Land use consists of approximately 24% forest coverage, 23% cropland, 6% other vegetation, and 47% developed (urban) land. Soil in the catchment and surrounding riparian zone consists primarily of loam and silty-loam and is dominated by Glenelg loam (Soil Survey, 2021). The surrounding geological basin falls under the Columbia Formation (DGIR) formed in the lower Pleistocene (Delaware Geological Survey). The Christina River is approximately 18 m across 200 m upstream of the dam, widens to 45 m at the dam, and returns to 18 m below the dam with the channel meandering downstream at varying widths and depths. Upstream of the dam is generally 4 m deep and downstream is approximately 30 cm deep immediately below the dam. Annual precipitation is 117 cm and average air temperature is 15.5°C (NOAA NCEI).

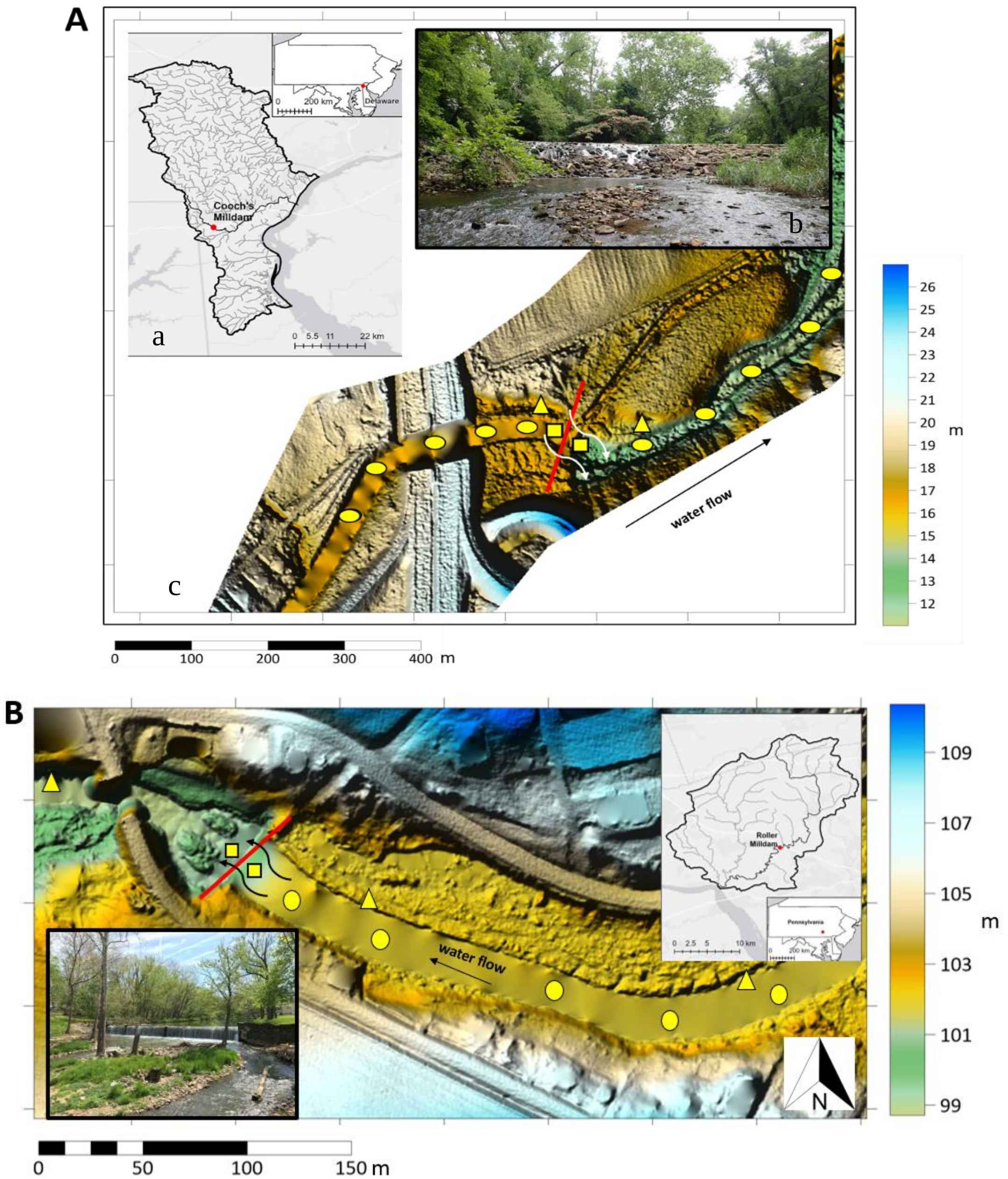

Figure 3.1: Christina River watershed and Cooch's Mill dam (39.645556°, -75.742500°) (A) and Chiques Creek watershed and Roller Mill dam (40.108306°, -76.443111°) (B). Watershed map inserts (a) indicating entire watershed outlined in black, individual streams in dark grey, main river stem highlighted, and red dot representing dam sites. Photos of dam looking upstream (b). Study site maps (c) overlaid on LIDAR digital elevation model indicating monthly stream grab sample collection above and below dam (yellow squares), seasonal streambed sediment core collection (yellow circles), and water quality sensors installed in the stream (yellow triangles).

Cooch's Mill dam, approximately 45 m wide and 4 m in height, was built in the 1792 by William Cooch, JR to power mill machinery to turn local wheat and corn crops into flour and cornmeal. Water was redirected from the Christina River through a raceway to a waterwheel attached to the mill. The mill survived two fires to remain operational until the 1980s (Delaware Division of Historical and Cultural Affairs). Today the mill is used for educational programming and tours (Delaware Division of Historical and Cultural Affairs) and is surrounded by property historically noted for the 1777 Battle of Cooch's Bridge, the only Revolutionary Battle fought on Delaware soil (RevolutionaryWar.US).

Figure 3.2: Images of water and large debris flowing over Cooch's Mill dam (left) and view of downstream of dam (right).

Roller Mill dam is located on Chiques Creek, a third-order or higher tributary to the Susquehanna River, in Lancaster, County PA and encompasses a drainage area of 127 km^2 (Figure 3.1B). Land use consists of ~24% forest cover, ~19% developed/disturbed (urban), ~56% agriculture, and < 1% open water (SRBC

Watershed Profile, 2022). The catchment and surrounding riparian zone consists mostly of silty-loam soils and is dominated by Glenelg loam (Soil Survey 2021). The geological basin is primarily limestone and dolomite in the Snitz Creek and Buffalo Springs Formations formed in the Cambrian age (PaGEODE). Mean basin elevation is 162.5 m and depth to rock 1.3 m (USGS StreamStats Report). Local topography consists of rolling hills and meandering streams. Channel width is approximately 21m above the dam, widens to 37 m immediately below the dam where it divides into three smaller streams around two islands, and then narrows to one channel 14 m across downstream of the dam. Upstream of the dam is generally 2.4 m deep and downstream approximately 0.9 m in depth immediately downstream of the dam. Annual precipitation is 111 cm and average air temperature 12.2°C (NOAA NECI 2020).

Roller Mill dam, built around 1730, is approximately 21 meters across and 2.4 m high. The dam was originally built to power the grist and sawmills of Scott Mill on the north side of Chiques Creek (also known as Chickies Creek/Chickesalunga Creek). A new mill was built in 1829 on the south side of the creek which became Shenck Mill and later Salunga Mills. A home is now built on the foundation of the old Scott Mill (the Barton House) (MillPictures.com). Roller Mill dam has been deemed compromised by the PA DEP Dam Safety and was slated to be removed in 2021 with efforts pushed back due to COVID-19 (American Rivers, 2020).

Figure 3.3: Roller Mill dam after a storm event above the dam (left) and below (right).

3.2 Experimental Design

To answer the questions previously addressed we studied hydrologic and biogeochemical conditions in the stream along with denitrification ability in stream sediments through the collection of physical, chemical, and biological data. Beginning November 2019, monthly stream grab samples from above and below the dams (~10 m from the dam itself in either direction) and were analyzed for dissolved nutrients and estimates of organic matter quality, as well as discrete measurements of common water quality metrics. Beginning January 2020, stream grab samples were also collected along a stream reach extending 300 m downstream at Cooch's Mill and 500 m upstream at Roller Mill and analyzed for nutrient concentrations including TN, NO_3-N, NH_4-N, and Cl^- Additionally, continuous monitoring of water chemistry from deployed water quality sondes and data loggers in the stream occurred from November 2019-September 2021 (Figure 3.4). Biweekly sampling was changed to monthly in March 2020 due to COVID-19 and for the remainder or the study period. Discharge and residence time were calculated above and below each dam using velocity measurements. Stream sediment cores were collected seasonally Fall 2019-Summer 2021 for a total of eight sampling sessions over two years. Sediment cores were analyzed for denitrification potential through denitrification enzyme assays (DEA), calculation of ash-free dry mass (AFDM), net nitrification, and net mineralization (methods described in detail below). A subset from Fall 2019-Fall 2020 was analyzed for particle size. Site layouts and sampling collection locations are detailed in Figure 3.1. Hydrologic and biogeochemical sampling are summarized by analytes and sample frequency (Table 3.1).

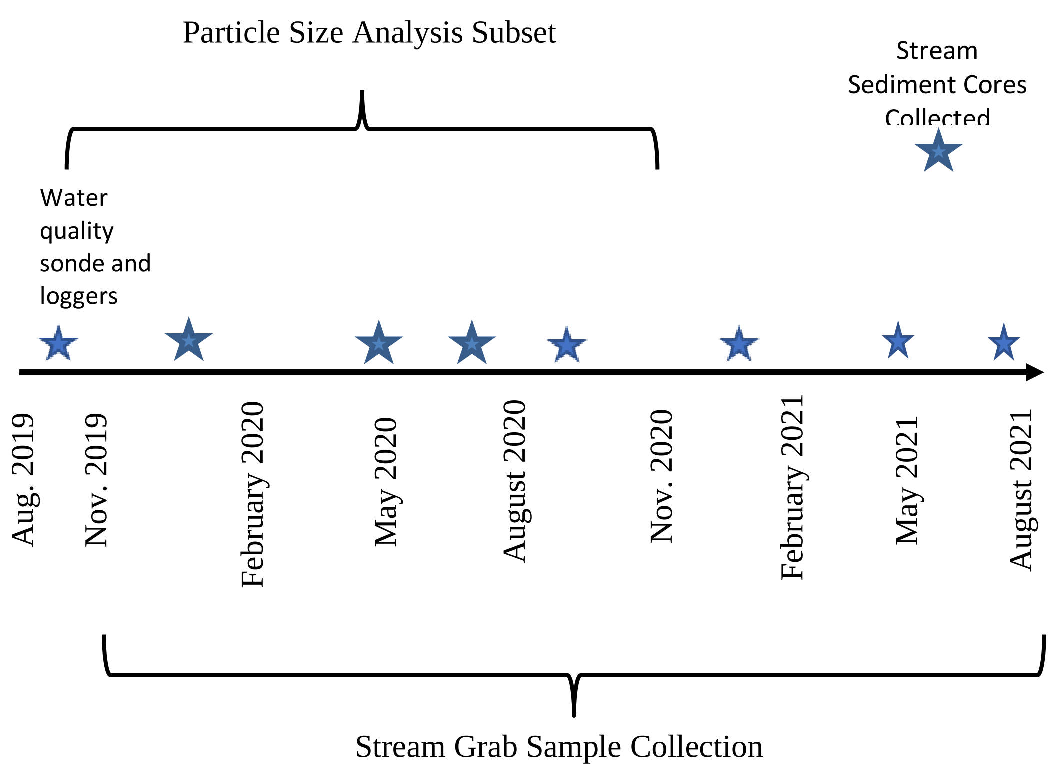

Figure 3.4: Timeline of site installation and sampling from August 2019-August 2021.

Table 3.1. Chemical and biological sampling (A) and high frequency data (B).

(A) Sample	**Analytes**	**Frequency**	**Laboratory**
Stream grab samples upstream and downstream of dam	TdN, DOC, NO_3-N, NH_4-N	Bi-weekly Nov. 2019- March 2020	UD Soils Lab
	SUVA		Inamdar Lab
Stream grab samples every 100 m for 500 m stretch	TN, $N0_3$-N, NH_4-N, Cl, SRP	Monthly March 2020- August 2021	Stroud Ecosystems Ecology Lab
Stream sediment cores every 100 m for ~500 m stretch	DEA, net mineralization, net nitrification	Seasonally Nov. 2019-August 2021	Stroud Ecosystems Ecology Lab
	Particle Size (percent sand, silt, and clay by both volume and surface area)	Subset of sediment samples from Nov. 2019-Oct 2020	UD ISE Lab

(B) Measurement	**Location**	**Frequency**
HOBO Loggers: SpC, stream level, DO	Cooch's Mill Upstream	30-minute intervals
HOBO Loggers: SpC, stream level AquaTroll Sonde: DO	Cooch's Mill Downstream	
HOBO Loggers: Stream level, SpC, DO	Roller Mill Upstream	
SRBC YSI Exo II Sonde: DO	Roller Mill Downstream	

3.3 Hydrologic Monitoring

Stream level loggers (Onset, HOBO U20L, Bourne, MA) were installed at each site and programmed to record stream height every 30 minutes. Loggers were suspended and encased in PVC pipes secured in stream bank to prevent biofouling and protect the instruments. Data was corrected with absolute pressure measurements from nearby installed atmospheric loggers in the adjacent riparian zone (Onset, HOBO U20L, Bourne, MA). In October 2019, Cooch's Mill had level loggers installed nearby to upstream and downstream of the dam (Figure 3.4b) and Roller Mill had two level loggers installed approximately 200 m (labeled RMST1), and 500m (labeled RMStream) upstream of the dam. Data was used from the Susquehanna River Basin Commission's (SRBC) YSI EXO II multiparameter sonde, pressure transducer, and discharge measurements located approximately 50 m downstream of the dam. SRBC high frequency stage data from January 1, 2018-August 31, 2021 and 42 discrete flow measurements collected from January 2018 to May 2021 were used to create a discharge rating curve for downstream of the Roller Mill dam (Figure 3.5). USGS stream gauge 50 m downstream of Cooch's dam provided discharge. Residence time was calculated using the following equations. Sampling length of 500 m was used and results in seconds were converted to hours.

$$Residence\ Time = Distance/Velocity$$

$$Q = A * V$$

Where Q =discharge in cubic meters, A = area in meters, and V = mean velocity in meters per second. For Christina River, upstream velocity was averaged from three runs using an Acoustic Doppler Current Profiler on May 19, 2021 under baseflow conditions. Downstream velocity was from USGS Christina River discrete flow measurements under similar baseflow discharge conditions (Gage located ~ 50 m downstream of dam, drainage area is within 4.6% of total drainage area of Cooch's dam.) For Chiques Creek, upstream velocity (V) was estimated using known SRBC bathymetry cross-sectional area (A) 100 ft upstream of the dam and known discharge (Q) estimated using a rating curve developed from continuous pressure transducer data and discrete discharge measurements. Downstream velocity was the daily flow average from SRBC pressure transducer data for the same day upstream measurements were taken. Velocities serve as measurements from a single day, and velocity can vary throughout stream reach and water column, so these values act as estimates based on respective daily conditions. Depth and widths of the streams above and below the dam were manually calculated using a measuring tape and yard stick. Velocity was also calculated above each dam as mean of three runs using an Acoustic Doppler Current Profiler (ADCP), presented below. The flow was frequently observed to be slow at both sites at baseflow. Handheld flow meters provided unreliable data not presented here.

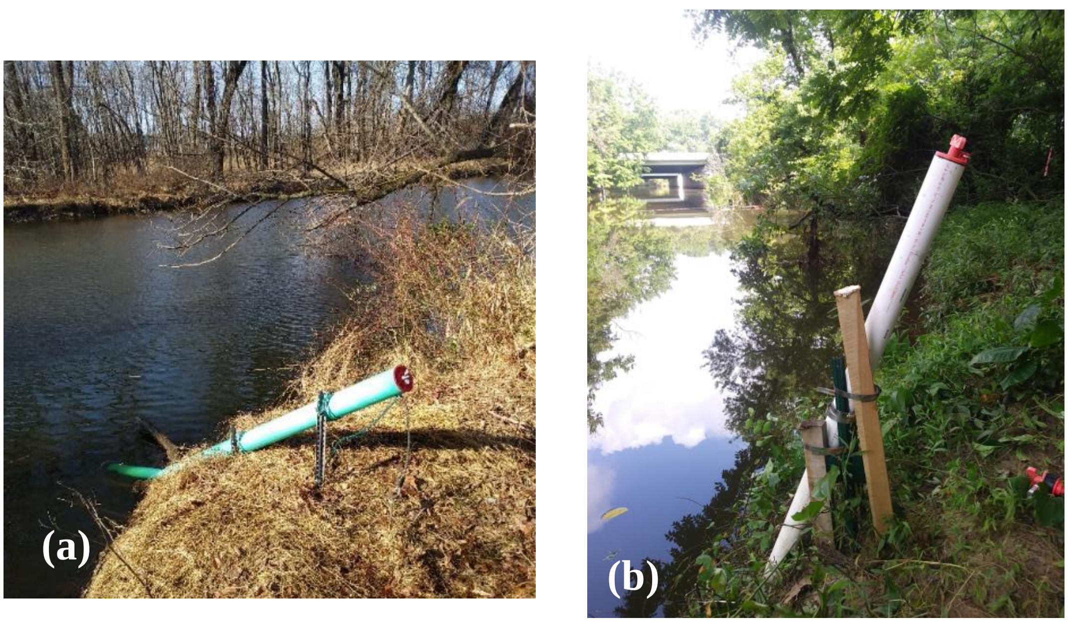

Figure 3.4: PVC pipe structures deployed at Cooch's Mill site to host water quality sondes (a) and conductivity and stream level loggers (b).

