THE EFFECT OF FLOODPLAIN CREATION ON SOIL BIOGEOCHEMISTRY IN AGRICULTURAL CHANNELS

by

Celena Alford

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STATEMENT OF COMMITTEE APPROVAL

Dr. Sara K. McMillan, Chair

Department of Agricultural and Biological Engineering

Dr. Laura Bowling

Department of Agronomy

Dr. Margaret Gitau

Department of Agricultural and Biological Engineering

Approved by:

Dr. Bernard Engel

Head of the Graduate Program

To my parents, for all of your support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Alford, Celena, A. MSABE
Institution: Purdue University
Degree Received: December 2018
Title: The Effect of Floodplain Creation on Soil Biogeochemistry in Agricultural Channels.
Committee Chair: Sara K. McMillan

In the agricultural Midwest, subsurface drainage allows excess water to drain into agricultural channels, which flows into rivers and streams transporting excess nutrients downstream. The construction of an inset floodplain within agricultural channels enhances sedimentation of particulate nutrients and sediment, provides stable conditions for vegetation to establish, increases rates of microbial activity, and promotes denitrification. Sediments were collected from floodplains of two-stage channels and naturally forming floodplain benches in conventional channels to determine the effect of floodplain creation on carbon and nitrogen cycling. Denitrification rates were seasonally measured across the floodplain width using an unamended acetylene inhibition technique (DNF_{AIT}). Composite respiration and denitrification rates were measured through sacrificial microcosms utilizing membrane inlet mass spectrometry (DNF_{MIMS}). While the two-stage reach showed a significant increase in soil organic matter (two-way ANOVA, $p < 0.001$) and respiration rates (two-way ANOVA, $p = 0.039$), there was no effect on DNF_{MIMS} rates (two-way ANOVA, $p > 0.05$). DNF_{AIT} rates at the two-stage reach only showed an increase at locations closest to the channel (two-way ANOVA, $p = 0.008$). Nutrient processing rates were most dependent on local environmental conditions, particularly organic matter and sediment grain size. This suggests that site-specific conditions may dictate the impact of floodplain creation on water quality. However, because of the increase in biologically active surface area, the net effect on water quality is likely greater for the two-stage channels.

INTRODUCTION

Background

Agriculture in the United States is the main source of non-point source pollution to rivers and streams (US EPA, 2017). In the Midwestern U.S., excess nitrogen (N) and phosphorus (P) from fertilizer application flows through subsurface drains into agricultural channels. These channels then flow into rivers and streams that transport excess nutrients downstream. During periods of high flow, greater loads of N and P are exported, contributing to eutrophication of lakes and coastal zones. This leads to many adverse impacts including oxygen depletion, algal blooms, and habitat loss (Carpenter et al., 1998). For this reason, research and management has focused on reducing agricultural nutrient loads through best management practices such as conservation tillage, reduced fertilizer application, and more recently, the construction of inset floodplains.

To effectively reduce nutrient loads, a range of practices are used to address the distinct hydrology of agricultural landscapes. Fields planted with row crop agriculture commonly have agricultural channels and piped subsurface drainage installed to reduce the time and extent of flooded soils and, therefore, improve productivity. This system has been common throughout the Midwestern U.S. since the late 1800's to offset the waterlogged clay soil of this region that results in poor natural subsurface drainage (Dinnes et al., 2002; D'Ambrosio, 2013). While subsurface drainage improves field conditions, it also facilitates rapid transport of nitrate rich soil pore water to surface water, and thus promotes N leaching from fertilizer (Fenelon and Moore, 1998; Randall and Goss, 2008).

Fertilizer application has been common place in the agricultural Midwest since the 1950's (Millar et al., 2010). Though it has been linked with an increase in productivity, it has also led to an increase in nutrient concentrations of fresh water bodies and greenhouse gas emissions. Most N fertilizers form ammonium (NH_4^+) in water (Butzen, 2011). It is readily available for plants and microbes and quickly nitrified into nitrite (NO_2^-) or nitrate (NO_3^-). During storm events, these highly mobile forms of N are transported through the soil pore water into subsurface drainage that leads to open channels (Fenelon and Moore, 1998). Phosphorus fertilizer is typically incorporated into the soil at planting (Mahler, 1999), but during rain events is transported through overland flow (Sharpley et al., 1996). The implementation of the two-stage channel has been proposed as a method to the reduce both N and P loads in agricultural channels, especially during storm events, while maintaining productivity.

Inset floodplains are designed to incorporate the functions of naturally formed floodplains, while still supporting the drainage capacity required by the farmer (Powell et al., 2007a). Natural floodplains convey and store water, recharge groundwater, control stream bank erosion, reduce sedimentation, filter nutrients and impurities, support and maintain biodiversity, and provide fish and wildlife habitats (FEMA, 2002). They also increase the surface area during high flow, allowing the velocity to decrease, suspended sediments to settle, and dissolved nutrients to be assimilated (Brinson et al., 1984; Maltby and Acreman, 2011; Tockner and Stanford, 2002; Cook, 2007). The two-stage channel is constructed with an inset floodplain to mimic many of the benefits of natural floodplains. They were designed to reduce the likelihood of flooding adjacent land (Powell et al., 2007a; Krider et al., 2017) and increase sedimentation by slowing water velocities at higher flow in the floodplain (Powell and Bouchard, 2010). Studies have shown that the floodplain can be effective at removing suspended sediments, similar to buffer strips (Barling and Moore 1994; Dosskey 2001; Tomer et al. 2003). Inset floodplains also increase channel stability (Rhoads and Massey, 2012) and, ideally, require little maintenance (D'Ambrosio et al., 2015).

The inset floodplains may also impact in-stream nutrient concentrations. In a two-stage channel, tile drainage flows directly onto the inset floodplain, allowing for greater physical and biological retention of nutrients (Powell and Bouchard, 2010). During low flow, water is stored within the soil pore space of the floodplain before percolating into the main channel, allowing for plant and microbial uptake and phosphorus sorption onto sediments (Hodaj et al., 2017). During high flow, the amount of surface flow interacting with the biologically active surface area of the channel increases. This has shown to increase potential NO_3^- and P retention within the channel (Davis et al., 2015; Hanrahan et al., 2018). While dissolved P can be temporarily stored through sorption or plant and microbe assimilation, NO_3^- can be permanently removed via denitrification (Reddy et al., 1999; Withers and Jarvie, 2008).

Denitrification is the process by which NO_3^- is permanently removed from waterways. It is an anoxic microbial process by which NO_3^- is converted into nitrous oxide (N_2O) which can be further reduced to dinitrogen gas (N_2). It is affected by factors such as oxygen concentration, organic matter content, temperature, residence time, and discharge (Arango et al., 2007; Roley et al., 2012). Inset floodplains increase the amount of vegetated surface within the channel, decreasing the flow velocity and increasing the residence time. It also increases the amount of dead plant material and trapped sediment collecting as organic matter (Hanrahan et al., 2018).

This can result in higher denitrification rates, which may be essential to agricultural channels as it can account for most, if not all NO_3^- reduction in rivers and streams (Hill and Sanmugadas, 1985; Seitzinger, 1988). Though the two-stage channel has been shown to increase denitrification rates, it has not translated into a reduction in nitrate loads (Davis et al., 2015; Hodaj et al., 2017). Therefore, more information is needed as to how inset floodplains function with regards to nutrient retention and transformation.

Naturally formed floodplains within unmanaged agricultural channels may function similarly to inset floodplains. Dredged agricultural channels tend to be incised and over-widened making them unstable. These unstable channels attempt to reach equilibrium by slumping and accumulating sediments to form small, unstable floodplains within the channel (Simon and Rinaldi, 2000; Powell et al., 2007a). Over time, the drainage capacity in these channels may be lowered due to a build up debris and sediment (Fausey et al., 1982). Farmers often dredge agricultural channels to remove deposited sediment. While channel dredging has the positive effect of stabilizing soil water throughout the growing season and reducing risk to farmers, it also facilitates rapid transport of nutrient-rich water to receiving streams. If left undredged, channels can naturally stabilize and form floodplains that begin to mimic those of natural streams (Magner et al., 2012).

More research is needed as to the potential of naturally formed floodplains for nutrient reduction and transformation in relation to inset floodplains. Studies have shown that both naturalized and inset floodplains provide an increase in organic matter and potential NO_3^- removal (Landwehr and Rhoads, 2003; Powell and Bouchard, 2010; Roley et al., 2012; Mahl et al., 2015; Hanrahan et al., 2018). When compared to each other, though they showed similar respiration rates, the inset floodplains accumulated more organic matter and had higher denitrification rates (Hanrahan et al., 2018). However, less is known as to how these floodplains compare in multiple channels and whether the relationship remains consistent throughout the year.

The objective of this research was to determine if the constructed floodplains of two-stage channels provide a benefit in carbon and nitrogen cycling over naturalized floodplains (slumped banks and accumulated sediments) at the stream reach scale. To address this question, water and soil samples were collected seasonally at three sites to (1) compare denitrification and respiration rates between constructed and naturalized floodplains; and (2) identify the relationships between these rates and environmental properties. To address the objective, two main methods were utilized to measure respiration and denitrification rates. Microcosm incubations were designed to measure net respiration and denitrification rates using a Membrane Inlet Mass Spectrometer (MIMS) and denitrification through the acetylene inhibition technique (AIT). The MIMS method allows us to measure changes in dissolved O_2 and N_2 concentrations within stream water of inundated soil samples. The AIT method allows us to measure changes in N_2O concentrations released in the headspace overlying inundated sediment slurries. The MIMS method is highly precise and more closely approximates in situ rates, microcosms must be sacrificially sampled at discrete time points to measure accumulation of dissolved N_2 and depletion of O_2 (Reisinger et al., 2016). The AIT method is more commonly used in agricultural systems and robust in systems with high nitrate levels as NO_3^- limitations are overcome, allowing the variability in this driver to be eliminated (Groffman et al., 2006). However, this method also depends on the use of acetylene as an inhibitor of the last step in the denitrification process (N_2O to N_2), which allows accumulated N_2O to be measured over time. It also artificially induces anoxia thereby inhibiting other important coupling processes such as nitrification. Due to the ease of sampling and analysis and the elimination of variability in NO_3^- and redox conditions, the AIT method was used to measure lateral variances in denitrification between channels seasonally. The MIMS method was used to compare differences in denitrification rates among channels containing different underlying geology as it better replicates in-situ solute delivery to soil microbes.

Hypotheses

Since respiration rates can be used as a proxy for microbial activity (Conkle and White, 2012), it was hypothesized that respiration and denitrification rates would follow similar patterns. Microbial activity generally increases with temperature (Pietikäinen et al., 2005), so it was expected that the highest respiration rates would be in the summer and the lowest rates in the winter. It was also expected that higher processing rates and channel nutrient concentrations would be measured in the spring, due to fertilizer application and higher soil moisture (Sexstone et al., 1985; Hanson et al., 1994). Since the inset floodplain is intentionally vegetated primarily to increase channel stability, it was expected that a greater build-up of organic matter in the inset floodplain would be a result of an increase in dead plant matter and sedimentation. This should lead to higher respiration and denitrification rates in correspondence with the higher organic matter, as carbon is a requirement for both reactions to occur (Robertson and Groffman, 2007).

