QUANTIFICATION OF IN-STREAM AND RIPARIAN DENITRIFICATION POTENTIAL AND ENVIRONMENTAL DRIVERS OF DENITRIFICATION FOLLOWING AGRICULTURAL STREAM RESTORATION IN THE PIEDMONT REGION OF NORTH CAROLINA

by

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ABSTRACT

MOLLY KATHERINE WELSH. Quantification of in-stream and riparian denitrification potential and environmental drivers of denitrification following agricultural stream restoration in the Piedmont region of North Carolina. (Under the direction of DR. SARA MCMILLAN)

Agricultural streams are subject to considerable disturbance including channelization, erosion, and sedimentation. Fertilizer is often applied to agricultural fields in excess of crop demand, and nitrogen (N) and phosphorus (P) can be transported to streams. When contaminated water reaches nutrient-sensitive aquatic areas, excess N and P cause eutrophication and often result in algal blooms, anoxia, and fish kills. Stream restoration has been performed to improve channel structure but also aims to improve multiple stream functions, including water quality. Through this research, I sought to characterize the impact of stream restoration on N removal via denitrification.

Denitrification is a desirable removal process for N in aquatic ecosystems because it transforms a biologically active form of N (NO_3^-) into gaseous forms (N_2O and N_2) which are returned to the atmosphere. Denitrification enzyme activity (DEA) was measured seasonally in riparian soil and floodplain and stream sediments in agricultural restored and unrestored stream reaches using the acetylene-block method.

Across all sites, DEA was significantly higher in floodplain and riparian zones than in-stream ($p < 0.001$) and dormant season DEA was significantly higher than growing season DEA ($p < 0.001$). These results highlight the importance of soil texture in stream sediments and percent moisture and organic carbon in riparian and floodplain areas in controlling DEA. This study also illustrates the importance of stream-floodplain connectivity and riparian buffers in improving water quality.

DEDICATION

This thesis is dedicated to my parents, Gail Martin and David Welsh, for their undying positivity and encouragement.

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CHAPTER 1: INTRODUCTION

1.1 Background and Study Rationale

Agricultural streams are subject to considerable disturbance including channelization, erosion, and sedimentation. Additionally, fertilizer is often applied to agricultural fields in excess of crop demand, and nitrogen (N) and phosphorus (P) can be transported to streams, particularly during storm events. When contaminated surface water reaches nutrient-sensitive aquatic areas, excess N and P can cause eutrophication, resulting in algal blooms, anoxia, and fish kills (Vitousek et al. 1997). Stream restoration practices such as Natural Channel Design (NCD) (Rosgen 2007) aim to mitigate physical impacts of agriculture via the installation of cross vanes, channel-spanning boulder structures, and re-vegetation of the riparian zone. Since in-stream and bank stabilization restoration approaches are generally designed for grade control, erosion control, and reduction of sediment transport, the effect of these restoration approaches on water quality via nutrient removal is unknown.

Therefore, at the watershed scale, it is important to gain a greater understanding of specific environmental factors that dictate the transport and fate of N in agricultural areas and quantify N removal mechanisms (Herrman et al. 2008, Alexander et al. 2009). Previous studies have shown that nitrate removal processes taking place in low-order streams may remove up to 50% of nitrate (NO_3^-) from agricultural runoff before it reaches coastal waters (Craig et al. 2008). Currently, multiple strategies exist that address nutrient retention (e.g., field-scale best management practices such as cover crops, no-till planting, and constructed wetlands) and stream restoration is one of many common strategies for reduction of NO_3^- loading via enhancing the denitrification process (Craig et al. 2008). Natural channel design is a stream restoration approach that aims to restore the fluvial geomorphology of a stream but is generally implemented with the broad goals of restoring ecological function but not specifically addressing nutrient removal. A cross vane is a dam-like grade-control structure that spans the active stream channel and is implemented to decrease near-bank shear stress, velocity, and stream power, shifting maximum flow velocity to the center of the channel (Rosgen 2007, Gordon et al. 2013, Zhou and Endreny 2013).

Gaseous dinitrogen (N_2) in the atmosphere can be converted to ammonia (NH_3) or other forms of reactive atmospheric gases via an energy-intensive process, nitrogen fixation. N is naturally fixed via lightning or via bacteria with an N-fixing enzyme. However, gaseous N is also fixed to soluble inorganic forms (ammonium, NH_4^+) via industrial processes and applied to cropland via fertilizers. Nitrification involves the oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) and finally to nitrate (NO_3^-) via chemoautotrophic bacteria. NO_3^- is the dominant product of nitrification reactions, as NO_2^- is highly chemically and biologically reactive. Nitrification is typically completed in oxygen-rich environments and often occurs in the field in agricultural operations. Plants will readily uptake NH_4^+ or NO_3^-, a process termed immobilization.