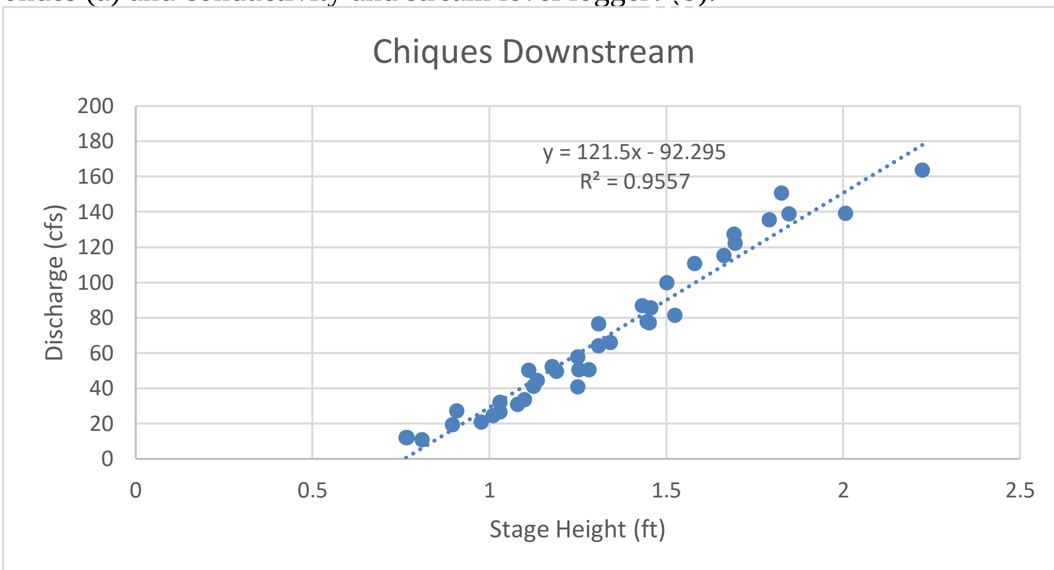

Figure 3.5: Discharge rating curve developed for downstream of the Roller Mill dam on Chiques Creek using SRBC high frequency stage height (ft) and discrete flow measurements (cfs).

3.4 Stream sample collection for nutrients

Nalgene bottles (250 mL) were rinsed three times with sample in the field before collection and immediately placed on ice. Samples were filtered within 24 hours with a Grade F Borosilicate glass microfiber filter (0.7 µm) (Sterlitech) using vacuum filtration. Filtered samples were sub-sampled, stored in amber bottles, and refrigerated until analysis on a Horiba Aqualog for specific ultraviolet absorbance values (SUVA, L/mg*m), calculated as the absorbance at the 254 nm wavelength (UV254, cm^{-1}) divided by dissolved organic carbon (DOC, mg/L) concentration for stream grab samples. SUVA values indicate if available carbon is recalcitrant or labile, indicating bioavailability for microbial processes (Cory 2011). All remaining filtered samples were then acidified with TraceGrade HCl 34-37% until the pH was ≤ 2 to prevent degradation of the sample and inhibit bacteria growth and consumption of N species. Samples were then processed by the University of Delaware Soils Laboratory for total dissolved N and dissolved organic carbon (DOC) as NPOC (measured by direct combustion and total organic carbon calculated by difference using an Elementar Vario-Cube TOC Analyzer, Elementar Americas, Mt. Holly, NJ), NH_4-N, NO_3-N measured colorimetrically using a Bran&Luebbe AutoAnalyzer 3 (Bran&Luebbe, Buffalo Grove, IL). Nitrate-N standards and replicates were randomly included to ensure accuracy. Any remaining sample was refrigerated for the rest of the study period.

Beginning January 2020, stream grab samples were collected along a ~500 m stretch downstream of the dam at Cooch's Mill and upstream of the dam at Roller Mill. Sampling locations were chosen due to property access and local river access. Stream grab samples are immediately placed on ice and frozen for future analysis by the Stroud Water Research Center Ecosystems Laboratory in Avondale, PA. Whole water samples were analyzed for TN and TP using a persulfate alkaline digestion and colormetrically analyzed using a SEAL Analytical AQ300 discrete analyzer (SEAL Analytical Inc., Mequon, WI). Dissolved nutrient samples were filtered on a 0.45 micron filter and also colometrically analyzed for NO_3-N, NH_4-N, and Cl^- using the same instrument.

3.5 In-Situ Water Quality Monitoring

To monitor hydrologic conditions in streams, water quality sondes were programmed to collect data every 30 minutes at each site. In November 2019 an In-Situ AquaTroll 600 multiparameter sonde (In-Situ, Fort Collins, Colorado, USA) was installed approximately 100 m upstream of the dam at the Roller Mill field site and secured to a tree branch near the streambank. Downstream water quality data was used with permission from SRBC who had a preexisting EXO2 YSI (Yellow Spring Instrument, Yellow Spring, Ohio, USA) sonde installed approximately 50 meters downstream as part of their Remote Water Quality Monitoring Network (SRBC Overview). In December 2019, another YSI EXO2 multiparameter sonde was installed approximately 30 meters upstream of the dam at Cooch's Mill and cased in a protective PVC structure to reduce biofouling and prevent instrument damage (see Figure 3.4a). Another AquaTroll600 sonde was installed downstream of the dam at Cooch's Mill July 2020 and became stuck in its position in the stream until the end of the study period due to a tree falling from Tropical Storm Isaias in August 2020. AquaTroll 600 sondes collected continuous data for pH, temperature (C), turbidity (NTU), conductivity (μS/cm), and dissolved oxygen saturation (%), and YSI EXO2 sondes collected the same parameters but dissolved oxygen concentration instead (mg/L). All sondes were routinely maintenanced and calibrated. However, we acknowledge issues in consistency with data collected from sondes at Cooch's Mill and upstream at Roller Mill. Two of the sondes had to be sent away for repairs during the study period resulting in lapses in data in addition to occasional unrealizable data due to biofouling and instrument malfunction while deployed. Additionally, sondes were not maintained during March-June 2020 due to disruptions caused by COVID-19.

To remedy lapses in data caused by sonde malfunction we relied on continuous high-frequency data collected by HOBO loggers deployed at each site. Conductivity loggers (Onset, HOBO U24, Bourne, MA) were deployed in the same PVC pipes as level loggers upstream and downstream of the dam at Cooch's Mill and upstream of the dam at Roller Mill. Dissolved oxygen loggers (Onset, HOBO U26, Bourne, MA) were installed upstream of the dams September 2020 and October 2020 at Cooch's Mill and Roller Mill, respectively. At Christina, a downstream conductivity logger was not installed until August 2020 and upstream conductivity is missing from July 12, 2021 onward is due to sensor malfunction. Data was downloaded every three months.

Lastly, monthly point measurements of water quality metrics were recorded from handheld meters placed in the stream and allowed to calibrate for two minutes within 10 m of the the dam upstream and downstream of each site. Metrics included temperature (°C), specific conductivity (SpC, µs/cm), oxidation reduction potential (ORP, mV) (Yellow Springs Instrument [YSI] Pro1030) and DO concentration (mg/L) (YSI EcoSense ODO200).

3.6 In-stream Denitrification Potential Assessment

Stream sediment cores were collected seasonally at each site from Fall 2019-Summer 2021 (n = 8 sampling seasons). Five sediment cores were collected upstream of the dam at Roller Mill via canoe with a 0.3 m hand auger inserted into the stream bed until resistance (bedrock) was felt, generally 10-20 cm, beginning 100 m from the dam and increasing at 100 m increments until all five are collected (Figure 3.6). Downstream coring was limited due to property access but starting Fall 2020 intermittent sediment samples were taken approximately 10 m downstream and approximately 100 m downstream of the dam from wading and manual collection. At Cooch's Mill 10 sediment samples were collected each sampling day, with 5 replicates upstream of the dam collected via canoe and 5 replicates downstream via manual grab sampling, both at the same 100 m increments.

Samples were analyzed at the Stroud Ecosystems Ecology lab using the DEA method modified from Tiedje et al (1989). Samples were kept in the dark from collection to analysis. Drained sediment is added to 250 mL media septa bottles and mixed with 20 mL of stream water collected from the same location. Amended bioassays receive 5 mL of DEA media (KNO_3 and $C_6H_{12}O_6$) to represent ideal nutrient conditions for denitrifying microbes. Both amended and unnamed bioassays receive chloramphenicol to inhibit protein synthesis to stop production of *de novo* nitrate-N reductase enzymes to create measurements close to *in situ* measurements (Murray and Knowles, 1999). Samples were then flushed three times with N_2 to create an anaerobic environment (Figure 3.7). Acetylene gas was added to each sample to block the conversion of N_2O to N_2 which allows N_2O to accumulate for measurement with gas chromatography (GC) (Merill and Tonjes 2014, Seitzinger et al., 1993) as pure nitrogen gas is difficult to measure due to naturally high atmospheric presence. Amended samples are shaken for three hours and N_2O gas samples are taken at .5, 1, 1.5. and 3-hour increments.

Figure 3.6: Collection of stream sediment cores upstream of the milldam using a coring rod on Chiques Creek in Manheim, PA.

Unamended samples were shaken for 6 hours and N_2O gas samples are taken at 1,2,4, and 6-hour increments. Vials are stored upside down until GC analysis. Denitrification is reported in both µgN/kg sediment/h^{-1} and as µgN/gAFDM/h^{-1} and AFDM (g) is also calculated per sample. Cores were collected October 2019, January 2020, May 2020, July 2020, September 2020, January 2021, May 2021, and August 2021 at Roller Mill and October 2019, December 2019, May 2020, July 2020, September 2020, January 2021, May 2021, and August 2021 at Cooch's Mill. Out of 124 sediment cores collected, 10 were not able to be completely analyzed and are not included in results. Reasons include insufficient sample volume or error while processing. Stream grab samples were also taken at the sites of cores at both sites and are analyzed for total TN, NH_4-N, NO_3-N, TP, PO_4-P, and Cl^- on the SEAL Analytical AQ300 discrete analyzer.

To further understand N processing in stream sediment, incubations were performed to calculate net nitrification net mineralization production in the sediment. 25 g aliquots of the dried and sieved sediment from DEA cores were used and mixed with 85 mL of associated stream water from the grab samples. Samples are shaken for 175 rpm for 5 minutes with 20 mL then filtered into a clean scintillation vial for future analysis of NH_4-N, NO_3-N, and soluble reactivephosphorus (SRP). Remaining samples were covered with tin foil and incubated in the dark at 135 rpm for four days with 20 mL being collected for analysis after one day and four days for abovementioned analytes (methods modified from Groffman et al. 2005 and Dodds et al. 2017). Additionally, sediment ammonium-N and nitrate-N concentrations were estimated from stream water nutrient concentrations at time zero.

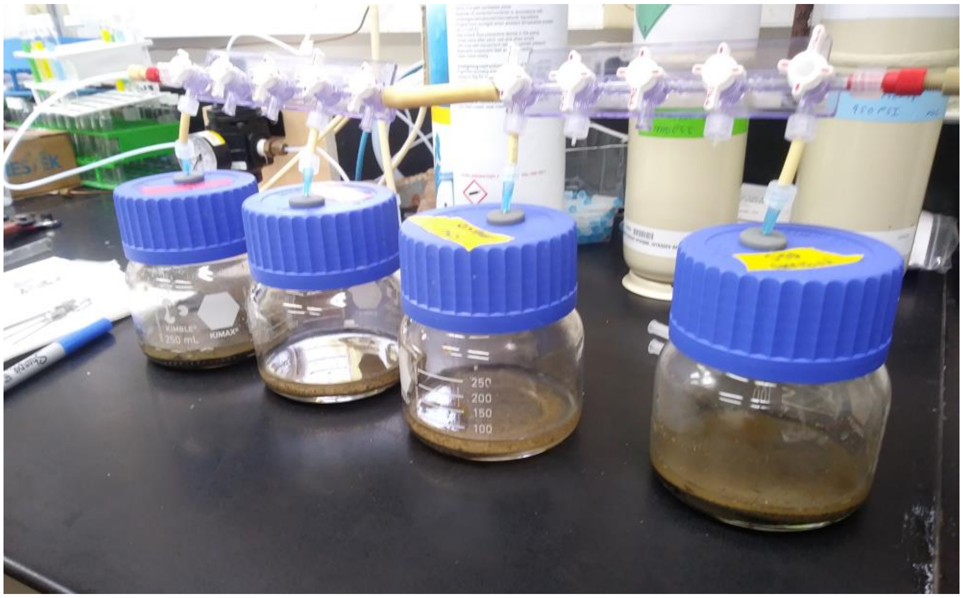

Figure 3.7: Streambed sediment samples being flushed with N_2 to produce an anaerobic environment for denitrification enzyme assays.

Figure 3.8: Images comparing streambed sediment from Chiques Creek (left) and Christina River (right) collected from various distances from dam and seasons. Note variation in sediment size and type.

3.7 Particle Size Analysis

Samples from Fall 2019-Fall 2020 (n=74) were analyzed for particle size at the University of Delaware's Interdisciplinary Science and Engineering (ISE) Laboratory using a using a Beckmann Coulter LS 13 320 Particle Size Analyzer ® (Indianapolis, IN) using laser diffraction to determine particle size distribution. Samples were disaggregated and sieved to 2000 µm as the particle size detection ranged from 17µm - 2000µm. Vials were cleaned and the column cleaned prior to each analysis and background checks run every 10 samples.

3.8 In-Situ Measurement Analysis

Continuous stream level data was plotted against stream conductivity to investigate dilution from storm events. Residence time was calculated and compared above and below each dam using velocity and assuming a sampling distance of 500 m. Daily changes in minimum and maximum variation of DO upstream and downstream of the dam were calculated and compared. Daily water temperature fluctuations were also calculated and compared above and below the dams. Calculations were done in Microsoft Excel, JMP Pro 15, and R version 4.1.1.

3.9 Stream Grab Sample and Sediment Core Analysis

DEA averages were compared above and below the dam and along the sampling gradient using a Student's t-test, ($\alpha = .05$). Bar graphs of DEA rates across seasons and site were created, and mean DEA rates were compared against %OM. Additionally, percent sediment particle size by both surface area and volume were calculated at 100 m increments by distance from dam for each sand, silt, and clay.

Stream grab samples were analyzed for temporal and spatial nutrient trends across each site and upstream and downstream of the dams. Average TN, NO_3-N, and NH_4-N were calculated for each site and location were presented in bar graphs along with temporal scatter plots of stream TN, NO_3-N, and NH_4-N values. SUVA values were processed and analyzed in Matlab using the N-Toolbox kit and were averaged by site location, plotted over time, and compared to DOC concentrations. Above and below dam means and standard error for stream nutrient concentrations, sediment incubations, and denitrification potential analysis were also calculated and summarized.

3.10 Statistical Analysis

An ANOVA was run to determine if significant differences existed between DEA sediment sampling locations. An ANCOVA was run to examine potential covariation among distance, date, and distance by date in stream nutrients (TN, NO_3-N, and NH_4-N) concentrations of stream grab samples collected along the 500 m sampling reach. A multilinear regression model ($\alpha = .05$) was run to understand potential controlling factors on denitrification in streambed sediment. The response variable was denitrification rate in µg N kg sediment h^{-1} and predictors included streambed sediment organic matter (%), stream concentrations of NO_3-N (mgL), NH_4-N (mg/L), TN (mg/L), SRP (mg/L), Cl (mg/L), and estimates of nitrate and ammonium sediment concentrations (mgN/kg/sediment) derived from net nitrification and mineralization incubation nitrate and ammonium concentrations at time zero, respectively. The model was run for individual and combined sites. Advanced modeling including a mixed-effects linear model did not result in higher explanatory power but did indicate season, sample date, reach, and treatment were not significant factors in explaining denitrification variability. Statistical analyses were performed using R version 4.1.1 and JMP Pro 15 software.

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Chapter 4

RESULTS

<u>Stream hydrology and water chemistry</u>

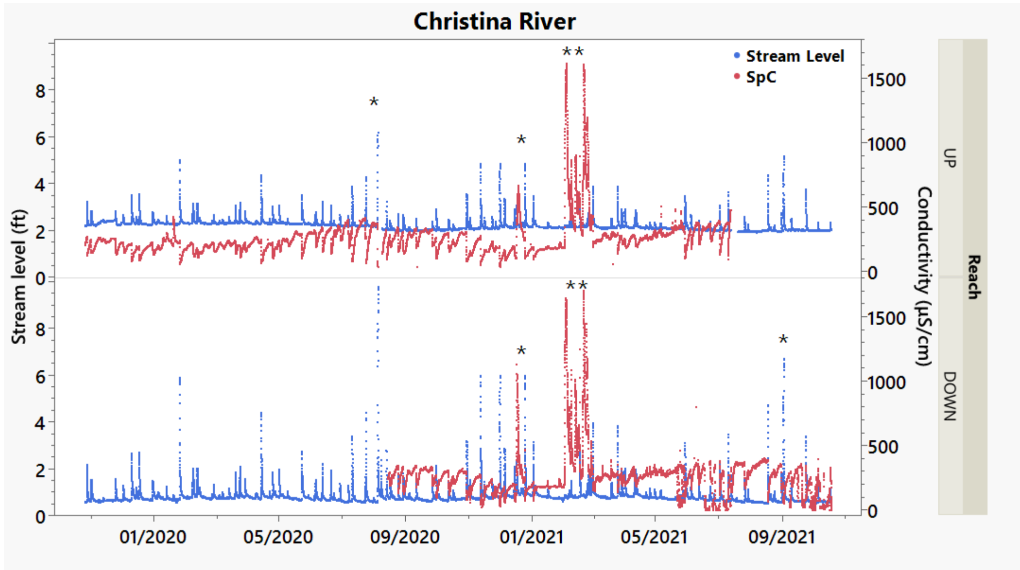
Stream level and conductivity

Upstream stage was consistently higher than downstream water levels at both sites. However, the timeseries of stream level and conductivity displayed similar patterns across the study period (Figure 4.1). Throughout most of the study conductivity was higher at Christina than Chiques, with similar conductivity values upstream and downstream of the dam regardless of site. A significant spike in conductivity occurred in Winter 2021 upstream and downstream of the Christina dam, likely due to salt runoff from nearby Route 95 highway.