METHODS

Site Description

The study was conducted at three agricultural channels in northern Indiana: Kirkpatrick Channel (KIRK), Shatto Ditch (SHA), and an unnamed channel at the Throckmorton Purdue Agricultural Center (TPAC) (Figure 1). Each channel is a first-order stream located in a watershed dominated by row crop agriculture in corn and soybean rotation (>70%). Paired reaches were selected at each of these three sites: a constructed two-stage channel and an agricultural drainage channel with naturalized floodplains (hereafter referred to as control) in the traditional trapezoidal design that is conventionally managed. The control reaches contained floodplains formed naturally through channel bank scouring, slumping, and sediment deposition (Figure 2). KIRK has an 800cm, one sided two stage channel reach with an average height of 45 cm above the channel thalweg; it was constructed in 2013. The vegetation on the silty clay loam of the floodplains is dominated by grasses and wetland species. The control reach is located adjacent to the two-stage and contains small, unstable, segmented floodplains. SHATTO's two stage channel reach was constructed in 2007 with a 600 m long inset floodplain at an average height of 27 cm above the channel thalweg. The vegetation on the sandy floodplains is dominated by canary grass and rice cutgrass. The control reach immediately upstream has relatively stable and connected floodplains that formed naturally during channel evolution processes however, the channel was dredged during the study in the summer of 2017. The two-stage channel reach of TPAC was constructed in 2012 with a 200 m long inset floodplain at a height of 30 cm above the thalweg. The floodplain soils are primarily clay and after construction, both the two-stage and control reaches were intentionally vegetated with five different mixes of sedges, forbs and grasses. The control reach is immediately upstream and contains well connected, vegetated floodplains.

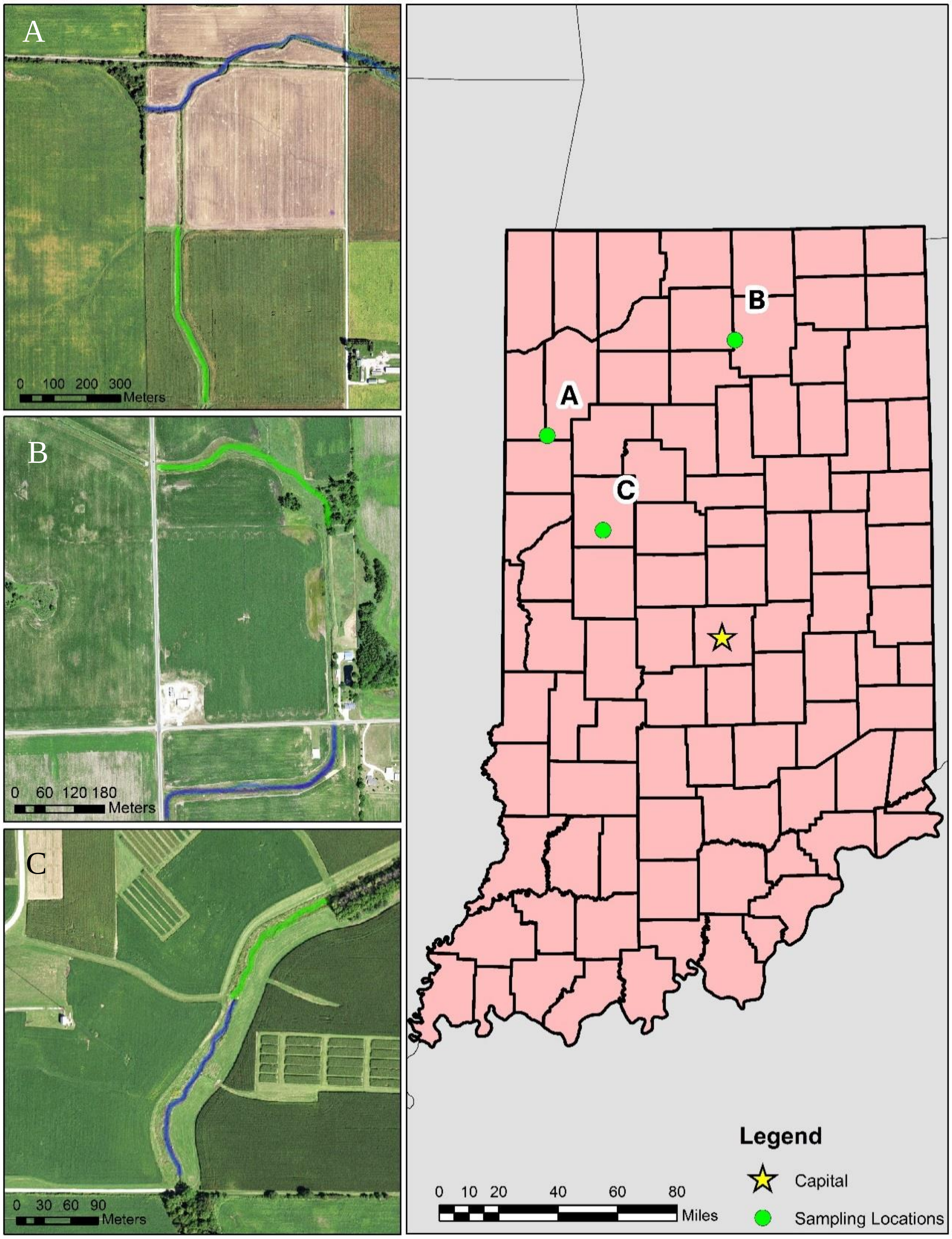

Figure 1 Map of study sites in Indiana with the control reach highlighted in blue and the two-stage reach highlighted in green. (A) Kirkpatrick channel located in Remington, IN. (B) Throckmorton Purdue Agricultural Center channel in West Lafayette. (C) Shatto Ditch in Harrison Township.

Site temperatures were obtained from NOAA, which maintains weather stations at local airports: Jasper County Airport, Warsaw Municipal Airport, and Purdue Airport. The Jasper County Airport is located 13.24 miles northeast of KIRK. The Warsaw Municipal Airport is located 11.25 miles northeast of SHA and the Purdue Airport is located 7.66 miles northwest of TPAC.

Sediment Respiration and Denitrification

Water was collected manually once per season simultaneous to soil sampling. Labelled five-gallon buckets were used to gather samples from the control and two-stage channel reaches of each site. Buckets were covered and kept out of direct sunlight until brought to the lab. Once transported, the covered buckets sat overnight to equilibrate and reach room temperature. Nutrient concentrations of stream water left overnight differed from concentrations in samples taken directly from the stream. Therefore, water samples for nutrient analysis were taken from the buckets immediately prior to conducting our experimentation to ensure accurate results. Buckets were well mixed prior to sampling and right before experimentation began. Samples were labeled, filtered in lab (0.7 micron pore size), and frozen the same day as collection until analysis could begin. Thawed samples were analyzed for soluble reactive phosphorus (SRP), nitrate, and ammonium concentrations on a SEAL AQ2 Auto Analyzer (USEPA 1993, APHA 2000). The detection limit for soluble reactive phosphorus (SRP), nitrate, and ammonium concentrations were 0.01 P-mg/L, 0.015 N-mg/L, and 0.02 N-mg/L, respectively. Samples measured as zero were set to half the lower detection limit for data analysis. Average nutrient concentrations were within the range of similar studies in-stream concentrations (Arango et al. 2007; Mahl et al., 2015; Hodaj et al., 2017), though the distribution of nitrate was positively skewed.

Floodplain soils were collected seasonally using a metal soil probe (2 cm diameter) from each site for one year (from Spring 2017 through Winter 2018). Floodplain soils were sampled to a depth of 5 cm because previous research has shown that denitrification rates decrease substantially below these depths in soils on similarly constructed floodplain benches (Roley et al., 2012). To account for spatial heterogeneity, 12 soil cores were collected at random along four, evenly spaced transects within the reaches of the two-stage channel (TSC) and the control (Figure 3). Soils cores were homogenized for each transect, as the transects were used in place of replicates.

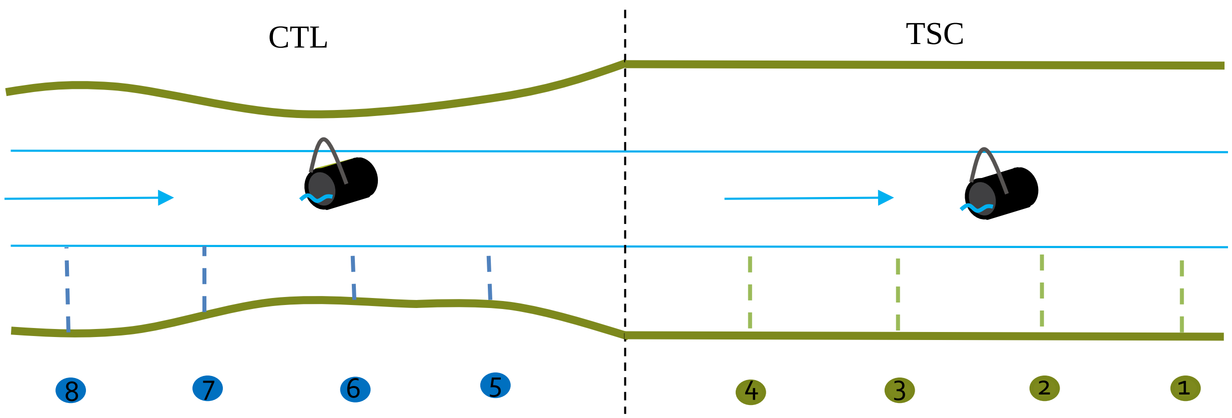

Figure 2 Sampling scheme for MIMS denitrification. Soil samples were taken from four transects within each reach. Water samples were taken mid-reach.

Homogenized soils from each location were hand textured and classified based on the modified guide to determine soil texture by feel (NRCS,1999). Split soil samples were used to measure dry mass and soil organic matter through the loss on ignition method (Dean, 1974). Soils were weighed, placed in the oven at 105 °C for at least 24 hours and weighed again to determine final dry mass. The sample was then placed in a muffle furnace at 550 °C for about 4 hours. Once cooled the sample was again weighed and organic matter was determined as the difference between the dried and burned mass normalized to dry mass.

Sacrificial microcosm incubations were used to evaluate net denitrification and respiration rates using a Membrane Inlet Mass Spectrometer (MIMS) to measure accumulation of N_2 during the incubation period (Reisinger et. al., 2016). The incubations were run for 8 hours in the spring, then shortened to 6 hours to reduce the likelihood of rate underestimation due to drops in N_2 concentrations. Concentrations were measured at five, evenly spaced sampling time points, with the first sample taken at the start of the incubation (t = 0 hrs).

To begin the experiment, 10 ± 0.05 grams of homogenized soil were placed into labeled 50 ml centrifuge tubes according to transect and homogenized with 10 milliliters of site water, except for the first timepoint, which contained no soil but was filled with site water only and sampled as the initial state of water column chemistry. Site water was added using a syringe to slowly fill each tube, capping underwater to eliminate headspace for a total of 5 tubes per site. Tubes were placed in a water bath the same temperature as the site water to stabilize temperatures as dissolved gas concentrations are sensitive to even slight fluctuations in temperature. One tube per transect was sacrificially sampled at each timepoint of 0, 1.5, 3, 4.5, and 6 hours (0, 2, 4, 6, and 8 hours in spring) and used to fill 12 milliliter exetainers. The exetainer was allowed to overflow to an equivalent volume of 24 ml (twice the 12 ml volume of the exetainer) before adding an antimicrobial zinc chloride solution and capping with no headspace. Dissolved O_2/Ar and N_2/Ar ratios were measured using a Membrane Inlet Mass Spectrometer (MIMS) (Bay Instruments, Easton, MD, USA) (Kana et al., 1994). Dissolved O_2 and N_2 concentrations were calculated as:

$$moles\ of\ N_2 = \frac{measured\ \left(\frac{O_2\ or\ N_2}{Ar}\right)}{standard\ \left(\frac{O_2\ or\ N_2}{Ar}\right)} * equilibrium\ \left(\frac{O_2\ or\ N_2}{Ar}\right) * equilibrium\ (Ar) * total\ volume$$

The $measured\ \left(\frac{O_2\ or\ N_2}{Ar}\right)$ is the ratio of gas to argon measured in each exetainer. Deionized water was used as a standard for background $\left(\frac{O_2\ or\ N_2}{Ar}\right)$ ratios. Due to the slight drift of the instrument over time, we interpolated what the $standard\ \left(\frac{O_2\ or\ N_2}{Ar}\right)$ would be at the same time the $measured\ \left(\frac{O_2\ or\ N_2}{Ar}\right)$ was taken using the $\left(\frac{O_2\ or\ N_2}{Ar}\right)$ ratios of the standard measured before and after. The $equilibrium\ \left(\frac{O_2\ or\ N_2}{Ar}\right)$ is the solubility of O_2 or N_2 and Ar at the experimental water temperature. From this, denitrification and respiration rates were calculated as:

$$Net\ Rate\ \left(\frac{g_{O_2\ or\ N_2}}{\frac{g_{DM}}{hr}}\right) = \frac{\Delta\ moles\ of\ O_2\ or\ N_2}{\Delta\ hr} * \frac{molar\ mass\ of\ \ O_2\ or\ N_2}{(10\ g_{soil} * dry\ mass\ \%)}$$

Rates $\left(\frac{g_{O_2\ or\ N_2}}{g_{DM}}\right)$ were expressed in three ways: per area (m^2), per gram dry mass (g_{DM}), and per gram ash-free dry mass (g_{AFDM}). Normalizing rates according to area allows us to observe the microbial activity across the length of each floodplain type. Expressing rates per dry mass allows us to measure the concentration of microbial activity within the soil and per ash-free dry mass allows us to look at the amount of microbial activity with regards to the amount of organic matter available within the soil.

Acetylene Inhibition Technique

Denitrification bottle assays were conducted using the acetylene inhibition technique in a similar procedure as denitrifying enzyme activity assays (Smith and Tiedje, 1979; Bernot et al., 2003; Royer et al., 2004). The incubations were run for five hours with five sampling time points, with the first sample taken at the start of the incubation (t = 10mins). Soil samples were collected at varying floodplain widths (0.5, 1.0, and 1.5 meters) at a depth of 5 cm along each transect to produce 12 samples per reach (Figure 3). Due to the instability and inconsistent floodplain width in the control reach of KIRK, denitrification rates measured using the acetylene inhibition technique at 1.0 and 1.5 m from the channel edge were analyzed using only SHA and TPAC data. Water samples were taken mid-reach using one-liter brown bottles. All soil and water samples were kept cool and out of direct sunlight during transport. Samples were stored in the refrigerator and used within forty-eight hours of being collected.

Split soil samples were used to measure dry mass and soil organic matter through the loss on ignition method (Dean, 1974). Soils were placed in the oven at 105 °C for at least 24 hours and weighed to determine final dry mass. The sample was then placed in a muffle furnace at 550 °C for about 4 hours. Once cooled the sample was again weighed and organic matter was determined as the difference between the dried and burned mass normalized to dry mass.

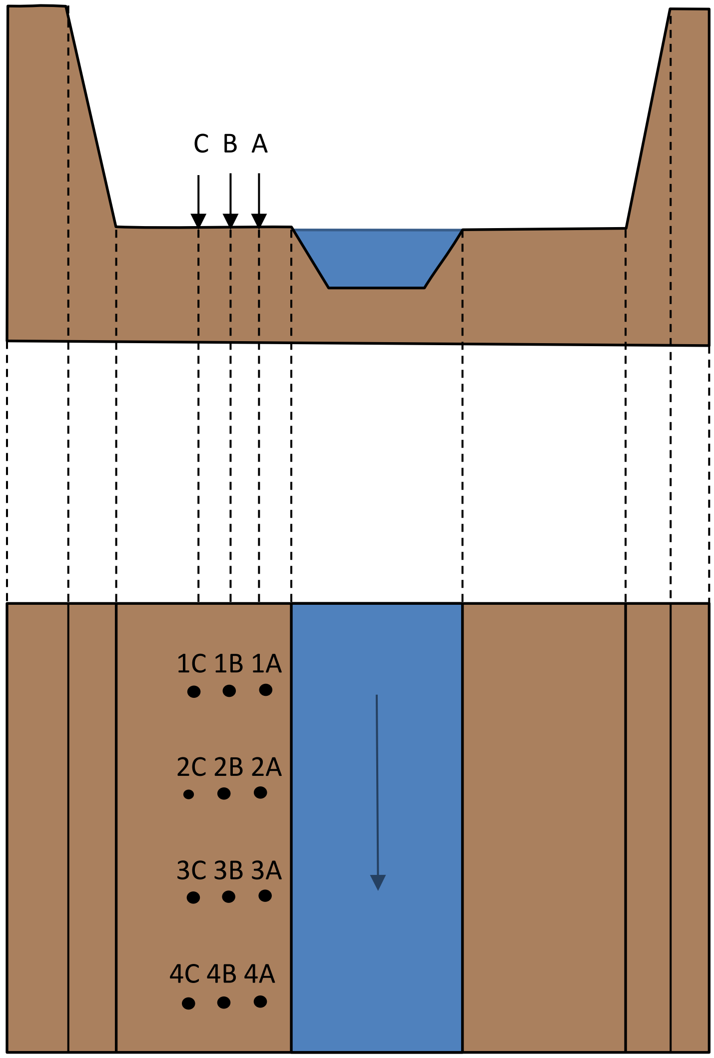

Figure 3 AIT Sampling in the two-stage channel. Letters A, B, and C represent sampling points 0.5m, 1.0m, and 1.5m, respectively, from the edge of the floodplain; numbers represent transects. Water samples are taken mid-reach.

Soil samples collected using the soil probe were first homogenized for each transect at each sampling width (1A, 1B, 1C, etc.). 160 ml glass bottles were labeled according to transect and sampling point (2 sites x 4 transects x 3 widths for n = 24). 25 ml of soil were then placed into labeled bottles according to transect and homogenized with 45 ml of site water to create a slurry. 5 ml of a 3.1 mM chloramphenicol solution was added to each bottle to inhibit the synthesis of new denitrifying bacteria (Smith and Tiedje, 1979). Bottles were purged with dinitrogen gas for 8 minutes then re-equilibrated to atmospheric pressure. 16 ml of headspace was removed and replaced with an equal volume of acetylene gas. Acetylene gas was used to prevent the microbial reduction of N_2O produced into N_2. An initial 5 ml sample of the headspace was taken 10 minutes after the gas addition to overfill a pre-evacuated 3 ml exetainer. The removed gas was replaced with a 5 ml mixture of 10% acetylene and 90% dinitrogen gas (N_2) in each bottle. This was repeated for each hour interval, with the time recorded for each sample taken. Each 3 ml sample was then run on a gas chromatograph (Shimadzu Corp., Kyoto, JP) to measure nitrous oxide concentration. Concentrations (ppm) were converted into mass using the ideal gas law and the Bunsen coefficient. Denitrification rates were calculated as the slope of the mass of N_2O versus time. Rates were expressed in three ways: per area (m^2), per gram dry mass (g_{DM}), and per gram ash free dry mass (g_{AFDM}).

Statistical Analysis

Water samples with undetected concentrations were set to half of the detection limit for all statistical analyses. Significant respiration and denitrification rates from the MIMS method were determined using linear regression. Transects that displayed a significant, linear decrease in O_2 or increase N_2 over the incubation period were considered to have measurable respiration and denitrification rates, respectively. Our alpha value was set to 0.25 to accommodate our low sample size (n = 5) and reduce the likelihood of a type II error (Reisinger et al., 2016). Insignificant values were considered to be below detection and were set to zero. Since this research is only concerned with the effective removal of nitrate from these channels, transects with a negative denitrification rate were also considered to have no measurable denitrification, as it would imply the dominance of other microbial processes (Hanrahan et al., 2018).

A two-way ANOVA with a 95% significance level was performed for denitrification, respiration, and organic matter data. This was to determine whether the two-stage floodplain in combination with either season or channel site (KIRK, SHA, or TPAC) had a significant impact on floodplain function. Reach (two-stage and control) and Season were the first fixed effects considered and included an interaction term. Significant main effects were reported and a pairwise Tukey–Kramer post hoc was used to compare means. For significant interactions, a one-way ANOVA with a Bonferroni corrected p-value was used analyze the seasonal variation of each reach. A pairwise Tukey–Kramer post hoc for each reach was used to compare the means of each season. A second two-way ANOVA was conducted with Reach and Site (KIRK, SHA, and TPAC) as fixed effects. For significant interactions, a one-way ANOVA with a Bonferroni corrected p-value was used analyze the reach differences of each site. A pairwise Tukey–Kramer post hoc for each site was used to compare the means of each reach.

The Spearman correlation coefficient was used to determine if any monotonic relationships existed between each of our environmental data (nutrient concentrations, organic matter, and temperature) and our measured respiration and denitrification rates. Local Regression (LOESS) was then run for significantly correlated data ($\alpha = 0.05$) and used to model the observed trends with a smoothing parameter of 0.25. Multiple regression analysis was used to determine whether our environmental data could sufficiently predict our denitrification and respiration rates. Resulting equations only containing significant predictors were reported below ($\alpha = 0.05$). Data were tested for normality using the Shapiro-Wilks test ($\alpha = 0.05$) and were transformed when necessary. All statistics were conducted in R (3.3.2; R Core Team 2016).

RESULTS

Soil and Water Quality

The inset and naturalized floodplains had different soil textures. The control reach for Kirkpatrick channel (KIRK) and the ditch at Throckmorton Purdue Agricultural Center (TPAC) had sandier soils than the two-stage reach (Table 1). There were also differences among sites. Shatto Ditch (SHA) contained the sandiest soils, with the texture in both the two-stage and control reaches categorized as sandy loam. Noticeable changes in the sand content of both reaches were observed after the summer (Figure 4). The clay content was highest in KIRK and TPAC had the highest silt content.

Table 1 The average background nutrient concentrations and soil texture in the two-stage and control floodplains. Each site had four water and twelve soil samples for each reach. The two-stage reach had no significant effect on soluble reactive phosphorus (SRP), nitrate (NO_3^-), or ammonium (NH_4^+) concentrations (two-way ANOVA, $p > 0.05$). The control reach for TPAC and KIRK had sandier soils than the two-stage reach.