Temperature fluctuation and stream flow

Differences in daily mean water temperature above the dams versus below indicates that Chiques Creek experienced more variation than Christina River over the study period (Figure 4.2). At Chiques, stream water above the dam was up to 2°C warmer than downstream, except during Summer of 2020 when this pattern was reversed. Contrastingly, stream water above the Christina dam was always warmer than below throughout the entire study period.

(a)



(b)

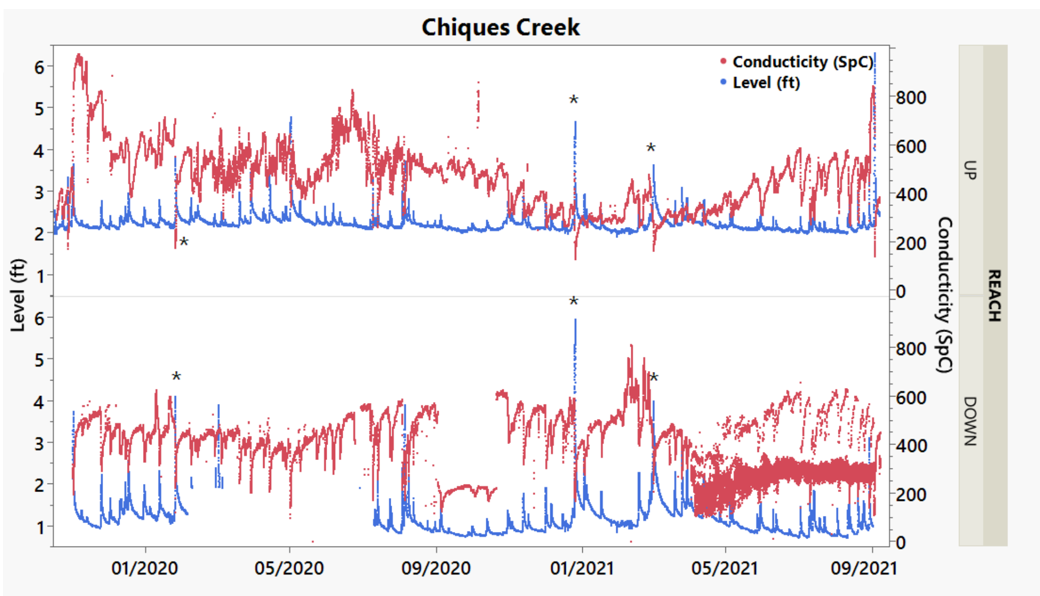
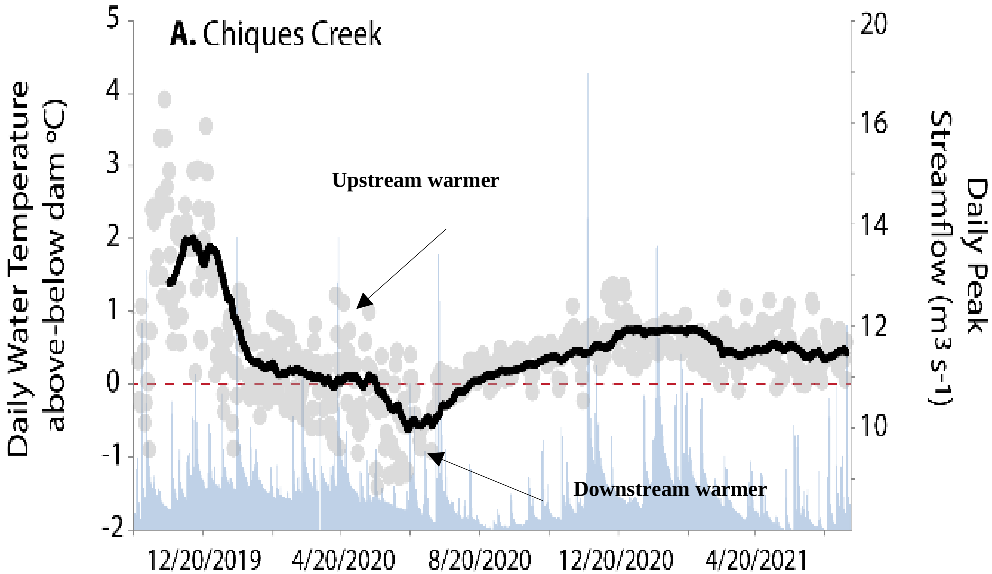


Figure 4.1. Temporal trends in stream level (ft) and conductivity (µS/cm) above and below the dams from Nov. 2019-Sept. 2021 for Christina River (a) and Chiques Creek (b). Dilution events in the streams due to rain events are denoted by (*). Conductivity spikes likely due to road salt runoff from a nearby highway at Christina denoted by (**). Note difference in Y axis scales due to higher conductivity at Christina.



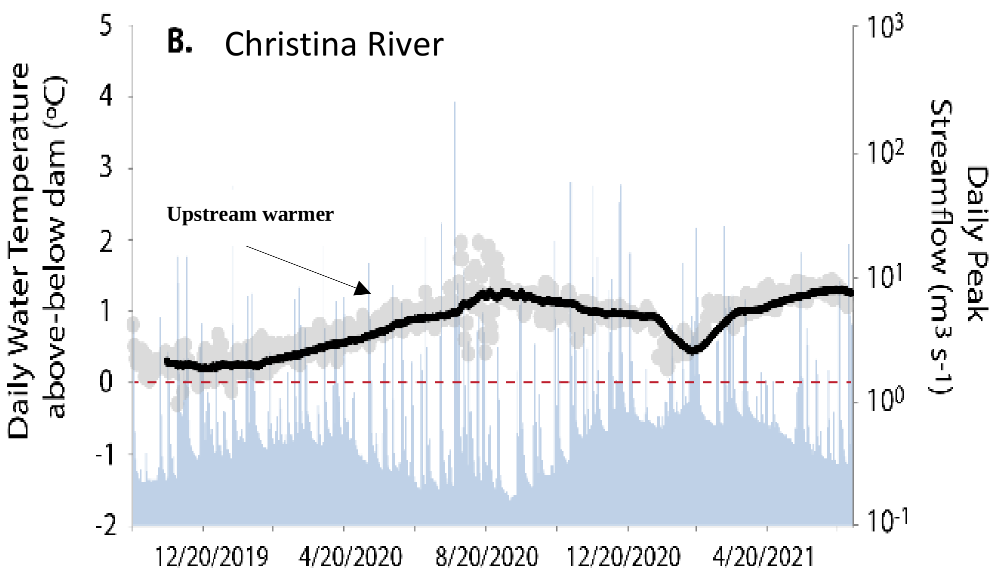
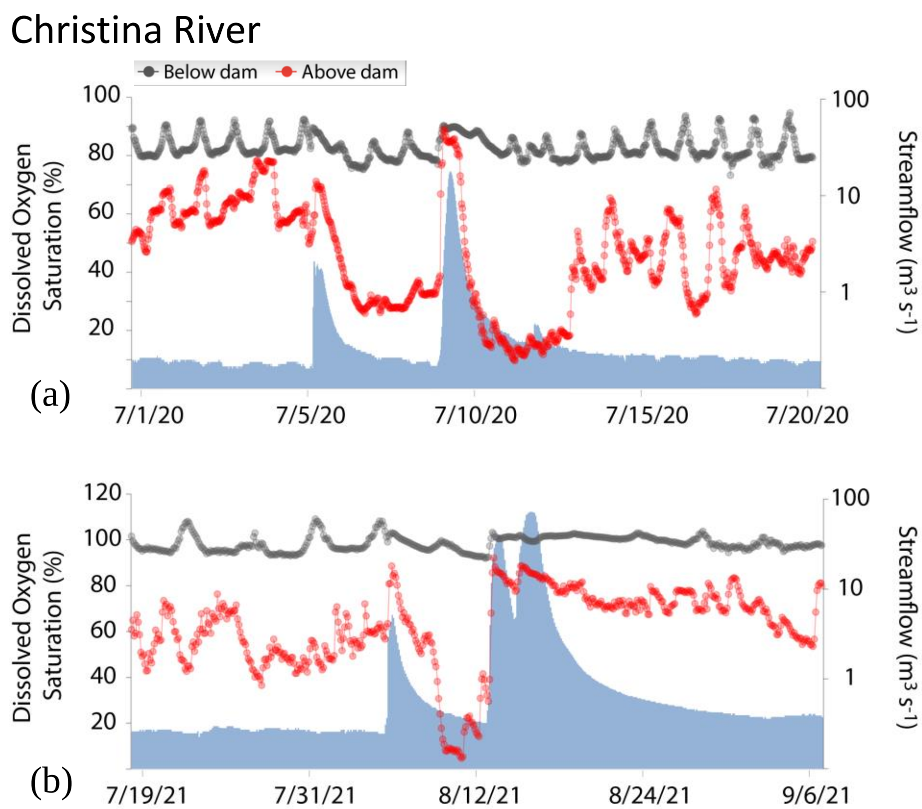


Figure 4.2. Average daily difference in temperature (°C) above the dam compared to below the dam plotted against streamflow (m^3) for Chiques Creek (A) and Christina River (B). Black line represents the moving average of daily difference in temperature.

Dissolved oxygen saturation versus stream flow

Dissolved oxygen saturation was almost always lower above the dams than below them, particularly in the Christina River. At both locations, DO saturation levels drop abruptly after rain events (e.g., July 8, 2021 at Christina; August 11, 2021 at Chiques), but not always during storm events. There were also recurrent hypoxic conditions (< 50% saturation, Carter et al. 2021) above the dam that contrast with well-oxygenated water downstream of the dam. (Figure 4.3 A-B).

A



B

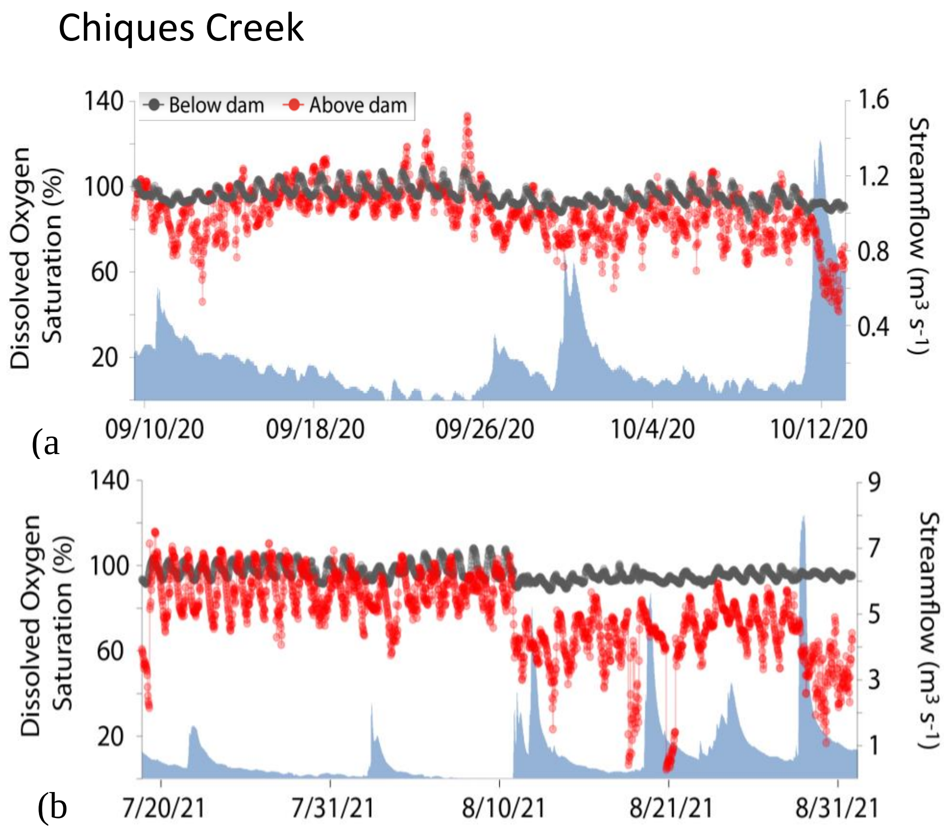


Figure 4.3. Daily percent dissolved oxygen saturation versus streamflow ($m^3\ s^{-1}$) for Christina (A) with values from July 1, 2020- July 20, 2020 (a) and July 19, 2021-September 6, 2021 (b), and Chiques (B) with values from September 9, 2020- October 12, 2020 (a) and July 20, 20921-August 31, 2021 (b) over selected time periods of best data available. Red points represent above the dam dissolved oxygen percent saturation; black dots represent below.

Residence Time above and below the dams

Water residence time above the Cooch dam was much longer than at Rollers dam, which is consistent with the size of each impoundment (i.e., dam height). At both study sites, water residence time was approximately eight times longer upstream of the dam than downstream (Table 4.1), reflecting significant effects of both dams on stream flow.

Table 4.1: Velocities (m/s), residence times (h), and difference in residence time above versus below each dam.

Stream	Location	Velocity	Residence Time	Ratio
Christina River	Above dam	0.013 m/s	10.68 h	8.5x longer upstream RT
	Below dam	0.110 m/s	1.26 h	
Chiques Creek	Above dam	0.046 m/s	3.02 h	7.7x longer upstream RT
	Below dam	0.355 m/s	0.39 h	

Stream nutrient concentrations above and below the dam

On average, dissolved (NO_3-N) and total nitrogen (TN) concentrations were not different above and below the dams (Christina: $p > 0.05$, n=24, Students T-test; Chiques: $p > 0.3$, n=22, Students T-test). At Christina, NO_3-N and TdN were slightly higher downstream of the dam and DOC slightly higher upstream, whereas NH_4-N and TdN concentrations were both slightly higher downstream of the dam and NO_3-N and C concentrations slightly higher upstream of the dam at Chiques (Figure 4.4).

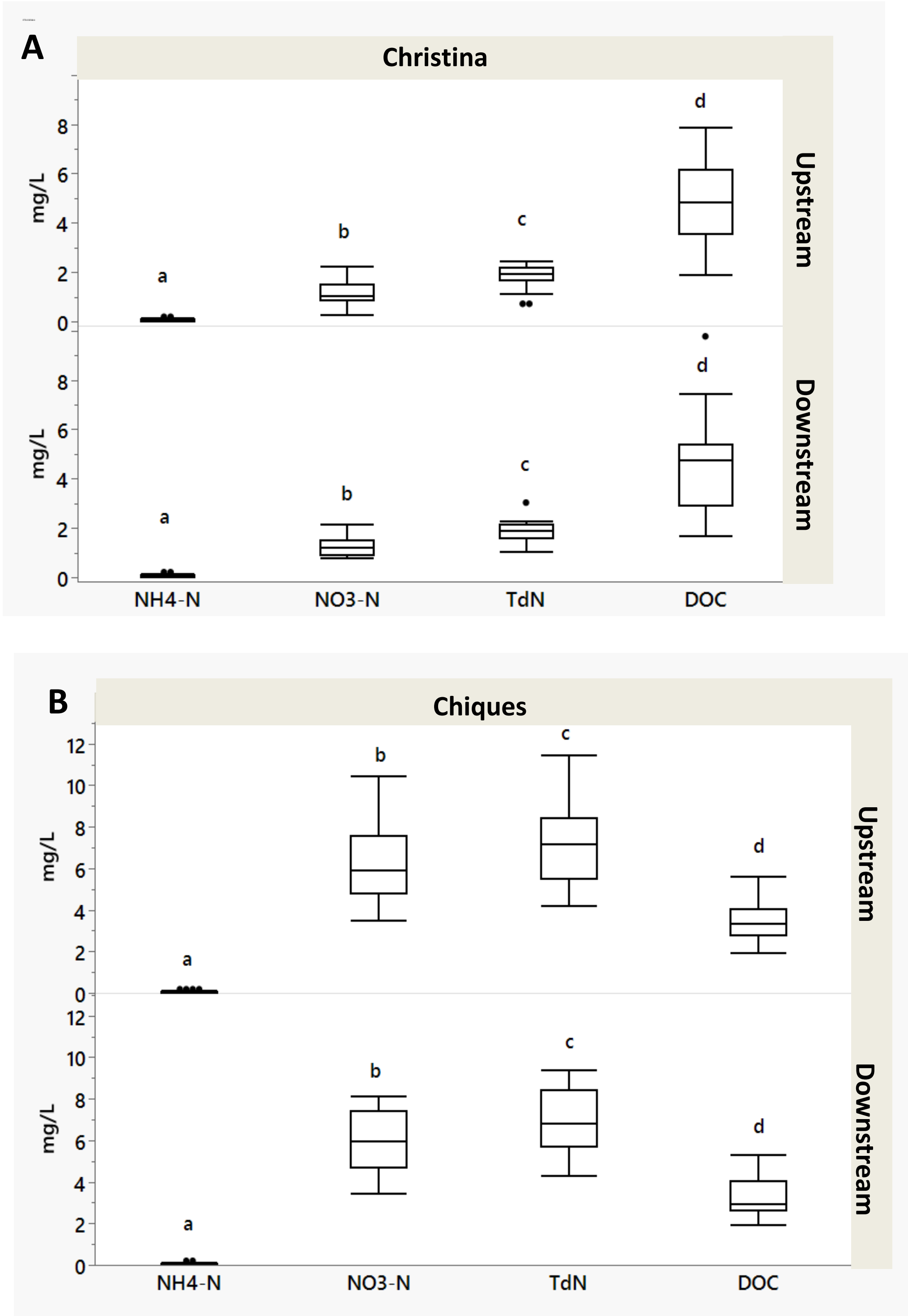
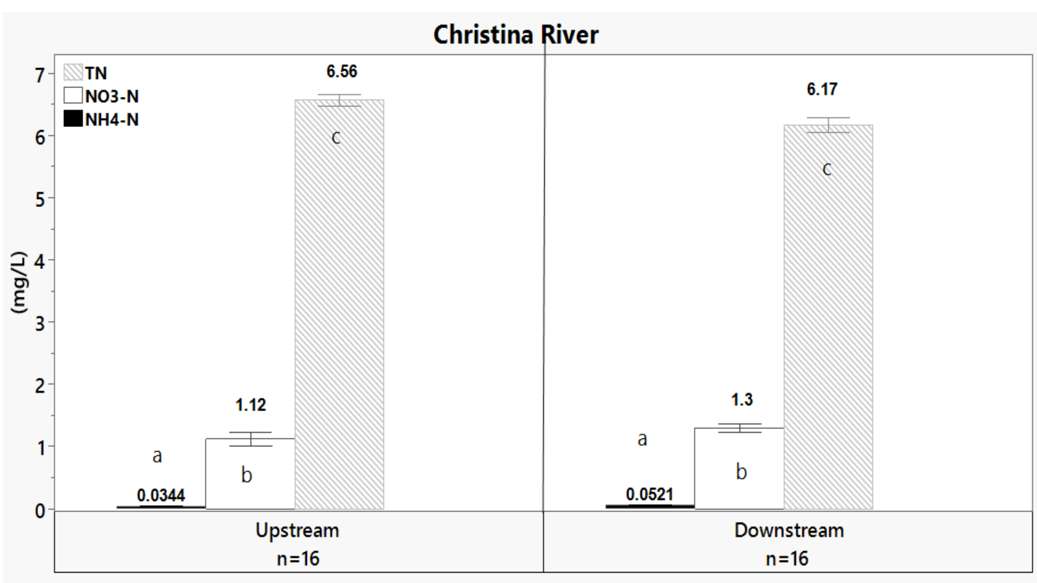


Figure 4.4: Stream nutrient concentrations upstream and downstream of the dam shown in boxplots for Christina (A) and Chiques (B). No significant differences were seen in nutrient concentrations upstream versus downstream of the dams at either site, $p > 0.5$. Matching letters indicate no significant differences at $\alpha = 0.05$.