REACH	**SITE**	**SRP (mg P/L)**	**NO_3^- (mg N/L)**	**NH_4^+ (mg N/L)**	**Soil Texture**
TWO-STAGE	KIRK	0.024 ± 0.016	9.66 ± 2.52	0.043 ± 0.015	Clay
	SHA	0.019 ± 0.014	6.19 ± 1.95	0.411 ± 0.334	Sandy Loam
	TPAC	0.049 ± 0.028	9.63 ± 2.22	0.063 ± 0.022	Silty Clay Loam
CONTROL	KIRK	0.055 ± 0.042	16.5 ± 7.43	0.210 ± 0.177	Sandy Clay Loam
	SHA	0.028 ± 0.017	6.75 ± 1.91	0.068 ± 0.040	Sandy Loam
	TPAC	0.044 ± 0.025	9.63 ± 2.24	0.063 ± 0.018	Loam

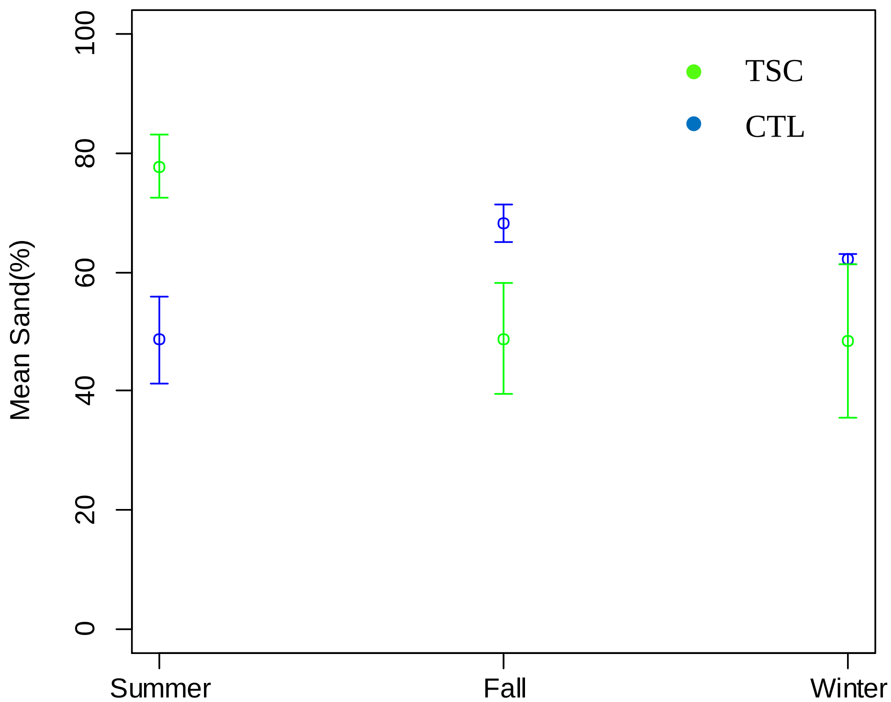

Figure 4 The average sand content of the two-stage (TSC) and control (CTL) reaches at SHA based on the NRCS soil texture classification (n=4). The standard error of the mean is plotted with each point. The data is shown to include the three seasons in which soils were hand textured.

Nutrient concentrations of stream water collected concurrently with seasonal measurements of ecological processes did not differ significantly by reach (one-way ANOVA, $p > 0.05$). Average SRP concentrations were the lowest at TPAC (0.02 ± 0.02 mg P/L) and the highest in KIRK (0.04 ± 0.06 mg P/L) (Table 1). KIRK also had the highest nitrate concentrations, with the control reach measuring the highest concentrations (range: 6.27 to 38.24 mg N/L). Average ammonium concentrations were highest in SHA (0.24 ± 0.45 mg N/L) and lowest in TPAC (0.04 ± 0.02 mg N/L). All concentrations were highly variable at each site, likely a result of the low sample size ($n = 8$). However, these data were primarily collected as potential explanatory variables for carbon and nitrogen processing rates (i.e., respiration and denitrification). Overall, soil organic matter did not vary significantly with floodplain width within a site but did vary between the control and two-stage (Figure 5). As lateral distance from the channel increased, differences between the two-stage and control became less significant. The constructed inset floodplains had significantly greater organic matter (two-way ANOVA; Reach; $p < 0.001$), averaging 7.47% ± 3.78 as compared to the 5.62% ± 2.71 of the naturally formed floodplains. Soil organic matter varied between the two-stage and control floodplains and among sites (two-way ANOVA, Reach x Site, $p = 0.003$). When sites were analyzed individually, SHA had significantly higher organic matter content in the inset floodplain (one-way ANOVA, $p = 0.005$). The control reach of SHA averaged 6.44% ± 4.12 while the two-stage reach averaged 11.02% ± 4.36, the highest percentage of any site reach. This site also had significantly higher organic matter content than KIRK (Tukey HSD, $p < 0.001$), but not TPAC (Tukey HSD, $p = 0.36$) at 8.73% ± 4.78. KIRK contained the lowest organic matter content at 4.32% ± 1.30 while TPAC averaged 6.62% ± 0.85.

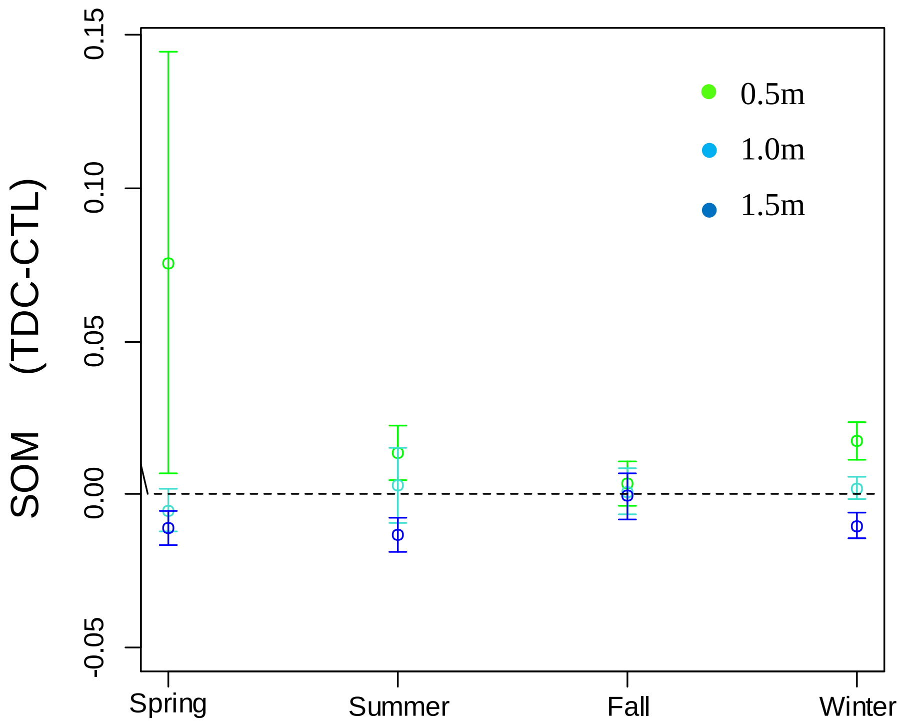

Figure 5 The difference in average soil organic matter (SOM) ratios from the two-stage to the control at TPAC. Points above the zero line represent locations in the two-stage that measured an increase in SOM over the control at a specific width (n=4). The standard error of the mean is plotted with each point. The relationships are shown to include the four seasons in which measurements were taken.

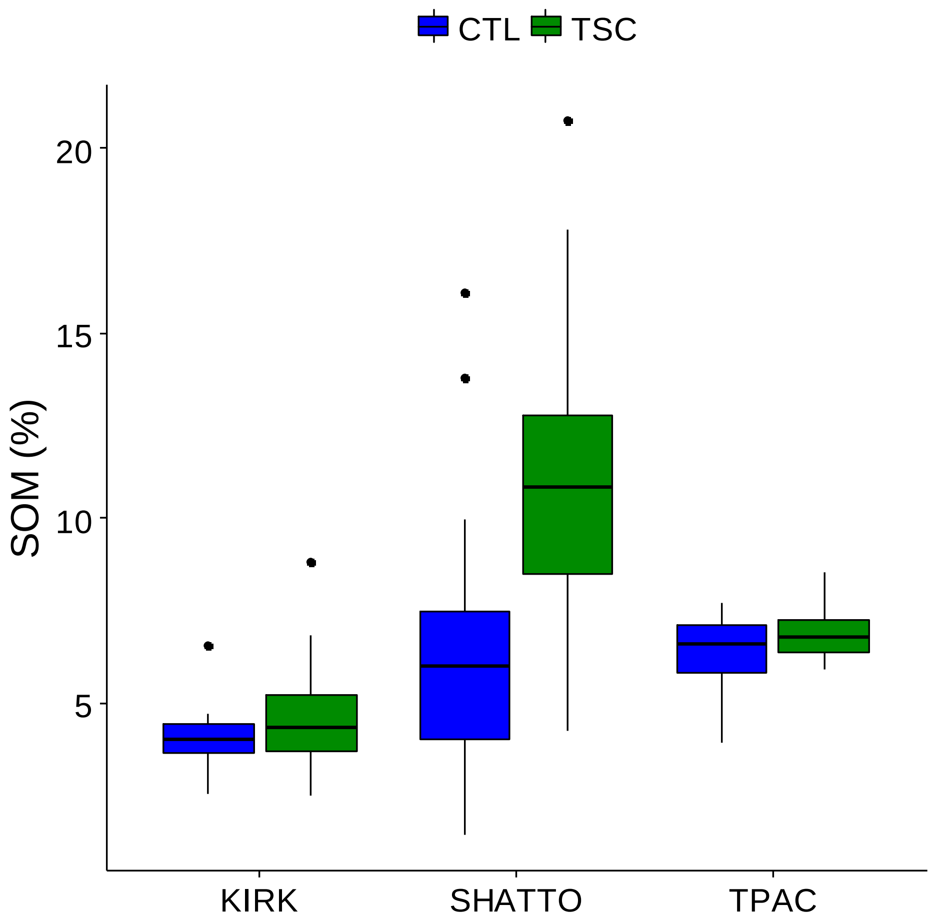

Figure 6 The average percent soil organic matter measured on MIMS soils (SOM_{MIMS}) between the slumped banks of the traditional ditch (CTL; n = 47) and the constructed floodplains of the two-stage channel (TSC; n = 48) for each site. The two-stage channel showed a significant increase in organic matter (two-way ANOVA, $p < 0.001$).

Respiration

The two-stage channel had a significant effect on respiration rates when expressed per dry mass (two-way ANOVA, Reach, p = 0.039) (Figure 7). Rates ranged from 0.36 to 6.28 ug-O/g DM/hr and were significantly higher in the inset floodplains in the two-stage channels compared to the control floodplains in the control reaches (TukeyHSD, p=0.033). Respiration rates were significantly different by site only when expressed per ash-free dry mass (two-way ANOVA, Reach, p = 0.0065). KIRK had significantly higher respiration rates than SHA (Tukey HSD, p = 0.0044), but not TPAC (Tukey HSD, p = 0.21).