Longitudinal stream water nutrient concentrations by distance from dam

No significant differences were seen in mean stream water nutrient concentrations (NO_3-N, NH_4-N, TN) above the dam (mean of 100 m, 200 m, 300 m, 400 m, and 500 m samples) or below the dam (mean of -100 m, -200 m, -300 m, -400 m, -500 m samples) at Christina (Student's T-test, α =0.05, Figure 4.5). Significant difference by sampling date was seen among all nutrient concentration at both sites, upstream and downstream of the dams (ANCOVA, α =0.05, Table 4.2). Only Christina downstream TN was significantly different by sampling distance from dam ($p < 0.01$, ANCOVA, α =0.05, Figure 4.6). Interaction of sampling date by distance was also significant for Chiques upstream NO_3-N, NH_4-N, TN and Christina downstream NO_3-N and TN (ANCOVA, α =0.05, Table 4.2). ANCOVA results indicate almost every sampling date at Chiques had a different slope, and slopes were a mix of positive and negative values. At Christina, most sampling date slopes were close to zero, with only a few strong positive and negative slopes found. The ranges of these slopes were smaller compared to the range of Chiques values (Table 4.2)



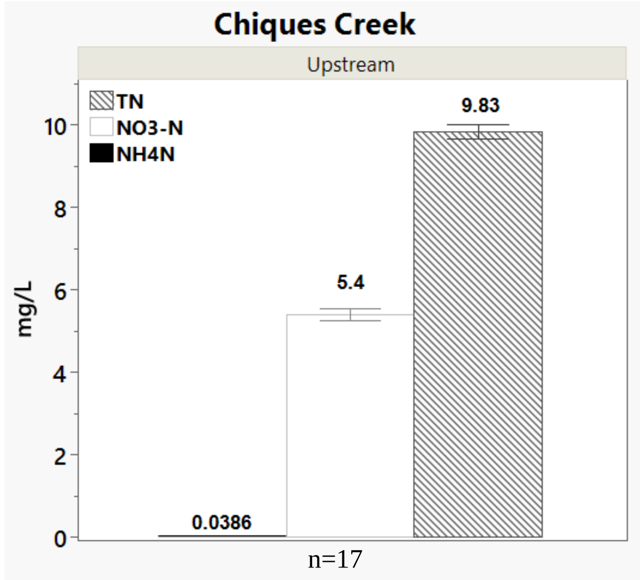
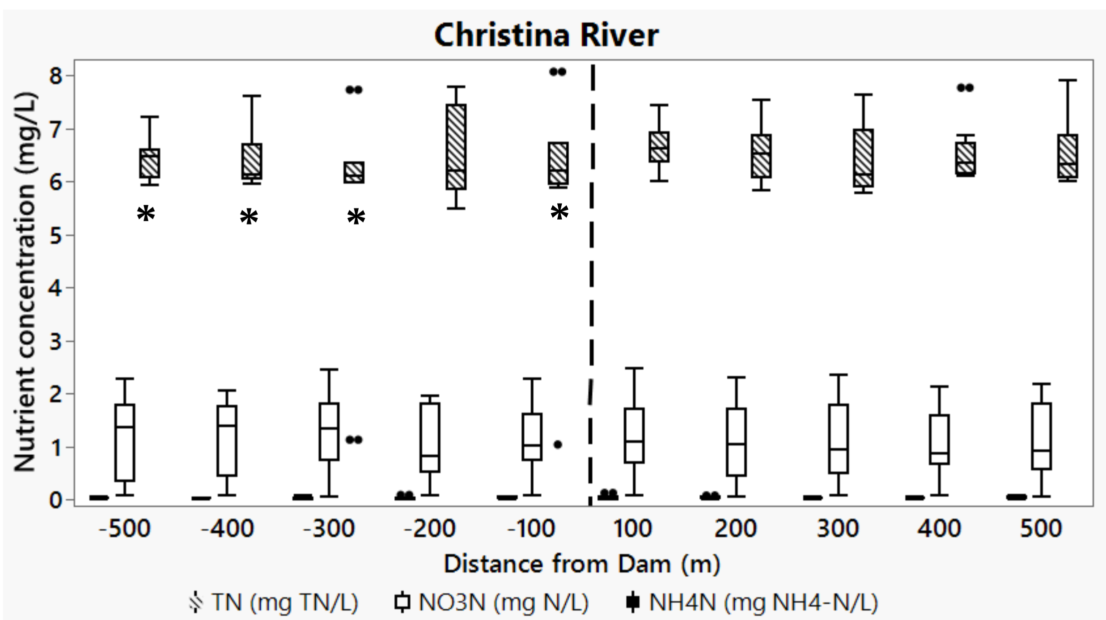


Figure 4.5: Average stream nutrient concentrations (NO_3-N, NH_4-N, TN) for upstream versus downstream of dam at Christina (above) and above the dam at Chiques (below). Matching letters indicate no significant difference. No significant difference was seen in above the dam versus below at Christina ($p > 0.3$, n=16, Student's T-test, $\alpha = 0.05$). Bars represent standard error.



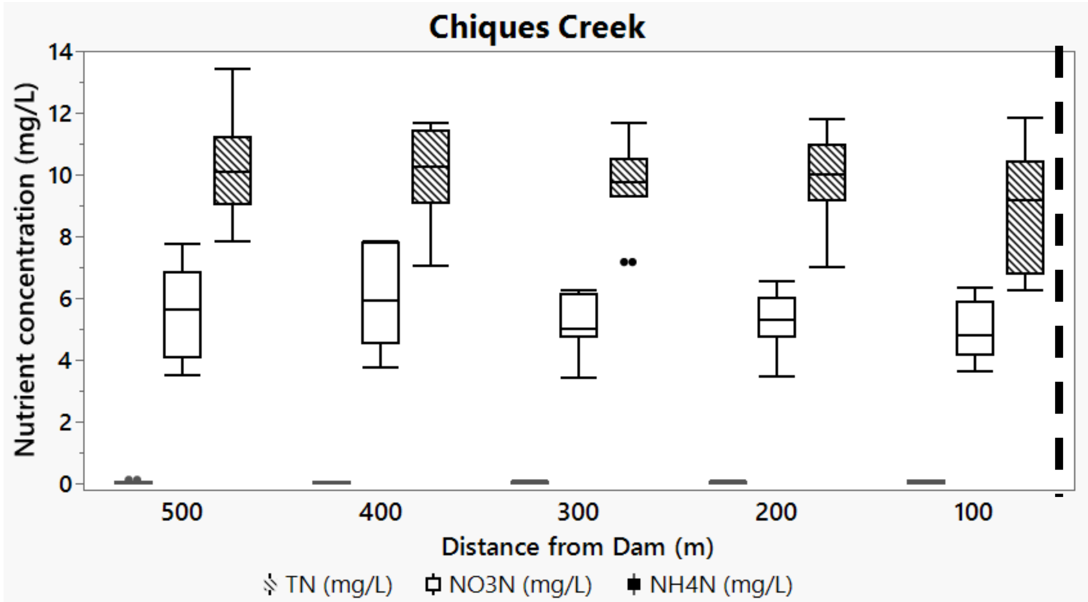


Figure 4.6: Boxplots of stream nutrients by distance from dam for Christina (top, n=16) and Chiques (bottom, n=17) over two-year study period. Positive distances indicate farther upstream, negative distances indicate farther downstream. Dam is represented by dashed line. Christina samples taken along a ~500 m reach upstream and downstream of the dam. Chiques samples taken along a ~500 m reach upstream of the dam. No significant differences were found among TN, NO_3-N, and NH_4-N by sampling distance at Chiques ($p > 0.5$, n=17, ANCOVA, α =0.05). A significant difference by distance was seen at Christina for downstream TN ($p < 0.01$, n=16, ANCOVA, α =0.05) but not for upstream TN or NO_3-N, and NH_4-N.

Table 4.2 ANCOVA results of stream nutrient concentrations by sampling date, distance along the 500 m sampling reach, and interaction of date by distance for Christina (top) and Chiques (below). Factors marked with * are significant at α =0.05.

Christina Upstream NO3-N	**Parameter**	**DF**	**p-value**	**R2 (whole model)**	**Slopes**
	Date	7	< 0.001*	0.99	Mix of positive and negative -3.2 to 3.6
	Distance	1	0.95		
	Date*Distance	7	0.39		

Christina Upstream NH4-N	**Parameter**	**DF**	**p-value**	**R2 (whole model)**	**Slopes**
	Date	7	0.04*	0.52	All near 0 -0.50 to 0.50
	Distance	1	0.33		
	Date*Distance	7	0.70		

Christina Upstream TN	**Parameter**	**DF**	**p-value**	**R2 (whole model)**	**Slopes**
	Date	7	< 0.001*	0.25	Mix of positive and negative -3.5 to 4.9
	Distance	1	0.17		
	Date*Distance	7	0.14		

Christina Downstream NO3-N	Parameter	DF	p-value	R2 (whole model)	Slopes
	Date	21	< 0.001*	0.89	Most near zero except 2 large negative slopes -1.9 to 1.4
	Distance	1	0.98		
	Date*Distance	21	< 0.001*		All near zero -0.007 to 0.007

Christina Downstream NH4-N	Parameter	DF	p-value	R2 (whole model)	Slopes
	Date	21	< 0.001*	0.76	All near zero -0.20 to 0.20
	Distance	1	0.92		
	Date*Distance	21	0.08		

Christina Downstream TN	Parameter	DF	p-value	R2 (whole model)	Slopes
	Date	21	< 0.001*	0.70	~ One-third of dates with clear positive or negative slope -3.0 to 2.5
	Distance	1	0.01*		Near zero
	Date*Distance	21	0.01*		All near zero

Chiques Upstream NO3-N	Parameter	DF	p-value	R2 (whole model)	Slope
	Date	19	<0.001*	0.76	Mix of positive and negative -3.2 to 3.6
	Distance	1	0.503		
	Date*Distance	19	<0.001*		All near zero -0.005 to 0.005

Chiques Upstream NH4-N	Parameter	DF	p-value	R2 (whole model)	Slope
	Date	19	<0.001*	0.54	All near zero -0.50 to 0.50
	Distance	1	0.99		
	Date*Distance	19	1.0		

Chiques Upstream TN	Parameter	DF	p-value	R2 (whole model)	Slope
	Date	19	<0.001*	0.9	Mix of positive and negative -3.5 to 4.9
	Distance	1	0.77		
	Date*Distance	19	<0.001*		All near zero -0.01 to 0.02

Temporal trends of stream water nutrient concentrations

Over the study period, TdN and NO_3-N were highest in winter, notably around February of each year across both sites. Nutrient levels then dropped during summer and began to rise again after autumn. Background concentrations of TdN and NO_3-N are higher on average at Chiques compared to Christina year-round. Ammonium-N levels are similar at both sites (Figure 4.7).

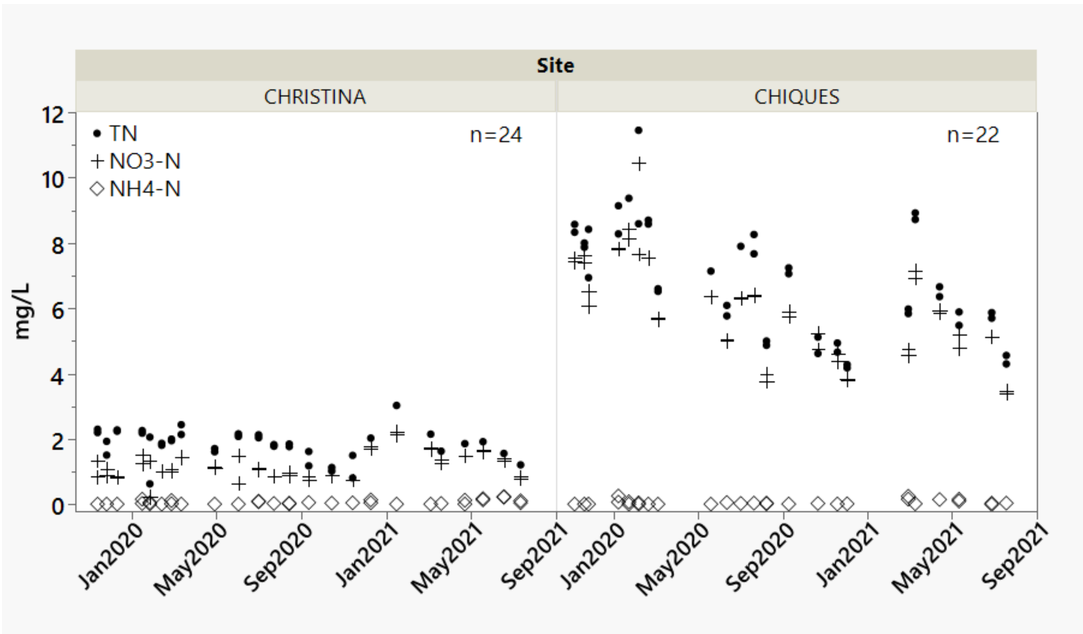


Figure 4.7: Temporal pattern of dissolved stream nutrients from grab samples collected above (open markers) and below the dam (closed markers) for Christina River (left, n=24) and Chiques Creek (right, n=22). No significant difference detected between upstream vs downstream samples at either site (α =0.05).

<u>In-stream N processes above and below the dam</u>

Spatiotemporal variation in sediment denitrification potential

We found substantial variation in DEA rates among site and treatment (Table 4.4 and Figure 4.8.) Overall, amended-DEA rates were significantly higher ($p < 0.002$)

than unamended rates indicating C and or NO_3-N limitation of denitrification rates in our study sites. At Christina, there were no significant differences in averaged DEA rates upstream vs downstream comparing amended samples, unamended samples, or combining treatment types. Similarly, no significant differences were seen in average net nitrification, net mineralization, or percent organic matter above or below dam. At Chiques, upstream DEA rates were significantly higher than downstream ones ($p < 0.001$), however the large difference in sample size (Table 4.3) gives a degree of caution to this interpretation.

Table 4.3: Average and standard error values for DEA rates (by treatment), % organic matter, net nitrification, and net mineralization of streambed sediments by site and sampling location. At Christina, no significant differences were seen above the dam versus below in DEA rates, OM%, or sediment incubations (all p values > 0.13). Overall, amended DEA samples were significantly different than unamended DEA rates, $p < 0.0007$.