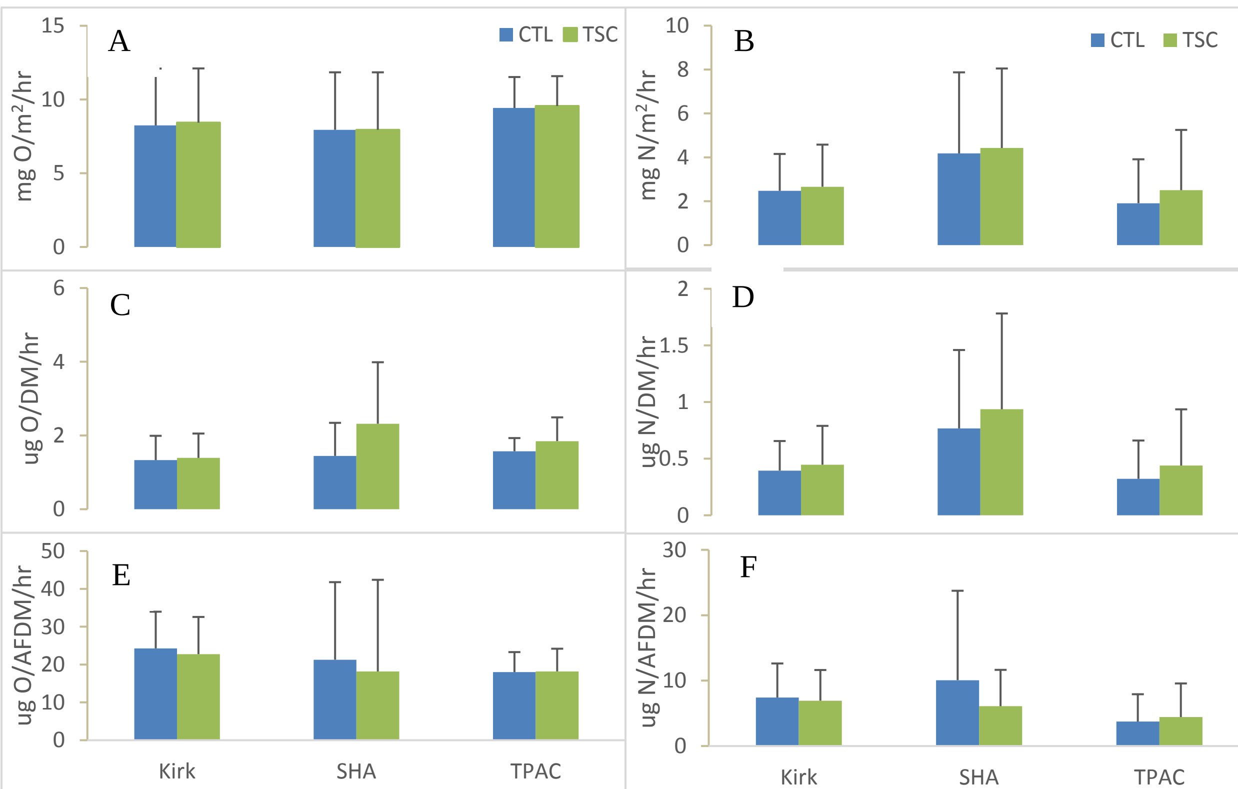

Figure 7. The average respiration (g O_2/hr) and DNF_{MIMS} rates (g N_2/hr; + standard deviation) between the naturally formed floodplains of the traditional ditch (CTL) and the constructed floodplains of the two-stage channel (TSC) for each channel. Rates were expressed per unit area (A,B), per gram dry mass (C,D), and per gram ash-free dry mass (E,F).

Respiration rates varied significantly by season when expressed per unit area (two-way ANOVA, Season, $p < 0.001$) per gram dry mass (two-way ANOVA, Season, $p < 0.001$) and per gram ash-free dry mass (two-way ANOVA, Season, $p = 0.0021$) (Figure 8). Generally, rates were highest in the summer (1.96 ± 1.15 ug-O/g DM/hr) and lowest in the fall (1.07 ± 0.49 ug-O/g DM/hr), however each site varied slightly from this overall pattern, especially when expressed per gram ash-free dry mass (two-way ANOVA, Reach x Season, $p = 0.01265$). When we separated the sites by reach, both the two-stage respiration rates (one-way ANOVA, $p = 0.0022$), and control reach rates varied significantly by season (ANOVA, $p = 0.035$). While both reaches had the lowest rates in fall, the two-stage reach had the highest rates in winter while the control reach had the highest rates in the summer.

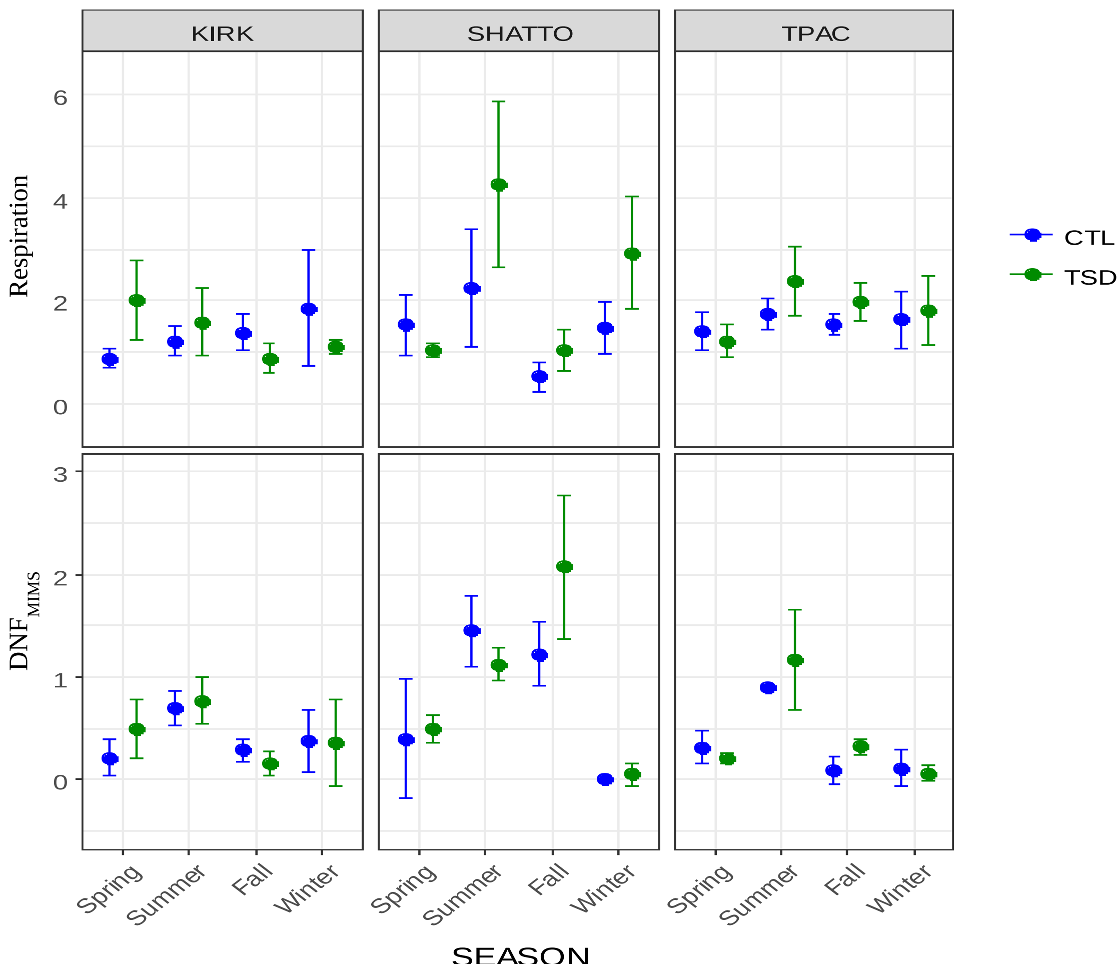

Figure 8 The seasonal average net respiration (ug-O/g DM/hr) and sacrificial microcosm denitrification (ug-N/g DM/hr; ± standard error) rates by reach for each site.

Denitrification

Denitrification rates and patterns varied depending on the method and rate expression. When expressed per unit area, rates measured using the acetylene block method averaged more than six times greater than those measured using the MIMS method. The acetylene block rates ranged from 0.18 to 50.07 mg N/m^2/hr while sacrificial microcosm rates ranged from 0 to 12.11 mg N/m^2/hr.

DNF_{MIMS} varied seasonally with highest rates observed in the warmer periods (summer and fall) and lowest in the winter (Figure 8, Tukey HSD, $p < 0.001$). This pattern was consistent regardless of how the rates were normalized. DNF_{MIMS} also varied significantly by site when expressed per gram dry mass (two-way ANOVA, Site, $p = 0.031$). KIRK and TPAC were the most similar (Tukey HSD, $p = 0.72$), with SHATTO averaging the highest rates (0.87 ± 0.75 ug-N/g DM/hr) and TPAC the lowest (0.41 ± 0.41 ug-N/g DM/hr). Similar site differences were observed when expressed per unit area.

A significant difference between DNF_{AIT} of the two-stage and control floodplains was only observed at the location nearest the channel (0.5 m) expressed per dry mass (two-way ANOVA, Reach, $p = 0.0438$; Figure 9). When expressed per gram dry mass, there was a significant interaction between reach and site at 0.5 m and 1.0 m. For both widths, the highest and lowest rates were found at SHA in the two-stage and control reaches, respectively. At all three widths across floodplain reaches, TPAC had statistically higher rates than SHA when expressed per unit area.

At a width of 1.0 m from the stream edge, DNF_{AIT} differed significantly by season when expressed per unit area (two-way ANOVA, Season, $p = 0.007$) and per gram ash-free dry mass (two-way ANOVA, Season, $p = 0.025$). At a width of 1.5 m from the stream edge, DNF_{AIT} differed significantly by season when expressed per unit area (two-way ANOVA, Season, $p = 0.024$), per gram dry mass (two-way ANOVA, Season, $p = 0.040$) and per gram ash-free dry mass (two-way ANOVA, Season, $p = 0.010$). There was a significant interaction between season and reach at this location, regardless of rate expression. The highest average rates were consistently found in the Summer at the two-stage reach and in the winter at the control reach.

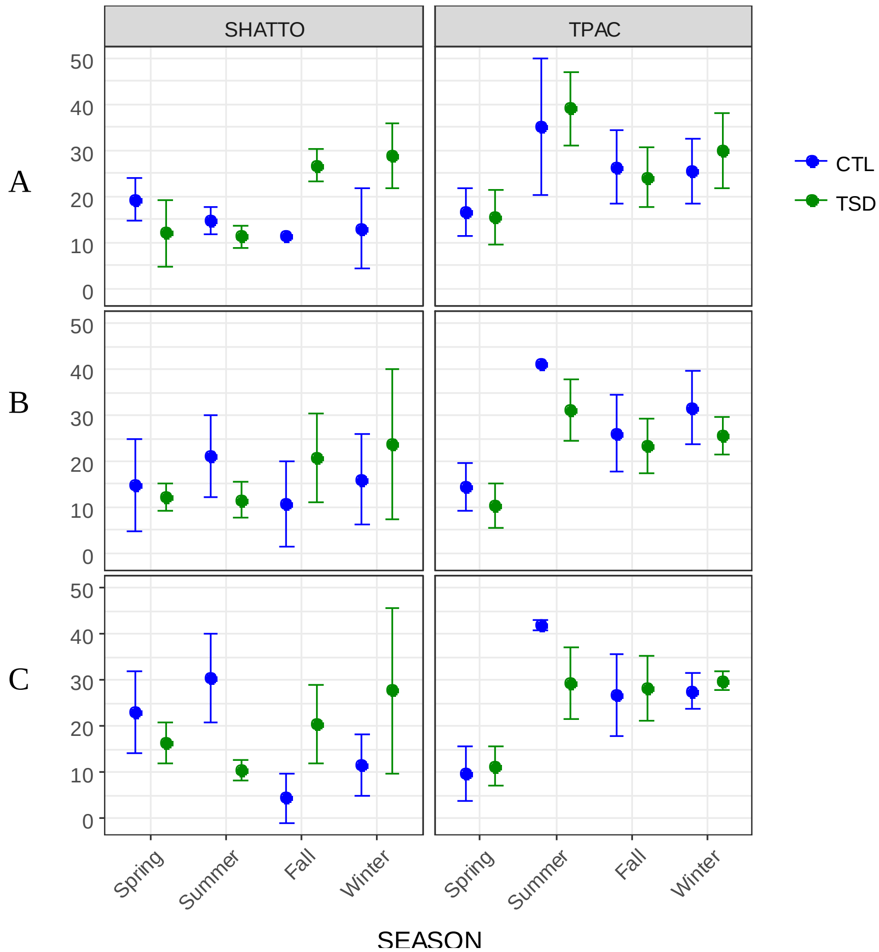

Figure 9 The average denitrification rates per unit area (± standard error) measured through the acetylene inhibition technique at: (A) 0.5 m (two-way ANOVA, Season, $p = 0.37$), (B) 1.0 m (two-way ANOVA, Season, $p = 0.007$) and (C)1.5 m from the channel edge (two-way ANOVA, Season, $p = 0.013$) by reach for SHA and TPAC.

Environmental Drivers of Microbial Processes

Respiration rates per gram dry mass were positively correlated with soil organic matter (ρ = 0.31, p = 0.002) (Figure 11). When separated by reach, a significant correlation was only measured in the control reach (ρ = 0.39, p = 0.007). Respiration rates displayed no observable pattern in relation to soil texture (Figure 12), but a distinct increase in DNF_{MIMS} rates was seen in soils with decreasing clay content (Figure 13). DNF_{MIMS} was not correlated with soil organic matter and was only positively correlated with respiration when both rates were expressed as per ash-free dry mass (ρ = 0.23, p = 0.025). When expressed per unit area, rates were positively correlated with local air temperature (ρ = 0.71, p < 0.001). DNF_{MIMS} was significantly negatively correlated with nitrate when expressed per unit area (ρ = -0.28, p = 0.006), per gram dry mass (ρ = -0.27, p = 0.008), and per gram ash-free dry mass (ρ = -0.22, p = 0.030).

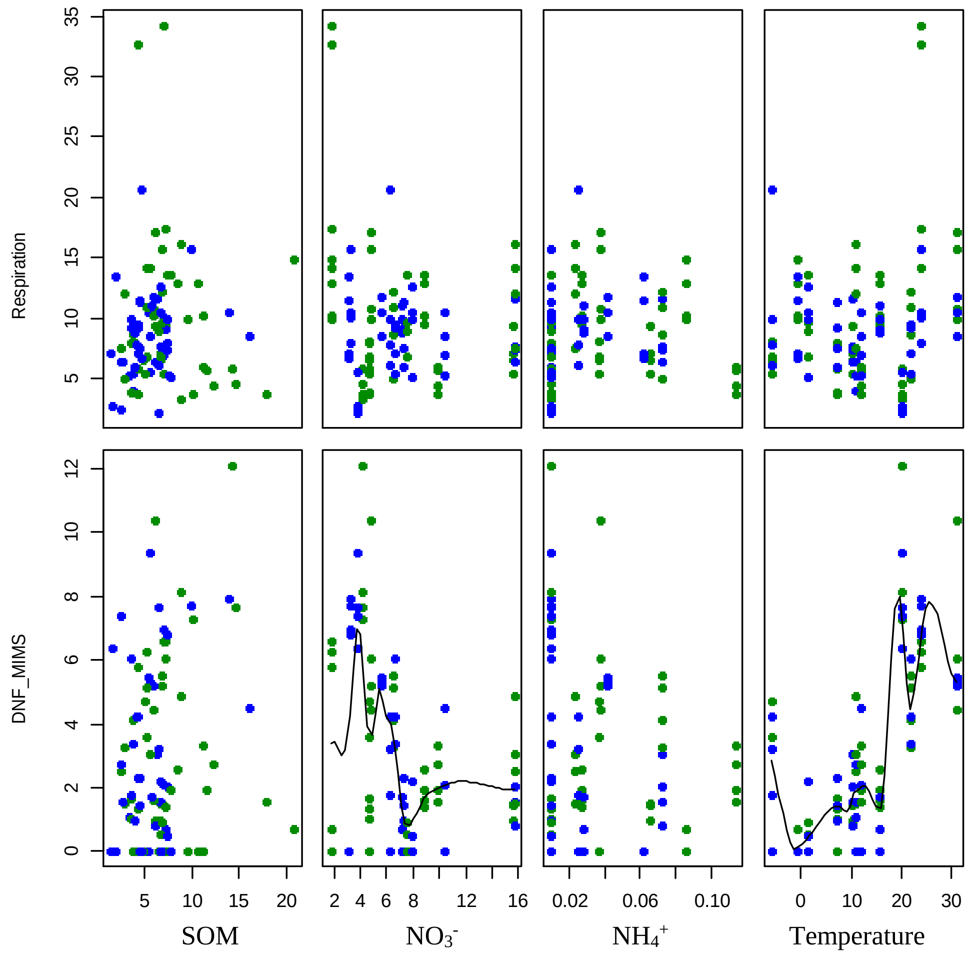

Figure 10 The relationships between net respiration (ug-O/g DM/hr), DNF_{MIMS} (ug-N/g DM/hr), temperature (C°), NO_3^- (mg N/L) and SOM (%) by reach. Respiration rates were positively correlated to soil organic matter ($\rho = 0.33$, $p = 0.001$) and DNF_{MIMS} was negatively correlated with nitrate ($\rho = -0.26$, $p = 0.010$) when outliers were excluded. Most relationships were driven by the two-stage channel. The LOESS regression was used to visualize data trends with a smoothing parameter of 0.25.

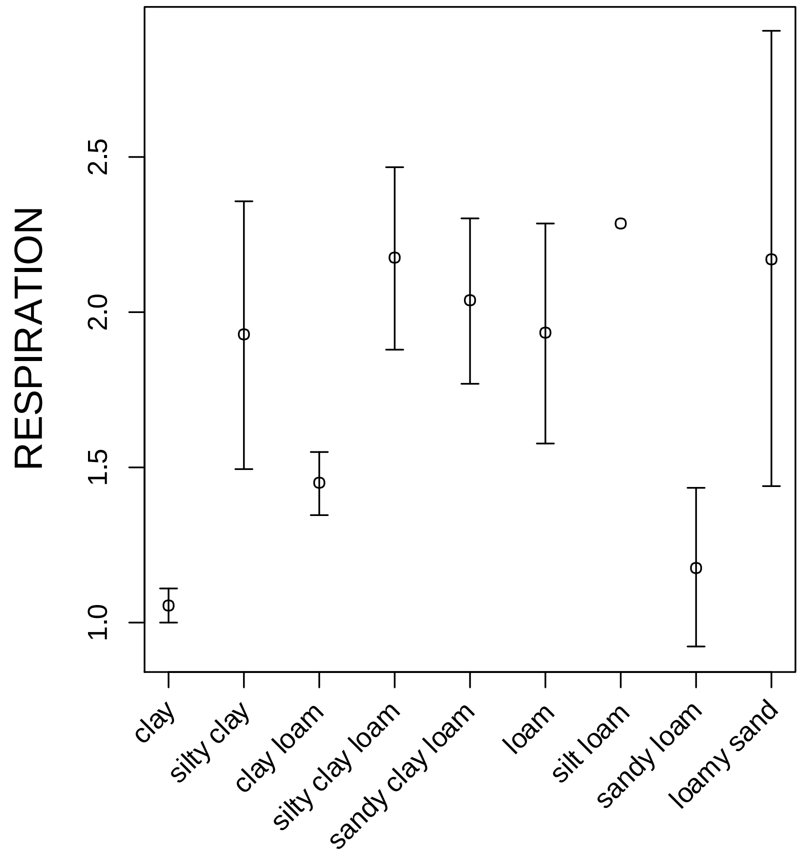

Figure 11 The relationship between floodplain soil texture classified based on the NRCS guide, and the average respiration rate (ug O^2/g DM/hr).. Soil texture is ordered according to decreasing clay content. Vertical bars represent the standard deviation of the mean; open circles without vertical bars represent a single sample classified as that texture.

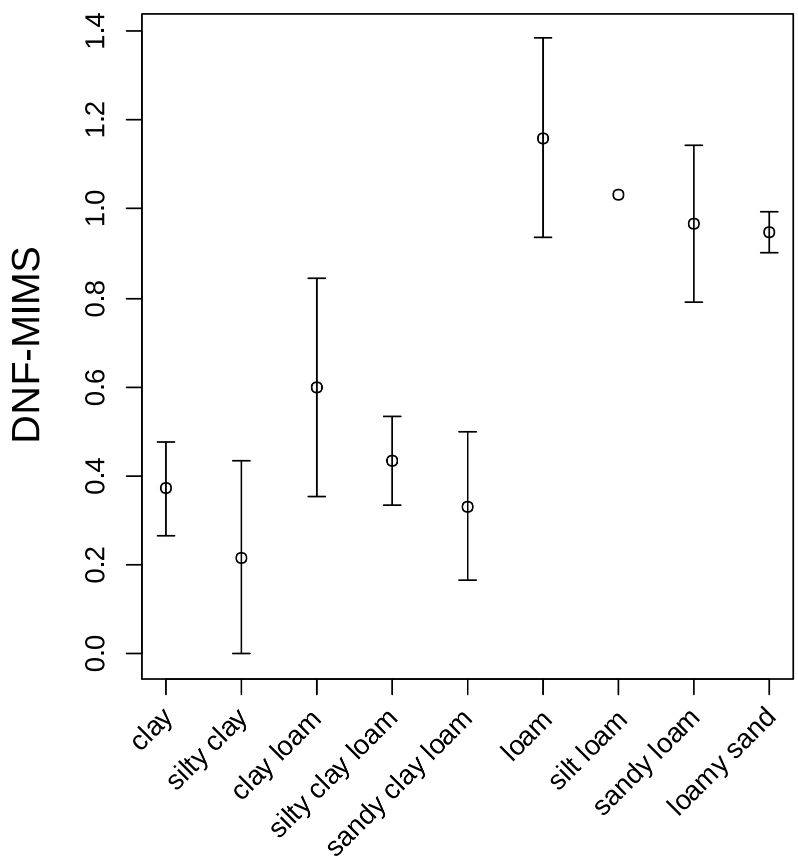

Figure 12 The relationship between floodplain soil texture classified based on the NRCS guide, and the average DNF_{MIMS} (ug N^2/g DM/hr). Soil texture is ordered according to decreasing clay content. Vertical bars represent the standard deviation of the mean; open circles without vertical bars represent a single sample classified as that texture.