	Christina Upstream n= 35	**Christina Downstream n=35**	**Chiques Upstream n=38**	**Chiques Downstream n=4**
Amended DEA (ugN/kg sed/hr^{-1})	19.3 (4.27)	23.5 (3.07)	24.8 (6.5)	0.61 (0.61)
Unamended DEA (ugN/kg sed/hr^{-1})	9.18 (2.16)	5.26 (1.30)	13.61 (3.38)	6.6 (3.96)
Organic Matter (%)	9.52 (1.6)	11.0 (1.87)	4.74 (0.44)	2.08 (0.67)
Net Nitrification (mg/L)	-0.01 (0.09)	0.11 (0.08)	0.25 (0.14)	0.41 (0.39)
Net Mineralization (mg/L)	-0.18 (0.09)	0.03 (0.11)	0.66 (0.16)	0.43 (0.14)

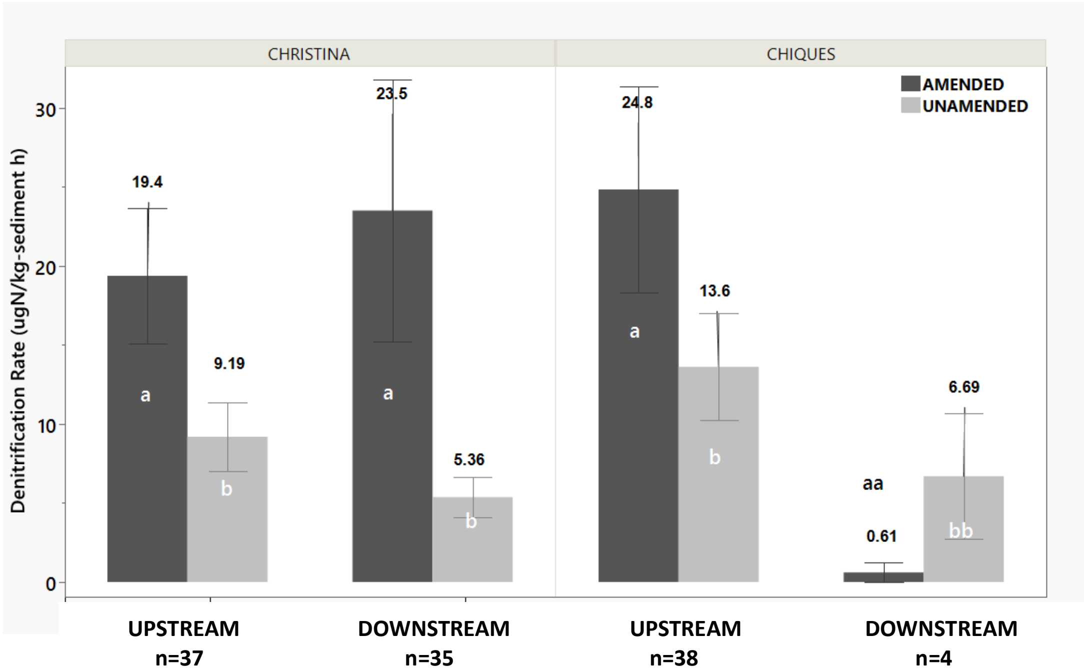
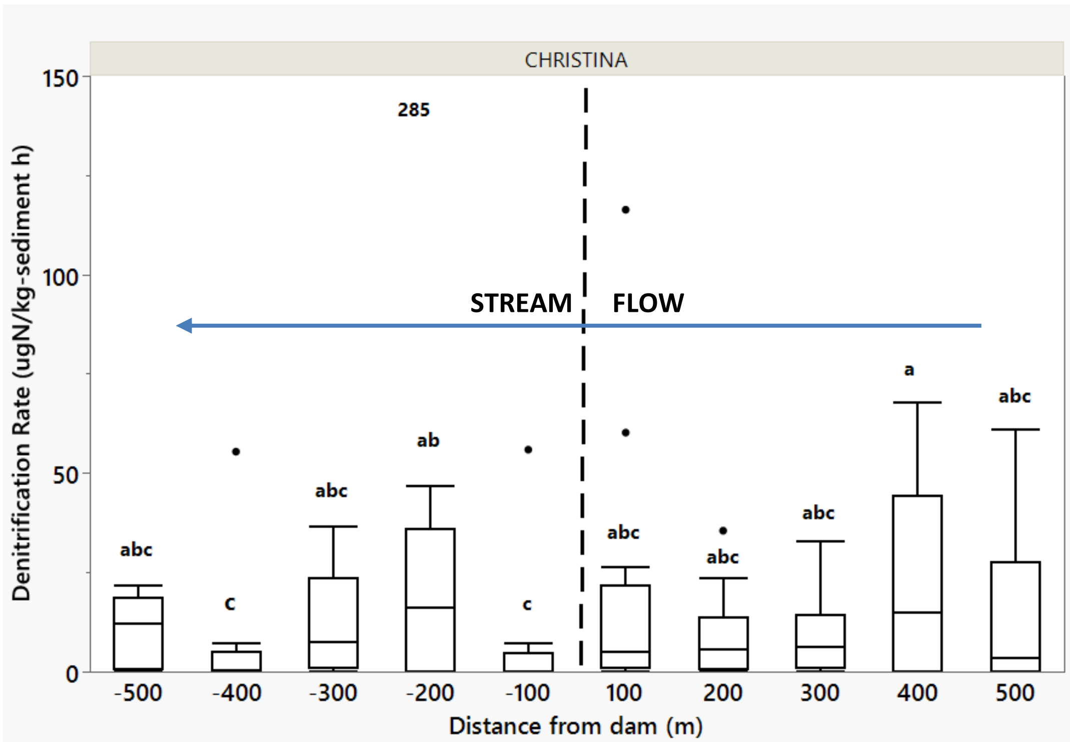


Figure 4.8: Average denitrification rate (ugN kg sediment h^{-1}) for each site by treatment (amended in black bars, unamended in gray bars) and sample location (upstream of the dam versus downstream). Bars represent standard error. A significant difference was found between upstream and downstream samples for both treatments at Chiques, $p < 0.0014$ as indicated by non-matching letters.

Longitudinal denitrification potential by distance from dam

We found significant differences in DEA rates across the longitudinal gradient above the dams (Figure 4.9) indicating the existence of spatial habitat heterogeneity in potential N removal. At Christina, four of the routine sampling locations above the dam (100 m, 200 m, 300 m and 500 m) were significantly lower than the 400m sampling location (Figure 4.9). Downstream of the Cooch dam, we found similar spatial variation among locations with -500m and -300 m sampling locations being significantly lower than the -200 m sampling location (which had the highest mean of all sampling locations), and also significantly higher than sampling locations at -400 m and -100m. Riffles, pools and runs were fairly evenly distributed downstream of the dam, except at 300 m that was wide and slower flow, and 500 m downstream being naturally dammed by a felled tree halfway through the study period with evidence of beavers along the banks. The upstream sampling stretch had slower flow, with drainage pipes noticeable across the 500 m stretch. At Chiques, two of the routine sampling locations above the dam (100 m and 300 m) were significantly lower than 200 m and 500 m above the Roller Mill dam, but were significantly higher than the 400m sampling location. The upstream sampling stretch was mostly slower flow without riffles/runs, but multiple drainage pipes likely from landowners' sewer and drainage systems were present.



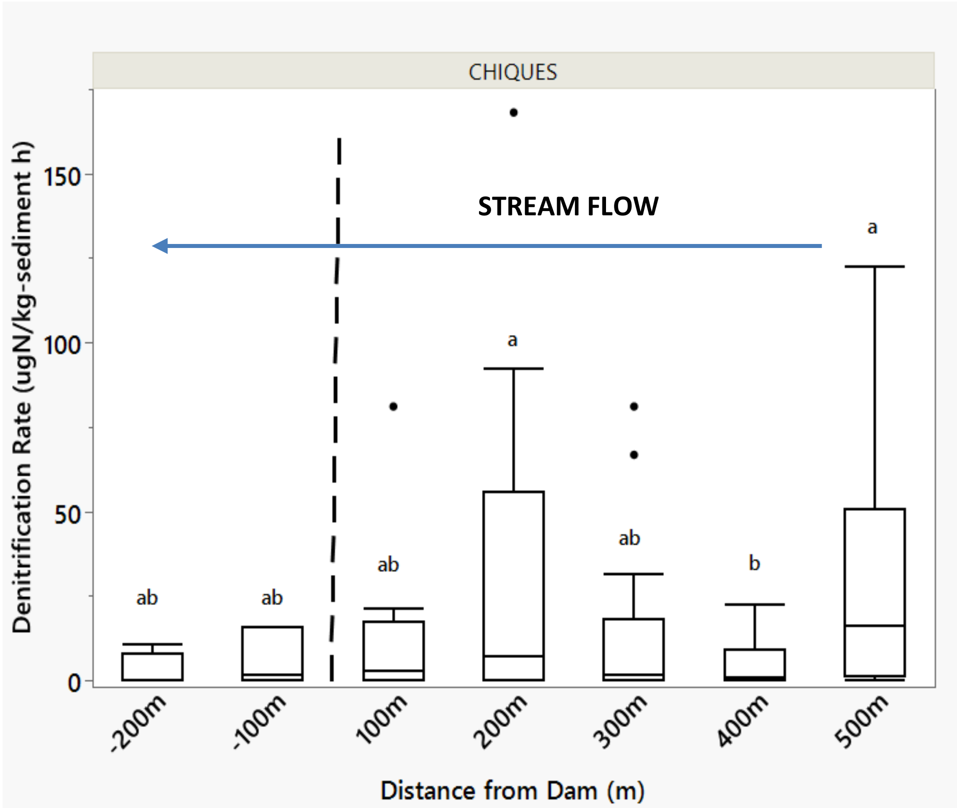


Figure 4.9. Boxplots of DEA rates by distance from dam for Christina (top) and Chiques (bottom). Dam marked by dashed line. N= 8 seasonal sediment core samplings from October 2019-Aug. 2021 for all Christina samples and all upstream Chiques samples, downstream Chiques samples n= 4. Boxplots with same letters are not significantly different at α =0.05. Values are amended and unamended samples combined. Note Y axes are different due to sampling distance differences among sites.

Temporal denitrification potential rates

Temporal patterns in DEA rates over the two-year study period were generally similar among unamended and amended rates at both streams (Figure 4.10). At each sampling date, DEA rates in Chiques were higher and more variable than at Christina with only one exception (Figure 4.10). At Chiques, ambient denitrification rates were highest in the winter and lowest in the summer, whereas amended DEA rates were also highest in both winter seasons and the summer of 2021 (Figure 4.10). At Christina, seasonal denitrification patterns were much less consistent than at Chiques (Figure 4.10). Instead, we observed that both unamended and amended DEA rates over the first year (2019-2020) were higher compared to the 2020-2021 sampling

(Figure 4.10). Amended DEA rates in the summer of 2021 were also much higher than unamended rates, similar to what we observed in Chiques. While DEA rates estimated in the Fall 2020 were the lowest that we observed at both sites (Figure 4.10), this suggests that large-scale conditions at that time were likely limiting for denitrification processes.

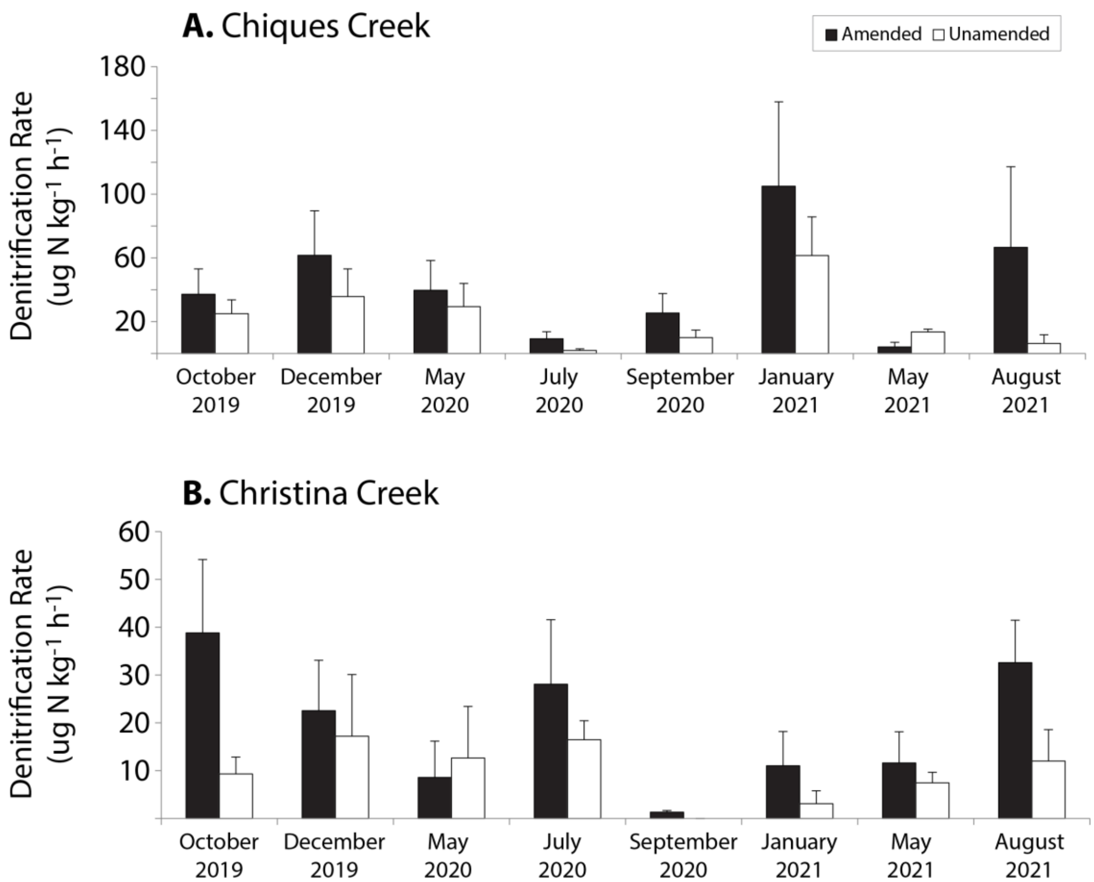
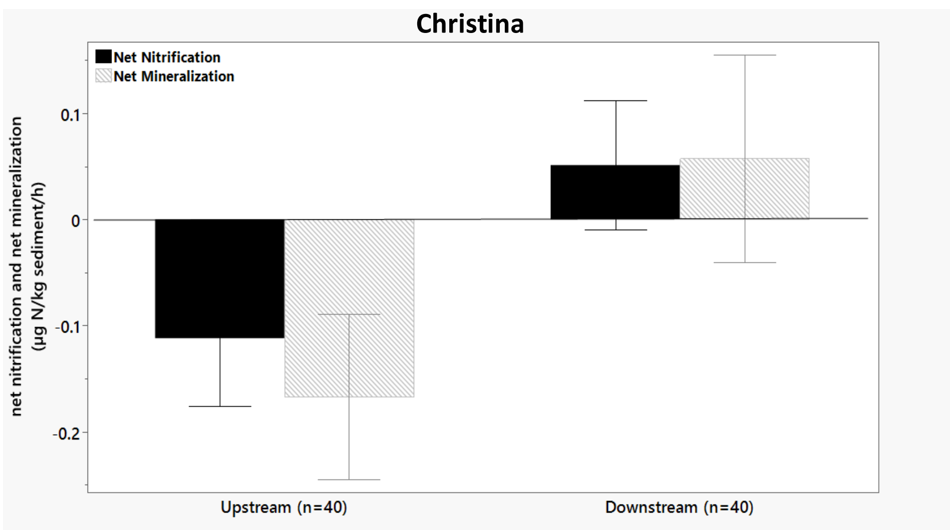


Figure 4.10: DEA rates (µg N kg sediment h^{-1}) by treatment (amended results in black bars, unamended in white) and sampling month for each site. Note Chiques (top) has a different Y axis than Christina (below) due to differences in overall DEA potential.

Net mineralization and nitrification above and below the dam

Christina streambed sediment experienced overall negative net nitrification and net mineralization for upstream samples over the study period (n=40 each) and positive net nitrification and net mineralization in downstream samples (Figure 4.11). This means that we consistently observed a net removal of both NH_4-N and NO_3-N in streambed sediments above the dam, while observing a net accrual of sediment N content below the dam (Figure 4.12). Average net mineralization and nitrification rates were always positive in Chiques, although similar directional change was found for net nitrification rates being higher below than above the dam (Figure 4.12). Nonetheless, caution must be taken when interpreting these patterns given the small sample size below the Rollers dam.



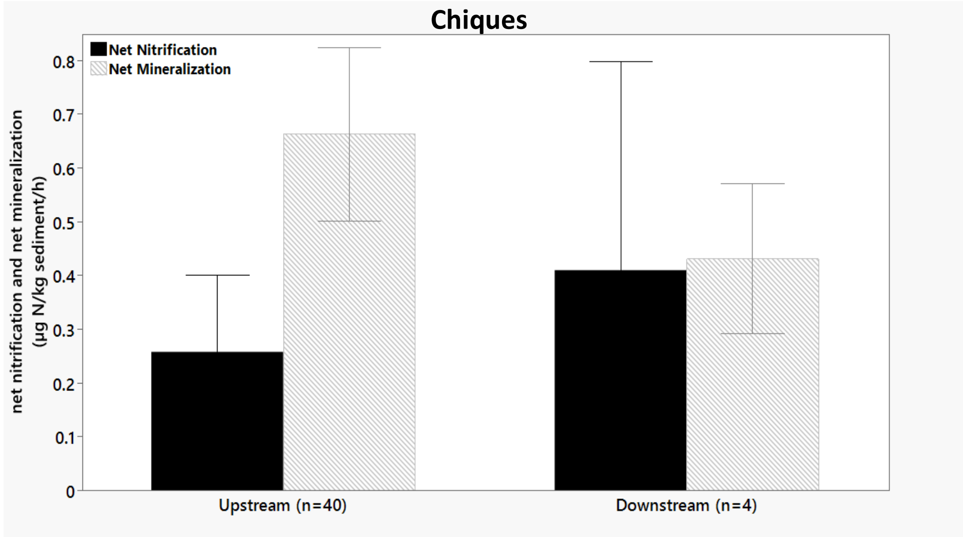


Figure 4.11: Average net nitrification and net mineralization for Christina (top) and Chiques (bottom). Bars represent standard error. Note different Y axis due to differences in over net N processing rates.

Denitrification potential versus net nitrification

Net nitrification rates are the result of both accumulation (from nitrification) and removal (from uptake) NO_3-N processes. When negative, net nitrification indicates that uptake is larger than nitrification, although the contribution of assimilatory and dissimilatory (denitrification) uptake processes is not determined. Over the study period, we observed positive net nitrification on average at Chiques creek (Figure 4.11), however, during both winter sampling and the Spring of 2020 we found negative net nitrification. On these same dates, unamended DEA rates were the highest (Figure 4.12) and similar in magnitude to net nitrification rates (Figure 4.12). Together, these results suggest that net removal of NO_3-N in Chiques is mostly attributed to denitrification processes in streambed sediment.