A multiple linear regression was calculated to predict respiration rates based on our measured environmental drivers. A significant regression equation was found for rates expressed per unit area ($F(5, 89) = 3.55$, $p = 0.0056$), with an R^2 of 0.12 (Table 2). The predicted rate was equal 2.79 + 0.073 (SOM) + 0.040 (NH_4^+) – 0.24 (NO_3^-) – 0.0017 (SRP) + 0.0077 (Temperature), where SOM was presented as the mass of organic matter over dry mass and Temperature is measured in degrees Celsius. Only NH_4^+ and NO_3^- were significant predictors of these rates, as respiration increased 0.040 mg O^2/m^2/hr and decreased 0.24 mg O^2/m^2/hr for each unit increase in NH_4^+ and NO_3^- concentrations, respectively. A significant regression equation was found for rates expressed per gram dry mass ($F(5, 89) = 6.638$, $p < 0.001$) , with an R^2 of 0.23. The predicted rate was equal 1.88 + 0.39 (SOM) + 0.040 (NH_4^+) – 0.27 (NO_3^-) – 0.0013 (SRP) + 0.0049 (Temperature). Significant predictors were identified as SOM, NH_4^+ and NO_3^-, with respiration increasing 0.39 and 0.40 ug O^2/g DM/hr and decreasing 0.27 ug O^2/g DM/hr for each unit increase in SOM, NH_4^+ and NO_3^- concentrations, respectively.

Table 2 The summary of multiple regression analysis for variables predicting respiration rates expressed per unit area (mg-O^2/m^2/hr) and per gram dry mass (ug-O^2/g-DM/hr). Both equations were statistically significant ($p < 0.05$).

	mg-O^2/m^2/hr			ug-O^2/g-DM/hr		
Variable	*B*	*SE B*	*p-value*	*B*	*SE B*	*p-value*
SOM	0.073	0.14	0.60	0.39	0.13	0.003**
NH_4^+	0.040	0.014	0.004**	0.040	0.013	0.002**
NO_3^-	-0.24	0.10	0.017*	-0.27	0.093	0.004**
SRP	-0.002	0.001	0.17	-0.001	0.001	0.26
Temperature	0.008	0.009	0.37	0.005	0.008	0.54
R^2	0.12			0.23		
F	3.55**			6.64**		

*$p < 0.05$. **$p < 0.01$.

A significant regression equation was also found for DNF_{MIMS} expressed per unit area ($F(5, 89) = 19.77$, $p < 0.001$) , with an R^2 of 0.50 (Table 3). The predicted rate was equal 0.14 + 0.14 (SOM) - 0.032 (NH_4^+) – 0.19 (NO_3^-) – 0.0018 (SRP) + 0.097 (Temperature). Only Temperature was a significant predictor of these rates, as DNF_{MIMS} increased 0.097 mg N^2/m^2/hr for each degree Celsius. A regression equation for DNF_{MIMS} expressed per gram dry mass ($F(5, 89) = 20.28$, $p < 0.001$) , with an R^2 of 0.51, was significant. The predicted rate was equal 0.17 + 0.079 (SOM) - 0.0066 (NH_4^+) – 0.054 (NO_3^-) – 0.0004 (SRP) + 0.022 (Temperature). Once again, only Temperature was a significant predictor of these rates, as DNF_{MIMS} increased 0.022 ug N^2/g DM/hr for each degree Celsius. A significant regression equation was also found for per gram ash-free dry mass rates ($F(5, 89) = 17.09$, $p < 0.001$) , with an R^2 of 0.39. The predicted rate was equal -0.29 - 0.050 (NH_4^+) – 0.096 (NO_3^-) – 0.0014 (SRP) + 0.13 (Temperature). Only

Temperature was a significant predictor of these rates, as DNF_{MIMS} increased 0.13 ug N^2/g AFDM/hr for each degree Celsius.

Table 3 The summary of multiple regression analysis for variables predicting DNF_{MIMS} rates expressed per unit area (mg-N^2/m^2/hr) and per gram dry mass (ug-N^2/g-DM/hr). Both equations were statistically significant (p < 0.05).

	mg-N^2/m^2/hr			ug-N^2/g-DM/hr		
Variable	*B*	*SE B*	*p-value*	*B*	*SE B*	*p-value*
SOM	0.14	0.18	0.44	0.079	0.041	0.059
NH_4^+	-0.032	0.017	0.066	-0.007	0.004	0.11
NO_3^-	-0.19	0.13	0.13	-0.054	0.030	0.070
SRP	-0.002	0.002	0.26	> -0.001	< 0.001	0.34
Temperature	0.097	0.011	< 0.001**	0.022	0.003	< 0.001**
R^2	0.12			0.51		
F	3.55**			20.28**		

$*p < 0.05$. $**p < 0.01$.

Denitrification rates measured through the acetylene inhibition technique at each width were positively correlated with every other width. DNF_{AIT} at each width were also positively correlated with soil organic matter, regardless of rate expression (Figure 14).

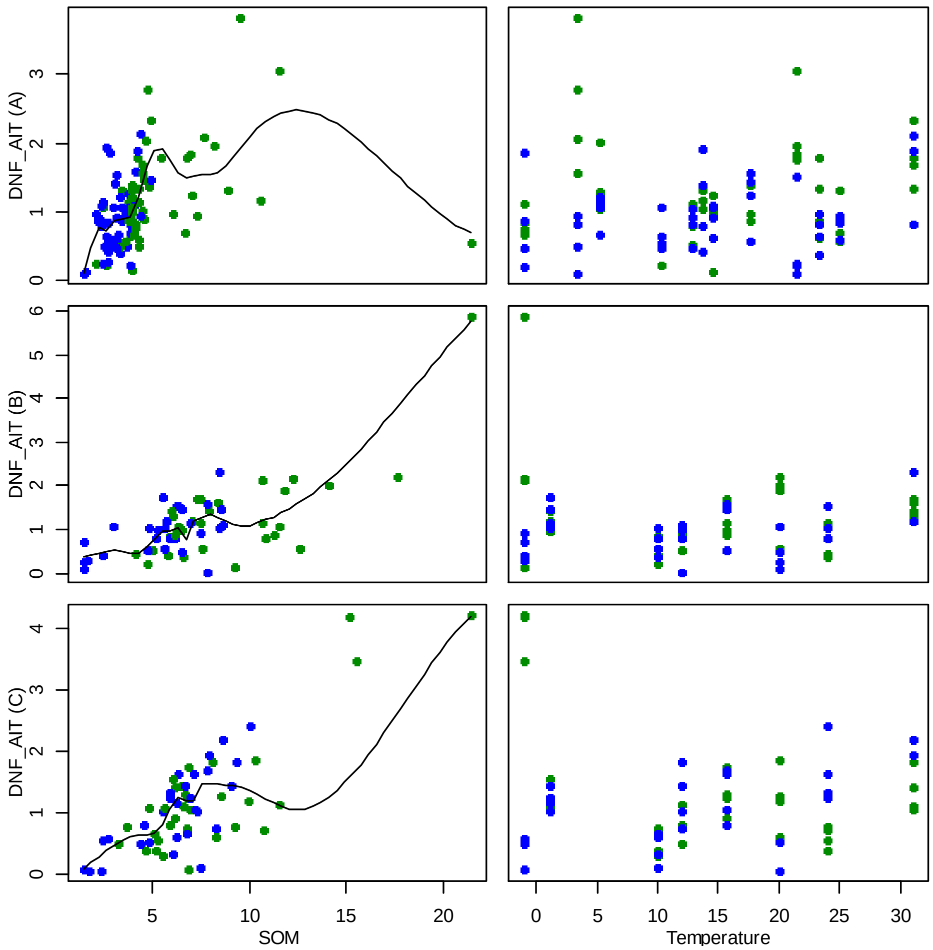

Figure 13 The relationships between DNF$_{AIT}$ (ug-N/g DM/hr), temperature (C^o), and SOM (%) for each distance by reach. At 0.5, 1.0, and 1.5 m from the channel edge (A, B, and C, respectively) rates were positively correlated with soil organic matter. (ρ = 0.40, $p < 0.001$), (ρ = 0.50, $p < 0.001$), (ρ = 0.64, $p < 0.001$). Only the location closest to the channel included measurements from KIRK. Most relationships were driven by the two-stage channel. The LOESS regression was used to visualize data trends with a smoothing parameter of 0.25.

DISCUSSION

Soil and Water Quality

The inset floodplain of the two-stage channel is designed to slow water velocities during storm flows and increase sedimentation (Powell et al., 2007a; Powell and Bouchard, 2010; Krider et al., 2017). Smaller particles are more likely to be transported to the floodplain during flooding (Walling and Moorehead, 1989). The slower velocity of water across the constructed floodplains allows suspended sediments in the overlying water to settle, resulting in finer soil textures. Floodplains that better trap and settle suspended sediments tend to have finer soil textures (Thonon et al., 2007; Davis et al., 2015). Due to this, we expected the two-stage channel reach to contain finer soil material than the control reach. This observation was true for KIRK and TPAC, but not for SHA. While two-stage channels are typically designed according to regional curves (Powell et al., 2007a), the inset floodplain of SHA was designed at the same height as the naturally formed floodplains within the control reach (Roley et al., 2012). Since the floodplains of the control reach are continuous, well vegetated and relatively stable, both reaches appear to experience similar inundation events likely leading to similar erosional and depositional processes.

Floodplains are activated during high flow events (Junk et al., 1989; Tockner et al., 2000). Since our samples were taken at baseflow and the two-stage and control reaches were within close proximity to each other, in-stream nutrient concentrations are representative of watershed conditions. Nutrient concentrations within the two-stage channel tended to be more similar to the control reach and followed the trend of their respective watersheds. The impact of the two-stage channel on nutrient concentrations was therefore expected to be minimal during low flow (Davis et al., 2015; Mahl et al., 2015; Hodaj et al., 2017). We observed no difference in concentrations between the two-stage and control reaches, though they did vary by site.

There was a significant difference in organic matter in the floodplains of two-stage and control reaches, though these differences began to favor the control reach as we moved further away from the channel. We predicted that the two-stage channel would promote the accumulation of organic matter due to increased vegetation, sedimentation and retention times. The inset floodplain of the two-stage channel was also planted following construction, increasing organic matter through biomass growth and accumulation and slowing water velocities to increase sedimentation (Osborne and Kovacic, 1993; Boyer and Groffman, 1996; Hernandez and Mitsch 2007). Increased sedimentation allows suspended materials to settle and accumulate within the floodplain (Powell and Bouchard, 2010) and this effect is likely to be more pronounced immediately adjacent to the stream. Size fractionation of floodplain soils has also been observed with coarse-grained soils nearer the channel and more fine-grained soils further away. Locations further from the agricultural channel are generally accessed only during the highest flow conditions (Thonon et al., 2007; Noe et al., 2013). We did not observe this differentiation likely because the inset floodplains are relatively narrow and engineered to have little topographic variability. Therefore, while the two-stage channel may exhibit an increase in organic matter in the two-stage channel (Powell and Bouchard, 2010; Roley et al., 2012; Mahl et al., 2015), the pattern may not extend laterally along the floodplain (Hanrahan et al., 2018). There was also a difference in organic matter among sites, with SHA containing the highest and KIRK the lowest organic carbon. We hypothesize that a combination of multiple factors, including channel age, floodplain height, bank stability, and vegetation density, contributed to this variability. The two-stage reach at SHA is the oldest of the three channels and was constructed at a lower floodplain height. This likely lead to an accumulation of organic matter and deposited sediments over time due to the more frequent inundation of the channel floodplains (Mahl et al., 2015). The stabilization and vegetation of floodplains formed through deposition of alluvial streamed material may take years to develop (Magner et al., 2012; Hanrahan et al., 2018). Alternatively, slumped banks are likely to have higher organic carbon because upper terrestrial soils simply fall into the channel. If they are vegetative, they often stay intact, however with lower plant diversity (Pedersen and Friberg, 2009; Kristensen et al., 2013; Mahl et al., 2015). We observed this in the slumped bank type floodplains in the control reach at TPAC. Whereas KIRK is the newest site, with less densely vegetated floodplains and soils high in clay content and low organic matter.