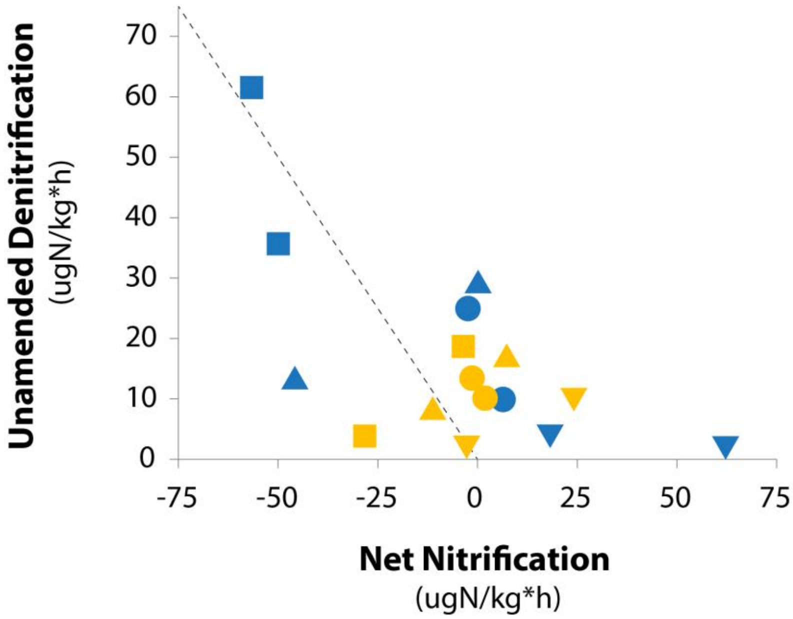


Figure 4.12: Unamended DEA rates (µg N/kg sediment/h^{-1}) versus net nitrification converted to the same units as DEA for comparison. Chiques is in blue, Christina in orange. Dashed line represents zero, or when net nitrification is equal in magnitude to unamended DEA. Winter is represented by a square, up triangle is spring, down triangle in summer, and circle is fall.

Controlling Factors of Denitrification in Streambed Sediments above dams

Percent organic matter per sediment sample plotted against DEA indicates the majority of samples had a lower percent of organic matter (OM) from 0-5%, and those above 20% OM had lower DEA rates compared to those from 5-15% OM (Figure 4.13a). Overall, Christina samples had higher organic matter compared to Chiques (Figure 4.13b).

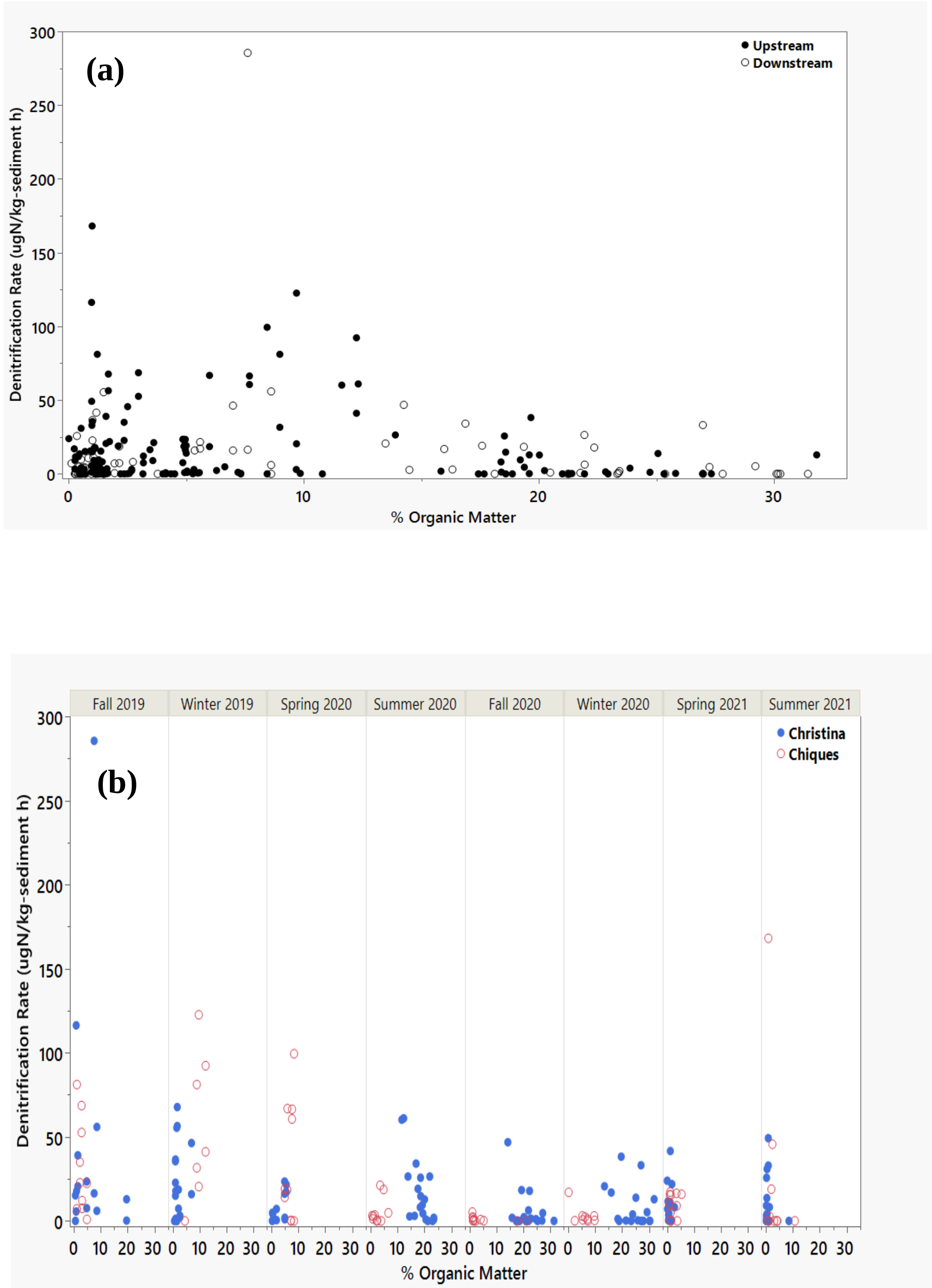
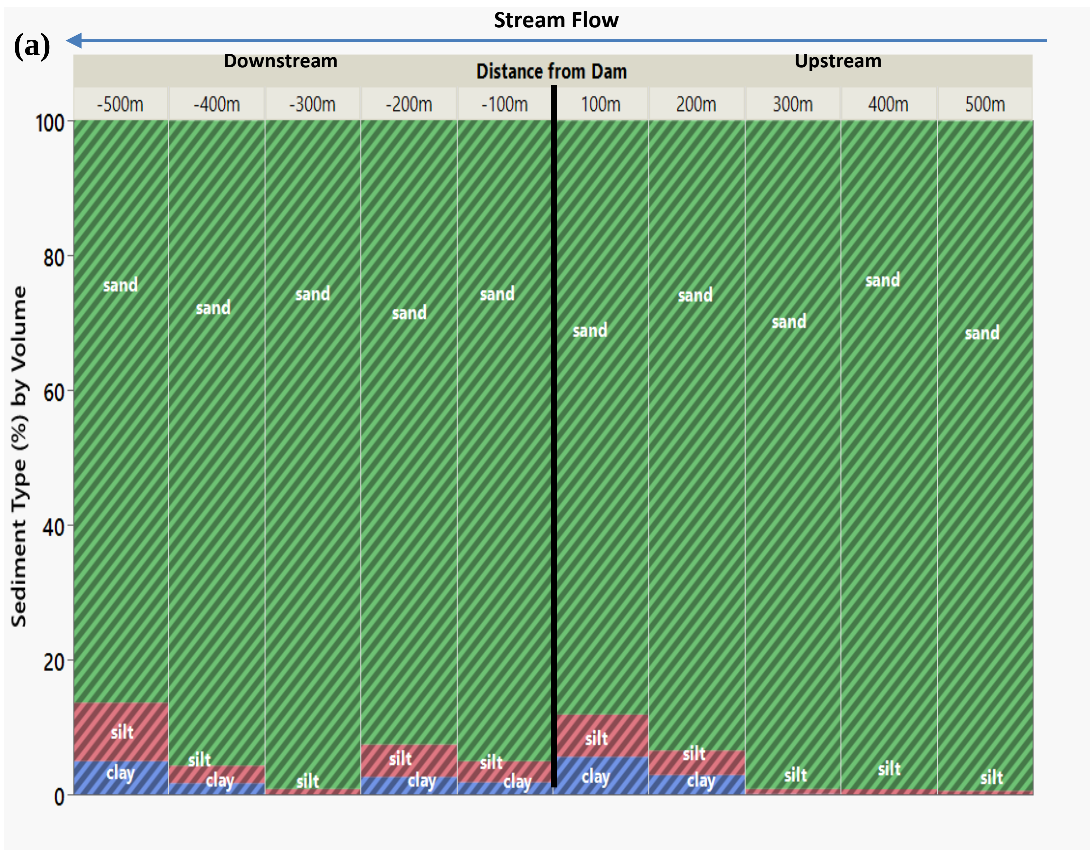


Figure 4.13. Scatter plots representing organic matter (%) per samples versus DEA rates (µg N kg sediment h^{-1}) by reach (a) with upstream in closed circles and downstream in open circles, and season (b) with Christina in blue closed circles and Chiques in open red circles.

Spatial distribution of sediment particle size by distance from dam

Sediment particle size was analyzed by volume (Figure 4.14) and surface area (Figure 4.15). By volume, both sites were dominated by sand (> 63 µ) across all sampling locations. Overall, Christina was dominated by sand compared to Chiques which had more mixing of clay followed by silt. By surface area, Christina was a mix of sand and clay (0-17µ) across all sampling locations except 100 m downstream of the dam which was dominated by silt (17-63 µ). At Chiques, all sites were dominated by clay with 200 m and 500 m upstream of the dam having the least amounts of clay.



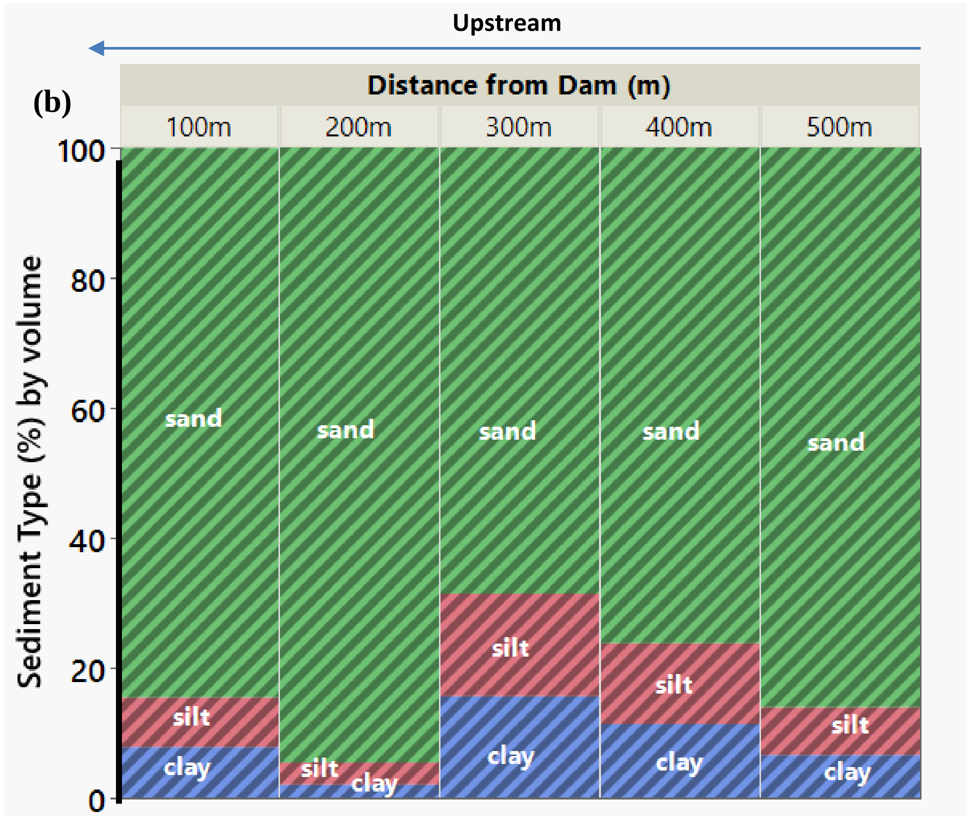
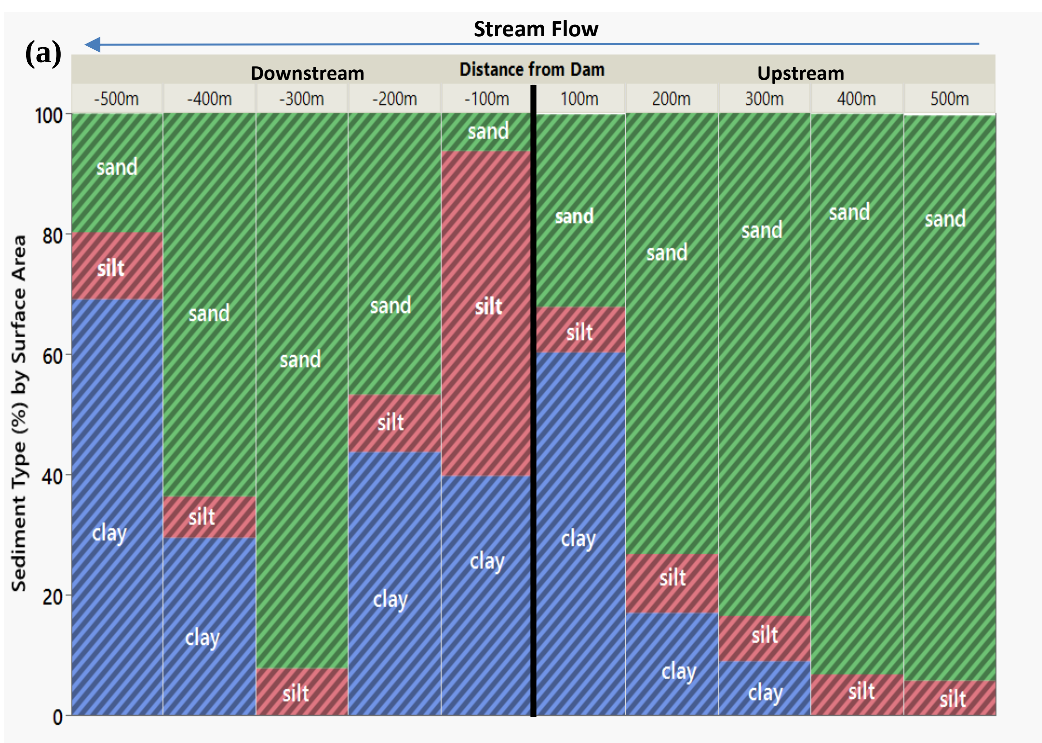


Figure 4.14. Percent sediment particle size by volume and by distance from dam for Christina (a) and Chiques (b). Dam represented by black line. No sampling was done for Chiques downstream.



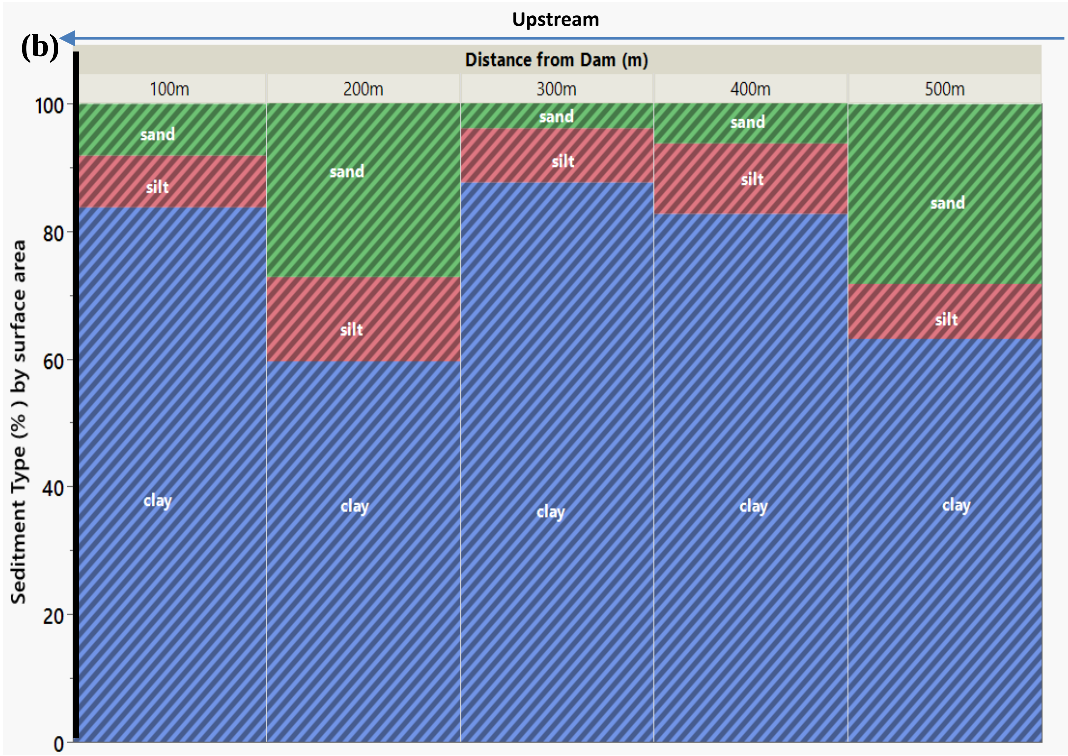


Figure 4.15. Percent sediment particle size by surface area and by distance from dam for Christina (a) and Chiques (b). Dam represented by black line. No sampling was done for Chiques downstream.

Multilinear Regression Model Results

Multilinear regression model examining predictors of DEA (µg N kg sediment h-1) for Christina alone and the regression had weak explanatory power (R2=0.06). Chiques had a higher explanatory power (R2=0.37) and percent organic matter and sediment ammonium-N emerged as significant contributing factors. Combined, percent organic matter and sediment ammonium-N also emerged as significant predictors (R2=0.33). No significant difference in predictor results was seen among treatment types therefore DEA rates were averaged in each model.

For Christina alone, the predictive model was:

$$Denitrification\ potential = (SRP)(0.03 \pm 0.02) + 7.75$$

R^2=0.06, SPR $p < 0.126$

For Chiques alone, the predictive model was:

$$Denitrification\ potential\ = (Organic\ Matter)(4.49 \pm 1.92) + (Sediment\ Ammonium)(1.65 \pm 0.06) + 1.54$$

R^2=0.36, Organic Matter $p < 0.025$, Ammonium $p < 0.0009$

For the two sites combined, the predictive model was:

$$Denitrification\ potential\ = (Organic\ Matter)(4.30 \pm 1.30) + (Sediment\ Ammonium)(1.36 \pm 0.45) + 3.29$$

R^2=0.33, Organic Matter $p < 0.001$, Ammonium $p < 0.003$

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Cory, R.M., Boyer, E. W., McKnight D.M (2011). *Spectral Methods to Advance Understanding of Dissolved Organic Carbon Dynamics in Forested Catchments*. Chapter 6. https:doi.org/10.1007/978-94-007-1363-5_6

DISCUSSION

This study showed that, overall, no significant differences were seen above or below the two low-head milldams in streambed sediment denitrification rates or in TN, NO_3-N, and NH_4-N concentrations for the two-year study period. Streambed ammonium concentrations and organic matter emerged as controlling factors on denitrification using a multilinear regression model. Temporal patterns of stream nutrient concentrations revealed increases in NO_3-N during the winter and drops in autumn at both sites, and DEA rates were highest Winter 2021 at Chiques and Fall 2019 at Christina. The following discussion elaborates on these observations by discussing key questions raised in the Introduction: (1) "How do milldams influence stream hydrologic conditions upstream and downstream of milldams?" and (2) "Do milldams create 'hotspots' of denitrification upstream of dams compared to below, and what are the controlling factors?"

Dam Effects on Physiochemical Responses and Variation Above and Below the Dams

As expected, increased stream levels and slower flow due to the dam resulted in an 8.5x increase in residence time above the dam versus below at Christina and 7.7x increase at Chiques (Table 4.1), although this was a one-time measurement and statistical analysis were not performed. Increases in residence time are commonly seen above dams when water is backed up including in reservoirs (Van Cappellen and Maavara 2016), small water retentions structures (Gómez-Gener et al. 2018) and run-of-the-river dams (Almeida et al., 2019). Large differences were not seen in stream conductivity above the dam versus below at our study sites, and conductivity values generally followed storm dilution patterns as expected. However, spikes observed in conductivity at Christina during winter months may be due to road salt applications (Figure 4.1), and the continuing salinization of freshwaters is an increasing topic of study (Hintz et al., 2021, Kaushal et al., 2018, Fay and Huang, 2013). Additionally, water temperature appeared to be almost always warmer above the dams, with the largest temperature difference 2°C at Chiques. Downstream water was only warmer at Chiques from ~June-August 2020 (Figure 4.2). This could be due to site morphology leading to higher water accumulation directly below the dam at Chiques compared to Christina. At Chiques, a steeper drop over the dams has created a deeper pool immediately below the dam, as opposed to Christina which has more evenly dispersed water and flow below the dam due to the dam composition of large rolling boulders and shallower slope of the dam. The pooled water at Chiques may more easily accumulate and retain heat in the summer months and increased residence time opposed to flowing water at Christina. While warmer water has been found downstream of dams (ranging from 0.21 of 0.21-5.24°C) due to reduced flow and loss of canopy cover downstream of the dam, the effects of dams on stream temperature are variable due to differences in dam and watershed characteristics such as dam height, impoundment volume, and residence time (Zaidel et al., 2021).

Dam Effects on Dissolved Oxygen Regimes

In general, at our study sites, DO was almost always lower above the dam versus below. Both sites experienced DO values below hypoxic levels above the dam (<50% saturation as defined by Carter et al., 2021 or < 2.0 mg/L CANR 2003), while

DO saturation did not drop below ~80% below the dam at either site (Figure 4.3). Streams may frequently experience periods of hypoxia in both dammed and free-flowing water, with lowest drops in DO occurring in pooled areas and dammed sites experiencing greater periods of hypoxia (Carter et al., 2021, Blaszak et al., 2018). These periods of hypoxia may be more common than previously estimated, and other studies have called for the use of high frequency data, in addition to moving away from a strict lentic versus lotic definition in fluvial systems, to better capture the true oxygen dynamics occurring in rivers (Carter et al., 2021, Blaszak et al., 2018). The high frequency data collected in this study showed fluctuations in DO levels that may have been missed using only point measurements.

Additionally, storm events appear to alter DO dynamics at our sites (Figure 4.3). At Christina, above-dam DO levels closely follow the rising and falling limb of storm peaks at Christina mid-July 2020 and beginning of August 2021. At Chiques, a storm event around August 12, 2021 appeared to mobilize anoxic waters at Chiques, as DO levels immediately drop both above and below the dam following the storm (Figure 4.3Aa). However, this is not apparent with all storms and the highlighted study periods represent short term DO dynamics of 5 weeks or length in length.

Temperature is known to affect DO saturation, as the solubility of DO decreases as temperature increases (Carter et al. 2021). Temperature and air pressure are also used to convert DO concentration (mg/L) to DO saturation (%), as was done in this study for DO data collected above the dam at Roller Mill and above the dam at Cooch’s Mill. Therefore, the changes in DO dynamics following storms may be related to changes in water temperature. Additionally, flood events can scour stream bottoms, removing substrate and biofilm for biota in addition to increasing turbidity, reducing photosynthesis and DO levels through reduced light penetration. Others have observed a similar decline to near 0% saturation in the weeks following a storm and prior to DO replenishment by new stormwater (Blaszczak et al. 2018, Carter et al. 2021, Dutton et al. 2018). Interestingly, a drop in DO and periods of hypoxia after storms was also seen by Dutton et al. (2018) in channel backwaters in East Africa beginning within minutes to hours after a storm. They hypothesized the drop in DO was due to the accumulation of organic matter from hippopotamus droppings accumulating in the streambed and lower water column which then lowered DO levels during decomposition, and the storms provided force to mobilize the waters and distribute them, ultimately resulting in fish kills downstream.

Magnitude and Comparison of Denitrification and Stream Nutrients Above and Below the Dams

Though numerous studies have described how localized areas of high denitrification activity may arise within a stream (Steinhart et al., 2000; Kreiling et al., 2019; Comer-Warner et al., 2020; Bettez and Groffman, 2012), aquatic sediment denitrification rates have high spatial and temporal variability dependent upon their location in the watershed and biogeochemical controlling factors (Comer-Warner et al., 2020, Korol et al., 2019, Kemp and Dodds, 2002, Steinhart et al., 2000, Wu et al., 2021). We had hypothesized that locations upstream of milldams would have higher DEA values due to increasing decomposition of organic sources such as disturbed biofilms, in addition to increasing turbidity residence time, accumulation of fine sediment, and lower DO. While we did find increases in residence time and lower DO above the dam, no apparent "hotspots" of denitrification emerged, and significant differences were not found in DEA averages above versus below at Christina (Figure 4.8). However, while numerous dams constructed during colonial times were effective at collecting and trapping sediment above the dam and preventing sediment and nutrients from continuing downstream (Walter and Merritts 2008), as dams age their sediment storage capacity lessens as reservoirs become full above the impoundments (Cero 2016). Dams that are full and at equilibrium are no longer trapping fine sediments, and therefore may not be as efficient at denitrifying and processing nitrate-N to N_2 gas as hypothesized in this study. Roller Mill Dam has been deemed to be at full storage, with a report by Stantec Consulting Services, Inc. (Preliminary Findings Report on Roller Mill Dam, 2020), indicating the dam has "been effective at trapping sediment in the upstream impoundment and is likely currently filled to capacity with sediment." This was also evidenced by majority sand composition in sediment above the dam at Roller Mill by volume (Figure 4.14).

In our study, DEA rates were not significantly different above and below the Cooch's Mill dam. Rates ranged from 0-285.5 μgN kg sed h^{-1} for amended and 0-67.7 for unamended samples, and Chiques DEA rates ranged from 0-168.1 μgN kg sed h^{-1} for amended and 0-81.1 μgN kg sed h^{-1} for unamended, both of which fall in line with other literature values (Table 5.1). At Christina, mean upstream DEA rates for amended samples were 19.4 μ N kg sed h^{-1} and 23.5 μgN kg sed h^{-1} downstream, while mean upstream unamended samples were 9.19 μgN kg sed h^{-1} and mean downstream 5.36 μg N kg sed h^{-1} downstream, although not significantly different for either treatment. At Chiques, mean amended rates were 24.8 μgN kg sed h^{-1} upstream and 0.61 μg N kg sed h^{-1} downstream, and mean unamended averages 13.6 μgN kg sed h^{-1} upstream and 6.69 μg N kg sed h^{-1} downstream, but as sample sizes were not equal (n=38 upstream, n=4 downstream), this should be interpreted with caution.

Previous studies have shown that denitrification rates can vary depending on their location in stream geomorphic structures and with the accumulation of organic matter content (Groffman et al., 2005; Steinhart et al., 2000; Jacinthe et al., 1998). DEA rates in high organic matter debris dams were higher than this study with values ranging from 185-4955 μg N kg^{1} h^{-1} in forested and suburban streams and were highest in high NO_3-N urban streams. Denitrification measurements near old and new beaver dams ranged from 27-79 μg N kg^{1} h^{-1}, which was more aligned with this study's findings (Lazar et al., 2015). Riffles and pools – seen mostly downstream of the dam at Christina at our study sites - had denitrification rates, respectively, between 7.9-219 μgN kg^{-1} h^{-1} and 7.6-73 μgN kg^{-1} h^{-1} (Groffman et al., 2005), similar to our results. Furthermore, Wall et al. (2005) found denitrification rates to be higher in a reservoir (0-63 ugN gAFDM h^{-1}), compared to riverine systems (0-12 ugN gAFDM h^{-1}), both higher than our sites' ranges of Chiques when expressed as ADFM (0.402-0.987 ugN gAFDM h^{-1}) and Christina (0.696-10.2 ug gAFDM h^{-1}).

Additionally, significant differences were not found in stream column nutrients (TN, NO_3-N, NH_4-N, DOC; Figure 4.8) or SUVA values (Appendix A.1) above or below the dams. This indicates that dissolved nutrient availability and DOM aromaticity (or labile C) were similarly available for denitrifying microbes above and below each dam. However, differences among study site watersheds were apparent, particularly in stream nitrate-N levels. Over the study period, Chiques had higher mean stream nitrate-N (6.46 mg/L) compared to Christina (1.74 mg/L), and Chiques also had higher mean DEA rates for both amended and unamended samples. The difference in stream nitrate-N could influence overall denitrification ability, as nitrate-N is a known limiting factor of denitrification below 0.88 mg NO_3-N/L (Inwood et al., 2005) or 0.4 mg/L (Wall et al. 2005). At Christina, nitrate fell below 0.88 mg/L 57 times and below 0.4 mg/L 13 times out of 198 samples compared to falling below 0.88 mg/L twice and never below 0.4 mg/L at Chiques out of 214 samplings. Chiques Creek - classified as one of Pennsylvania's most impaired watersheds (SRBC, 2019) - sits in more agriculturally and receives high fertilizer runoff during storm events, while Christina River is a mixed land use watershed. Our findings align with observations from Inwood et al. (2005) and Kemp and Dodds (2002) who also found higher denitrification rates correlated with higher nitrate inputs in agriculturally influenced streams compared to forested or urban watersheds. However, Kemp and Dodds (2002) also found higher denitrification rates downstream of agriculturally influenced watersheds on streams without dams.

Our two study sites also differed in that Chiques is a more biogeochemically active stream compared to Christina when introduced to excess nitrate-N, as indicated by the relationship between denitrification and net nitrification (Figure 4.12). Chiques either accumulates nitrate when denitrification is very low (i.e., in summer), or shows net removal of nitrate when denitrification is very high (i.e., in winter). This relationship suggests that during winter months, most of the net nitrate removal at Chiques can be attributed to denitrification as opposed to biological assimilation or uptake. Christina, on the other hand, is a more “neutral” stream that neither accumulates nor removes nitrate with great variation across seasons. However, while Chiques is more active in removing nitrate from the system than Christina, the rates are low compared to engineered bioreactors that have been proposed as means to remove large amounts of NO_3-N from agricultural systems with rates exceeding our study at up to 20 mg N kg^{-1}substreate h^{-1} over a period of 180 days (Zhang et al., 2017).

Dam Effects of Longitudinal Variation of Denitrification and Stream Nutrients

While certain factors may be correlated with high denitrification potential at small scales, they are often not at larger spatial scales as these factors become more variable with distance (Korol et al., 2019). Our findings show significant differences in streambed denitrification were seen along the longitudinal gradient by distance from dam across both sites (Figure 4.9) but may only be applicable to the length (500 m) of our study reach and not relevant to entire length of the stream due to natural streambed variability and dilution occurring from local drainage pipes emptying along

Table 5.1: Denitrification rates from this study compared to other literature values, converted to µgN kg^{-1} h^{-1} if applicable.

Reference	Method	Ambient/ Unamended Denitrification	Carbon Source and Nitrate-N Amended	Units	Stream Nitrate (mg/L)	Habitat
This Study: Christina	Chloramphenicol, Acetylene inhibition, nitrate and glucose amended	Upstream 9.18 (2.16) Downstream 5.26 (1.30)	Upstream 19.3 (4.27) Downstream 23.5 (3.07)	µgN kg sediment^{-1} h^{-1}	1.74	Semi-forested and urban stream, DE
This Study: Chiques	Chloramphenicol, Acetylene inhibition, nitrate and glucose amended	Upstream 13.61 (3.38)	Upstream 24.6 (6.5)	µgN kg sediment^{-1} h^{-1}	6.46	Agriculturally influenced stream, Lancaster, PA
Groffman and Dorsey 2005	Chloramphenicol, Acetylene inhibition, nitrate and dextrose amended	NA	0.7 (0.3) -11 (2.0) 0 (0.0)-180.0 (39)	µgN kg^{-1} h^{-1}	NA	Urban streams MD, US
Lazar et al. 2015	^{15}N and ^{15}N-N_2O mass balance mesocosms	27-79 (24.7)	NA	µgN kg^{-1} h^{-1}	0.21-0.90	3 active beaver ponds in RI (US)
Kaushal et al. 2008	^{15}N-enriched nitrate-N “push-pull”		3.2 ± .53 (restored reach) 1.45 ± .33 (unrestored reach)	µg N kg soil $^{-1}$ h^{-1}	1.47 ± 0.05 (unrestored reach) 1.15 ± 0.04 (restored reach)	Geomorphic restoration of riparian-zone-stream interface of an urban stream (MD, USA)

the streambanks. While variation in streambed denitrification was present, an outlier of 285 µgN kg^{-1} h^{-1} that occurred 200 m downstream of the dam likely influenced the categorization of the reach at Christina, creating five statistically significant different groupings. Without the outlier, all DEA rates were below 75 µgN kg^{-1} h^{-1}.
At Chiques, all DEA rates were below 150 µgN kg^{-1} h^{-1} and were separated into three statistically significant groupings. Despite these differences, no apparent trends emerged in rates with distance from the dam at both sites. Overall, a stream may have patches of higher denitrification potential that are dispersed throughout the streambed. This could be due to natural streambed heterogeneity and is reflected in the chance of missing a patch of denitrification ability using point core collection (Steinhart et al., 2000).

Variation in longitudinal stream nutrients along the 500 m stretch highlights additional differences between our two study sites. ANCOVA results indicated sampling date was significant at all sites, and the interaction of sampling date and distance was significant for Chiques upstream NO_3-N and TN, and Christina downstream NO_3-N and TN. However, individual slopes of dates indicate that while both Chiques upstream NO_3-N and Christina downstream NO_3-N are significant by interaction, Chiques dates had a different slope for almost every date, with both positive and negative ranges, indicating conditions change rapidly over time at Chiques as N is accumulated or removed. Christina, however, had only a few dates that had clear negative slopes, indicating only a few sampling days showed N removal, while most sampling days did not show a large change in net N with slopes near zero. Chiques upstream TN followed a similar pattern and had more variation in slopes compared to Christina downstream TN.

Temporal Variation of Denitrification and Stream Water Nutrients Above and Below the Dam

We had hypothesized that instream denitrification rates would vary seasonally and be highest during the summer due to increased temperatures promoting microbial activity. While temperature is a known controlling factor of denitrification (Steinhart et al., 2000, Wall et al., 2005), we observed highest DEA rates during January 2021 at Chiques (in both amended and unamended samples) and during October 2019 for Christina amended samples and December 2019 for unamended samples (Figure 4.10). Stream nitrate levels also varied seasonally (Figure 4.7) and were highest during winter months, with levels dropping in late spring and decreasing in the fall, before accumulating in winter again. This is a well-known pattern of stream nitrate-N that occurs in forested and semi-forested streams due to increased biotic uptake during the growing season versus dormant season, and allochthonous leaflitter nutrient inputs stimulating microbial activity in the fall (Ledford et al., 2016, Wall et al., 2005).

While our study found highest DEA to occur during fall and winter, denitrification rates are known to vary seasonally and can be highly site specific. Wall et al. (2005) found highest denitrification during spring when NO_3-N levels were greater than 0.88 mg/L in a midwestern US reservoir-rive continuum. David et al. (2006) saw a correlation with stream nitrate levels and denitrification rates, with highest rates in spring and early summer in a 44,000-ha US midwestern reservoir corresponding with high nitrate concentrations (10-14 mg/L). Lazar et al (2015) also observed seasonal denitrification variation in Rhode Island beaver ponds with rates significantly lower in spring compared to the fall and summer. This was possibly due to higher spring soil microbial biomass C indicating immobilization was competing with denitrification.