Respiration

We observed significantly higher respiration rates in the two-stage channel compared to the control, which is likely a reflection of its positive correlation with soil organic matter. As organic matter increases, labile organic carbon is more likely to be available for microbial processes (Groffman et al., 2005; Welti et al., 2012). The two-stage reach contained greater supply of carbon for microbes to decompose during respiration and a smaller mean particle size which provides greater surface area for microbial colonization. However, the relationship between the two was shown to be more significant in the control reach, which may indicate greater dependence of these microbes on the quantity and quality of carbon. As organic matter decreases, available carbon becomes more critical to microbial functions (Schimel and Weintraub, 2003).

Differences in respiration rates of the two-stage and control reaches were mainly driven by SHA, which contained the highest rates of any channel in its two-stage reach and the lowest in its control. The disparity in organic matter between constructed and naturally formed floodplains at this site, which likely led to the disparity in respiration rates, can be attributed to channel maintenance. The control channel at SHA was dredged and regraded in the late summer/early fall of 2017. When the data was analyzed to include only rates occurring before this event, there was still a statistical difference between respiration rates in the two-stage and control reaches, but only when expressed per unit area. After this event, average organic matter content in the floodplains of the control reach dramatically decreased from 9.29% ± 3.75 to 3.58% ± 1.94. The two-stage channel content remained more consistent (9.56% ± 4.49 to 12.5% ± 3.96). This pattern was also reflected in respiration rates, likely contributing to the disparity between the two-stage channel and conventional channel design.

Higher concentrations of ammonium may also be impacting measured respiration rates. Though differences in concentrations were not significant, ammonium concentrations measured in stream water collected concurrently with seasonal measurements of respiration were higher in the two-stage channel and were positively correlated with measured respiration rates. The positive correlation between soil respiration and ammonium concentrations may point to the presence of nitrifying bacteria, which oxidize ammonium to nitrate (Master et al., 2003). This could result in an observed decrease in dissolved oxygen that may be reflected in our measured respiration rates because the method is using a net oxygen removal rate to calculate respiration. Other studies have also shown ammonium concentrations to be a significant predictor of respiration rates, possibly indicating high rates of decomposition and nitrification (Scott-Denton et al., 2003), though nitrification has been shown to be negligible as conditions approach anoxia (Kremen et al., 2005).

Overall, respiration rates showed significant seasonal trends, with the lowest rates occurring in fall. Although our respiration rates were positively correlated with soil organic matter and ammonium, neither exhibited seasonal trends. Instead, respiration rates may be dependent not just on organic matter quantity, but also its quality (Tank and Webster, 1998; Hoellein et al., 2009). Fall is typically when organic matter accumulates through leaf litter; soil samples were taken after surface litter was gently removed. Only organic matter incorporated within the soil contributed to respiration rates and much of the processed organic matter during fall typically consists of coarse, refractory particles (Fuss and Smock, 1996).

Denitrification

We measured rates within the microcosms of the acetylene inhibition technique that were up to six times higher than those of our sacrificial MIMS microcosms, which was expected because of the difference in methodology. The acetylene inhibition technique optimizes redox conditions by purging microcosms of oxygen and consistently incorporating the nitrate rich stream water in the soil through homogenization via vigorous shaking. This increases the anoxic conditions, resupplies nitrate to soil microbes, and releases gases produced within the soil column throughout the experimental incubation time (approximately 6 hours). This method is particularly useful when comparing across sites because NO_3^- and redox limitations are overcome allowing the variability in these drivers to be eliminated.

The sacrificial MIMS technique more closely approximates in situ rates and is also capable of concurrently measuring net respiration rates via decreasing dissolved oxygen concentrations. Microcosms are not purged of oxygen and, therefore, are more likely to promote aerobic processes. The stream water was only gently mixed with the floodplain soil during the initial composition. During the experiment, microcosms were not shaken, which limited transport to diffusive flux of solutes (e.g., NO_3^-) and gases (e.g., N_2 and O_2) in/out of the soil surface. Both the presence of oxygen and the transport limitations combine to suppress denitrification rates in the MIMS microcosms when compared to slurry-based methods like the acetylene inhibition technique (Hanrahan et al., 2018).

We expected to observe enhanced microbial function in the floodplains of the two-stage channel as compared to the naturally formed floodplains of the conventional channel. When comparing the MIMS denitrification rates, there were no differences between the two types of floodplains nor was it correlated with organic carbon. However, there appeared to be a threshold effect when DNF_{MIMS} was plotted versus soil texture. As particle size increased, we would expected to see a decrease in denitrification rates. Larger particles have less pore space, a lower water holding capacity, and less surface area, limiting microbial populations and their activity (Sexstone et al., 1985; Pinay et al., 2000; Zhang et al., 2016; Mori et al., 2017). However, in this study, clay-rich soils exhibited low rates, while sandier soils had higher rates. We hypothesize that this is likely the result of methodological limitations of diffusional processes limiting the flow of solutes and gases in the MIMS microcosms. Finer textured soils have a lower permeability, limiting the depth at which microbes are supplied with nitrate. As microbes convert nitrate to N_2 in the surface soils, a concentration gradient develops between nitrate in the water column and the soil.

Reddy et al. (1978) showed that denitrification in the soil layer often occurs faster than the diffusive flux supplying nitrate to the soil layer. As microbes deplete the nitrate present in the overlying water, denitrification slows to accommodate the lack of readily available nitrate. Any N_2 produced would also be limited in its release from the soil as gas builds up in the undisturbed soil. Though the control reaches had sandier soils in general, the sandiest soils were overwhelmingly located in the two-stage and control reaches of Shatto Ditch which is representative of the underlying geology of the region (Davis et al., 2015). The sandy soils of this channel combined with the higher organic matter content likely led to the higher measured DNF_{MIMS} rates. Previous studies focusing on this channel have shown denitrification rates to be dependent on organic matter and nitrate concentrations (Roley et al., 2012; Mahl et al., 2015; Hanrahan et al., 2018).

Overall, DNF_{MIMS} rates showed significant seasonal trends, with the highest rates occurring in the warmer months, and the lowest rates in winter. The two-stage and naturalized floodplains had similar trends throughout the year. Warmer environmental conditions provide energy to microbes that allow them to become more active and populations to grow. In the MIMS microcosms, microbial populations and activity may be critical, as rates are limited by redox conditions and solute transport. This is supported by the significant positive correlation between the air temperature and DNF_{MIMS} rates. The negative relationship between denitrification rates and nitrate concentrations within the MIMS microcosms may be reflective of its relationship with temperature. Our data and others show that warmer, dryer months are typically when in-stream nitrate concentrations are the lowest (Neill,1989; Reynolds et al., 1992). During seasons with higher concentrations, complete denitrification may also be inhibited as high nitrate concentrations have been shown to inhibit the conversion of N_2O to N_2 and the method measures dissolve N_2 thereby potentially underestimating rates under high NO_3^- conditions (Weier et al., 1993). However, within the MIMS microcosms, solute transport limitations would minimize the influence of nitrate concentrations, leading to an increase in denitrification rates as a result of warmer temperatures in spite of the lower nitrate concentrations. This is reflected in our multiple regression analysis, which resulted in temperature as a much stronger factor in predicting DNF_{MIMS} rates than nitrate concentration.

The acetylene inhibition technique also showed no difference in denitrification rates between the two-stage and control reaches, except at locations nearest the stream channel (0.5 m) expressed per gram dry mass. This is likely a reflection of soil organic matter which was positively correlated with DNF_{AIT}. As organic matter increases, labile organic carbon is more likely to be available for microbial processes (Groffman et al., 2005; Welti et al., 2012). However, since differences organic matter concentrations between the two-stage and control reaches became less distinct with increasing distance from the channel, DNF_{AIT} rates followed a similar pattern. Organic matter is a well known control of denitrification rates in agricultural channels (Welti et al., 2012; Roley et al., 2012), but soil texture may also be important. As the soil organic matter and moisture contents increase, labile nutrients become more available to microbial populations. This allows them to grow more quickly and become more active (Sexstone et al., 1985; Letey et al., 1980). In soils with fine textures, the amount of surface area is increased, allowing these soils to better support larger microbial populations (Pinay et al., 2000; Mori et al., 2017). This supported by our observation of larger DNF_{AIT} rates measured in the silty soils of TPAC as compared to the sandy soils of SHA.

Additionally, there was a decrease in DNF_{AIT} in the conventional channel reach of SHATTO after the summer. The channel was dredged and regraded in the late summer/early fall of 2017 in the conventional channel reach of SHATTO. Soils that are in high disturbance areas have been shown to contain less microbial activity due to the instability and lack of readily available nutrients (Rey et al., 2011; Hladký et al., 2016). Microbes rely on carbon as an electron donor for aerobic and anaerobic respiration and soil organic matter is a major source of carbon in agricultural channels (Groffman et al., 2005; Hanrahan et al., 2018). When the channel was regraded, live vegetation, accumulated organic matter, and a portion of the soil surface was stripped away. Though rates are not typically limited by carbon in these systems (Roley et al., 2012; Mahl et al., 2015), microbes likely had to adjust to the reduced carbon supply as they competed for the limited resource. Microbial populations were also likely removed with the upper soil layer during regrading. The removal of these microbes may have been especially crucial considering the sandy soils of this channel. Sandier soils have less surface area for microbial populations to inhabit and larger pores that drain faster and reduce the residence time of nutrient rich water (Sexstone et al., 1985). After this event, soils became sandier and floodplains soils were more exposed due to the removal vegetation. This likely resulted in the decrease in denitrification rates after the construction.

Significant seasonal trends were measured in DNF_{AIT} rates, with the trends strengthening with lateral distance from the channel. The lower moisture content of the soils further from the channel may have led to a greater response to seasonal variations in weather. As the soil organic matter and moisture contents increase, labile nutrients become more available to microbial populations. This allows them to grow more quickly and become more active (Sexstone et al., 1985; Letey et al., 1980). The moisture in the soil also regulates soil temperatures, causing soils with more moisture, i.e. closer to the channel, to be less influenced by temperature fluctuations (Al-Kayssi et al., 1990).

CONCLUSION

Overall, our results showed some interesting differences between the floodplains of the two-stage and control reaches. In general, the two-stage contained a coarser soil material, higher organic matter content, and higher respiration rates. Denitrification rates were only higher in the two-stage at locations closest to the channel. Though respiration rates and denitrification rates were measured using the same method (Sacrificial Microcosms), they did not exhibit the same seasonal patterns or correlations with organic matter. This was likely due to transport limitations within our experimental set-up, as observed in the relationships between these rates and soil texture.

The objective of this research was to determine if the floodplains of two-stage channels provide a benefit in carbon and nitrogen cycling over naturally formed floodplains within agricultural channels. The two-stage reach significantly increased respiration rates and organic matter content but had no impact on denitrification rates. However, the increased surface area and carbon availability of the inset floodplain may increase the reach-scale floodplain function. Over time, the naturally formed floodplains will likely approach the floodplain function of the two-stage channel as they build up soil organic matter and become more stable. Agricultural channels containing clay rich soils may have more difficulty forming natural floodplains, as soils with a smaller particle size tend to form more stable aggregates (Idowu, 2003). The high clay content present in these channels may also limit the ability of microbes to denitrify NO_3^-. For these channels, the construction of the inset floodplain may prove the most beneficial in nutrient retention.

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