Controlling Factors of Streambed Denitrification

Multilinear regression models for our sites did not reveal any one significant controlling factor for streambed sediment denitrification but instead highlighted multiple factors that influenced denitrification. This is supported by current literature that acknowledges nitrate-N is a main limiting factor of denitrification, but many potential controlling factors exist (See Table 2.1). Controlling factors at Chiques (R^2 = 0.36) were organic matter ($p < 0.0025$) and sediment ammonium-N ($p < 0.0009$), while the same model had minimal explanatory power for Christina (R^2 =0 .06), possibly due to the lower nitrate level and overall nitrification rates. Lower DO is also a known controlling factor on denitrification, and since denitrification is an anaerobic process, the high predicting power of sediment ammonium-N may be due to ammonium often accumulating under low DO (Fitzgerald et al., 2015.) Additionally, Wall et al. (2005), found that above the 0.88 mg/L nitrate threshold, other factors may have more control over denitrification potential including temperature, C availability, and DO, while also finding a positive correlation between DEA and sediment OM and N content.

While sediment type and size are highly variable and localized (Steinhart et al., 2000, Inwood et al., 2005), smaller particles (clay < 17 µm, silt 17- 63 µm) allow for more organic matter to accumulate due to increased surface area. At Chiques, DEA rates were higher when sediment samples had a higher percentage of clay particles as opposed to sand particles, and DEA rates were higher at Chiques compared to Christina where sediment consisted of mostly clay by percent surface area opposed to sand at Christina. This was also seen by Comer-Warner et al. 2020, who found sand sediment to have almost 10x higher denitrification rates than larger gravel sediment across all treatments, and finer sediments had both highest NO_3-N reduction and N_2O concentrations compared to coarser gravel sediments. Findlay et al. (2011) also found sand dominated sediments to have higher denitrification potential compared to larger particles across 65 streams surveyed, along with fine benthic organic matter (FBOM) and epilithic algae showing high denitrification potential.

Historic Fluctuating Lentic/Lotic Characterization of Streams and In-stream N Processing

Prior to colonists constructing milldams in high densities along the eastern US (up to one dam every 2.4-5 km in some Eastern US streams according to research by Walter and Merritts, 2008), natural stream systems were more connected and flowed continuously without disruption by dams. This pre-1800's system was more lotic in nature, often consisting of valley-bottoms with emergent wetlands and connected streams (Elliott et al. 2016, see Figure 5.1A) with vegetation that stored large amounts of organic C despite little sediment accumulation (Walter and Merritts, 2008). As damming occurred, the stream system moved to more numerous fragmented reaches with lentic features present in each segment of stream (Figure 5.1B). This damming of water from milldams disconnected streams and affected upstream tributaries with individual and cumulative effects on watersheds, although the spatial extent of these effects is dependent on channel geometry, gradient, and number of dams upstream (Fencl et al. 2015). This lotic-lentic stream evolution likely affected N processing in streams by altering biogeochemical processes including denitrification. Fragmented streams systems with slower flow, deeper water levels, and increased residence time promote conditions that facilitate denitrification, potentially serving as N sinks and removing nitrate-N from the stream system.

By the late 1800s many milldams were abandoned, in addition to over-trapping of beaver populations, resulting in the removal of dams on waterways (Harvey and Schmadel, 2021, Naiman 1988). However, some evidence suggests that in the 1900s smaller dams and reservoirs were again created - spurred by the need for drinking water and irrigation- switching some waterways back to a more lentic and stagnant system (Harvey and Schmadel, 2021). Since the early 1900s, though, more than 1700 dams have been deliberately removed since due to safety and environmental concerns (American Rivers 2020, Tschantz 2014, Bellmore et al 2019). This frequent fluctuation of lotic/lentic like conditions has consequences on individual streams and watershed health.

Today, lentification has also been seen in the Amazon River due to deforestation and creation of small (cattle ponds) and large (hydroelectric) dams, creating more lentic water bodies which also negatively affect biodiversity (Schiesari et al., 2020). As low-head milldams are being removed at increasing rates across the US Mid-Atlantic region and nationally (Tschanz 2014, Bellemore et al., 2019), these fragmented stream systems are slowly allowed to transition back to a more lotic-like systems to some extent, although not truly anabranched and as free-flowing as before. It is important to better understand this transition to a new regime, where in-stream N processing may be altered by dam removal as streams become more lotic in nature.

A

B

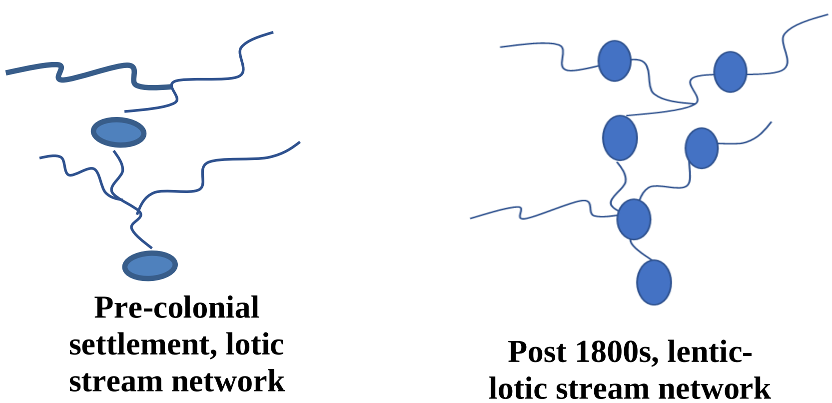

Figure 5.1: (A) Artist's rendering of Pennsylvania valley bottom pre-colonial settlement based on plant macrofossils from Elliott et at., 2016. (B) Creation of milldams on stream networks changed waterways from a more open and connected flowing system (bottom left) to a disconnected and fragmented ponded system (bottom right.)

Environmental Impacts and Management Implications

While site-specific investigations have been called for to better predict how N flux will change in individual watersheds post dam removal (Gold et al., 2016), better understanding the effects of low-head milldams on N processes is integral as dams continue to age and reach full sediment capacity above the impoundments. The change in in-stream trapping ability may alter N consumption and storage, as biogeochemical nutrient cycling is altered by changes in stream velocity, water level, and reduction in fine sediment accumulation over time (Figure 5.2). Additionally, the effects of milldams on riparian zones N processes are largely understudied (Inamdar et al., 2020). Inamdar et al. (2020) highlight the knowledge gap of how milldams and their removal may affect riparian N processes and propose the riparian discontiuum concept, where milldams may disconnect hydrologic and biogeochemical processes. While dams at dynamic equilibrium may not be acting as in-stream N sinks, other studies have found that the associated increase of the water table in the riparian zone may be indirectly reducing N flux from the watershed (Inamdar et al. 2020, Lewis et al., 2021). Riparian zones upstream of dams may be act as N sinks due to buried organic horizons of legacy sediment (C) and anerobic soils promoting denitrification, an increase in dissimilatory nitrate reduction to ammonium (DNRA), or elevated plant N uptake due to shallow ground water flow (Inamdar et al. 2020).

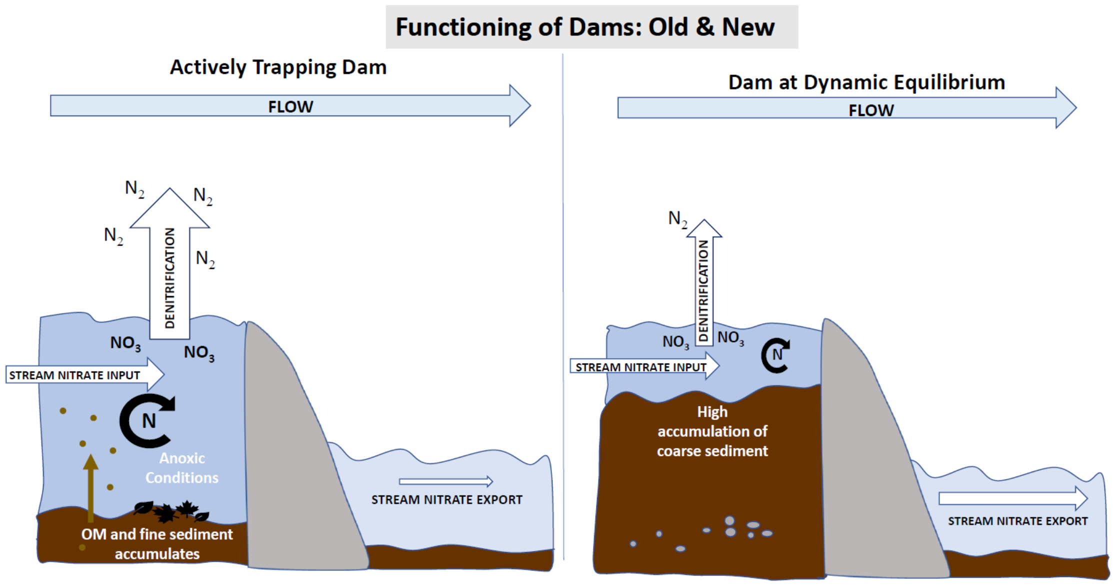

Figure 5.2: Schematic indicating biogeochemical processing ability as it relates to denitrification potential on a dam that is actively trapping sediment (left) and one that is full above the impoundment and at dynamic equilibrium (right).

Additionally, stream nutrient loading and pollutants have changed over the last two centuries, and it is important to consider what contaminants might be trapped in sediment above the dam. Contaminants from the 1800s were mainly from untreated sewage or mill runoff resulting from adjacent mills including tannery, paper mill, and sawmill waste. Today, inputs include excess fertilizer runoff, heavy metal contamination, and chemical contaminants. This is of more concern in urban streams where metal and chemical contaminants may become mobilized under anoxic conditions (Pennino et al., 2017, Vidon et al., 2010), or be released when the dam is breached or removed. An analysis of Roller Mill sediment impoundments found similar concentrations in TN, TC, and TP compared to other Lancaster County, PA former millponds, and levels of polychlorinated biphenyls (PCBs), lead (Pb), and mercy (Hg) were below detection or accepted safety limits (Stantec, 2020), indicating sedimentation may be an issue upon removal but PCBs and heavy metal leaching is unlikely. Furthermore, dams at full storage capacity may become hot spots for methane emission due to sediment accumulation, contributing to greenhouse gas emissions (Maeck et al., 2013).

Akbarzadeh et al. (2019) found that globally, large dams (such as hydroelectric dams) and reservoirs reduce the loading of TN in rivers through denitrification and burial, acting as a sink with removal or burial outpacing N fixation. They found in 2000, worldwide denitrification and burial eliminated 7%, or 270 Gmol yr^{-1}, of global N loading to river networks. While this was a conservative estimate, it indicates that large reservoirs cumulatively have an impact on eliminating N from a system. Interestingly, while colonial-era milldams are being removed across the US due to safety or ecological concerns (Hart et al., 2002, Bellemore et al., 2017a), larger dams are being constructed at increasing rates globally to supply waterpower (Akbarzadeh et al. 2000, Zarfl et al., 2015). This shifting trend highlights the need to better understand the ecological impact of historic milldams to improve stream health in a time of growing hydropower construction on global waterways. Future studies are needed to examine how potential cumulative benefits of milldam removal compare to ecological impacts of large dam construction on a global scale, particularly in N processing across watersheds.

Results from this study can help inform watershed managers and decision makers on how current low-head milldams may be altering stream N processing, what factors help control these processes, and how dam removal may or may not alter N flux through the watershed. Bellmore et al. (2019) have called for clearer communication with all stakeholders involved in the dam removal process to better manage expectations post-removal, and this study and others can contribute to the current body of knowledge to better predict consequences.

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CONCLUSION

This study showed that two low-head milldam sites in northern Delaware and south-eastern Pennsylvania did not appear to alter streambed denitrification rates upstream versus downstream of the dam. This was despite higher water levels, warmer average daily temperature, increased residence time, and lower DO concentrations upstream versus downstream of the dam. This could be due to the temporal and longitudinal variation that occurs naturally in streambed denitrification, the smaller size of our dams' impoundments compared to larger reservoirs that are known to increase denitrification, and/or the age of our dams resulting in impoundments that are filled with sediment above the dam.

Key findings from this study include:

1. No significant differences in denitrification were found upstream versus downstream of the dam at Christina and rates from both sites were in line with literature values from natural dams and agriculturally influenced streams.
2. Sediment percent organic matter and sediment ammonium-N concentration accounted for 33% of denitrification variability across both sites using a multilinear regression model.
3. No significant differences were seen in dissolved nutrient concentrations (TdN, NO_3-N, NH_4-N, DOC) above the dam versus below, and a significant difference along a 500 m

sampling reach was only found for Christina downstream TN.

4. Highest DEA rates occurred in Fall 2019 and Winter 2021 and were correlated with higher stream level nitrate-N concentrations and overall were correlated with higher percent organic matter and percent clay particles by surface area.

Improvements to this study include looking at sediment and sediment-bound nutrients that may be flushed out after dam removal, in addition to the studied dissolved stream water nutrients. Additionally, equal sample sizes of streambed sediment cores taken above and below the dam at Chiques would be beneficial, and future studies should ensure sampling locations are chosen with adequate access before and after dam removal occurs.

Results from this study can be used to inform watershed managers about the impacts of dams on N processing in streams, a topic of growing concern especially as aging dams reach equilibrium in storage ability, in addition to being removed at increasing rates. Future studies are needed to monitor how streambed sediment denitrification ranges change over time after dam removal, particularly when sediments deposited in the dam reservoir are released and redeposited.

SUPPLEMENTARY

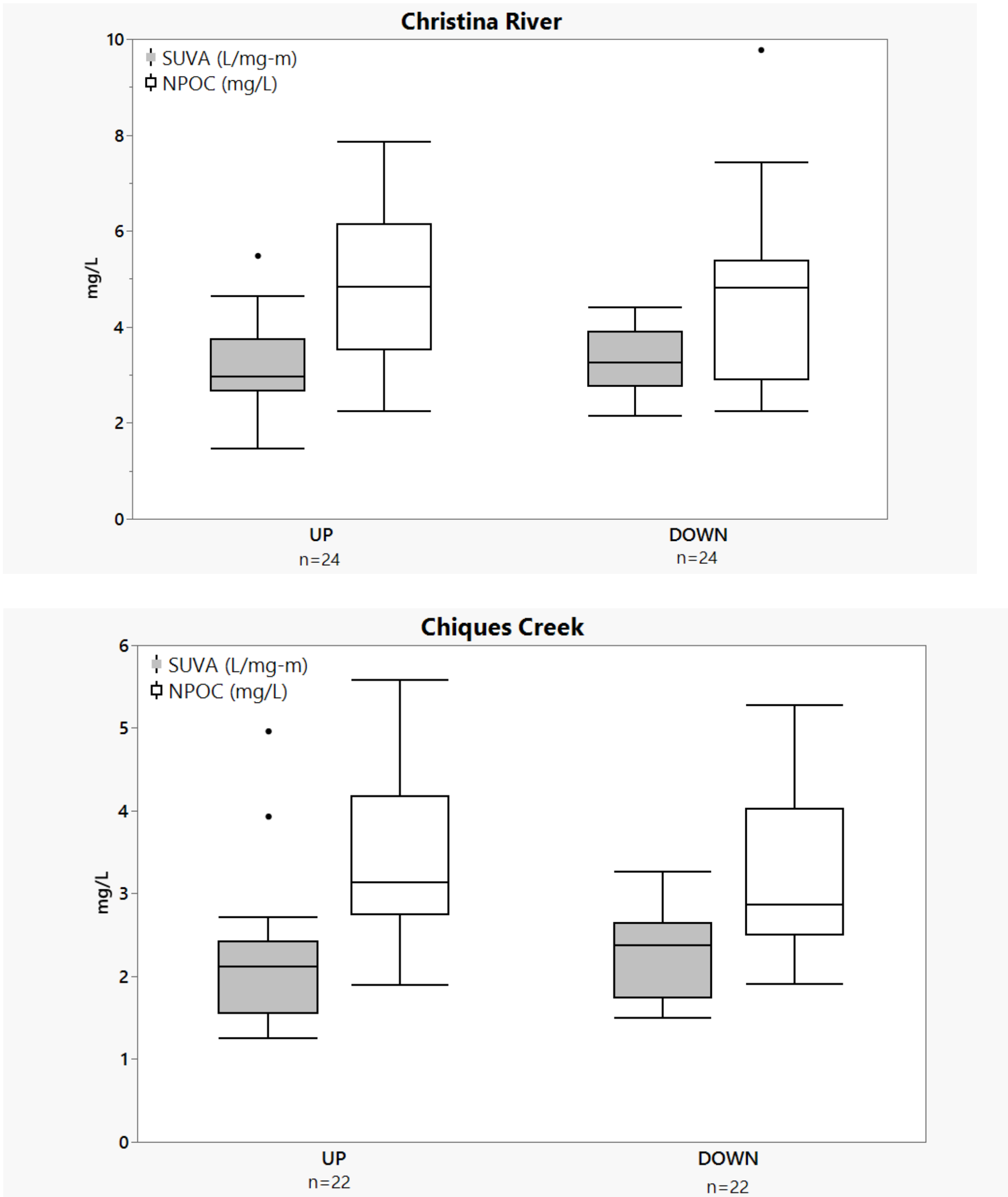

Figure A.1: Boxplots of SUVA values (L/mg*m) and DOC (mg/L) for Christina (top) and Chiques (below) by sampling locations. No significant differences were found across sites (Christina: $p > 0.3$, n=24, Students T-test, $\alpha = 0.05$; Chiques: $p > 0.7$, n=22, Students T-test, $\alpha = 0.05$).