Denitrification is a permanent removal process for N in aquatic ecosystems because it transforms a biologically active form of N into gaseous forms (N_2O and N_2) which are lost to the atmosphere. Plant uptake is another removal process for N, however it results in N being returned to the system upon plant decomposition. Denitrification is an anoxic oxidation-reduction process wherein NO_3^- is converted to nitrous oxide (N_2O) and dinitrogen gas (N_2) via the transfer of electrons (Groffman et al. 2006, Beaulieu et al. 2011). This process requires organic carbon, which serves as an electron donor as it is oxidized (Zumft et al. 1997) and is also a food source for denitrifying bacteria. Carbon also increases heterotrophic respiration, promoting anoxia (Craig et al. 2008). NO_3^-, the electron acceptor, becomes reduced to N_2O or N_2 (Zumft et al. 1997). Though denitrification effectively reduces in-stream NO_3^- concentrations, N_2O, a by-product of denitrification, is a potent greenhouse gas and contributes to stratospheric ozone depletion. Studies have shown there is a positive relationship between N_2O emissions and stream NO_3^- concentrations (Baulch et al. 2011, Beaulieu et al. 2011).

The denitrification process, facilitated by facultative heterotrophic bacteria, generally occurs as NO_3^- moves through anoxic riparian soils or floodplain and stream sediments (Seitzinger et al. 1988, Kellman et al. 1998). The riparian zone is defined as the vegetated land adjacent to a stream that serves to facilitate transport of water and dissolved solutes between terrestrial and aquatic ecosystems via overland, subsurface, and deep hydrologic flowpaths (Dahm et al. 1998, Dosskey et al. 2010, Vidon et al. 2010). The riparian zone plays an important role in in moderating water and chemical exchange between precipitation and storm water runoff, groundwater, and stream water (Dosskey et al. 2010). Though riparian zones are a small part of the landscape, they exert a disproportionate influence on water and solute fluxes to streams (Gold et al. 2001). Intersecting hydrologic flowpaths within the riparian zone produce dynamic moisture and biogeochemical conditions, allowing riparian zones to function as "hot spots" for biogeochemical cycling (Vidon et al. 2010). "Hot spots" are areas with disproportionately high denitrification rates in relation to the surrounding area (McClain et al. 2003). The top meter of riparian soil has high root density of vegetation, organic-rich soils, and high microbial activity, leading to both denitrification and plant uptake (Gold et al. 2001). Accordingly, Cooper et al. (1990) found that riparian soils only comprised 12 percent of a stream's border but were responsible for 56 to 100 percent of NO_3^- loss.

Hydrologic connectivity of a stream to its floodplain and riparian zone is important, as it can enhance delivery of nitrate to floodplain and riparian areas rich in organic matter to stimulate denitrification (Wolf et al. 2013). The hyporheic zone is an area of surface water-groundwater mixing beneath and adjacent to the stream that often extends into floodplain sediments (Hester and Gooseff 2010). Hyporheic zones may bring high dissolved organic carbon concentrations in surface water in contact with high nitrate concentrations from subsurface water and groundwater, facilitating denitrification. Hydraulic conductivity of hyporheic zones can be important; though low hydraulic conductivity increases residence times, it can also restrict the amount of groundwater carrying nutrients that can interact with sediments and be removed from groundwater (Devito et al. 2000). Disconnection of the stream from the riparian zones, which occurs during channelization due to urbanization or agricultural activities, leads to decreased nutrient transformation and essentially allows the stream to act as a pipe for rapid surface water transport (Dahm et al. 1998). In an analysis of various stream types, Dahm et al. (1998) found that channelized, canalized, constrained, and disconnected streams have the lowest potential for nutrient retention.

Denitrification can also occur in stream sediments. Duff et al. (2008) assert that hydrologic and geomorphic channel characteristics exert considerable control on N transport and retention, and understanding hydrologic and physical constraints for biological N processing in low order agricultural streams is crucial. Important hydrologic controls on NO_3^- removal include hydraulic resistance, water residence time, nutrient load, and percent of N load delivered in high flow versus baseflow conditions (Hanson et al. 1994, Craig et al. 2008). Different geomorphic features have differing oxygen availability, water retention times, and available carbon, all important factors for denitrification (Opdyke et al. 2007). For example, riffles, areas of faster-flowing, oxygenated water, can favor nitrification, the conversion of ammonium to nitrate in the presence of oxygen. Pools, which are areas of ponded, low-velocity water, should promote denitrification due to high retention times, which increase contact time between nitrate in water and denitrifying microbial communities in the anoxic sediment. Due to the low water velocities in pools, fine sediment tends to settle out into these areas, providing favorable areas for enhanced microbial colonization. Further, pools may be potential “hot spots” of denitrification due to high organic matter content (Hill et al. 1988, Craig et al. 2008). Channelization, common in agricultural streams, destroys in-stream geomorphic complexity, such as the presence of pools, riffles, and point bars.

Nitrate processing can also be affected by sediment size on the streambed. Fine streambed sediment, such as clay and silt, has been demonstrated to promote higher rates of denitrification than coarse sediment, such as sand (Cooke and White 1987, Smith et al. 2009). Smaller pore size in fine-textured soils may be conducive to denitrification due to facilitating greater opportunity to create anaerobic conditions (Groffman and Tiejde 1989).

In-stream denitrification and microbial assimilation may also be limited by carbon availability (Weier et al. 1993, Craig et al. 2008). Additionally, high benthic organic carbon supply may stimulate elevated denitrification rates in the presence of excess NO_3^- (Arango et al. 2007). Various studies have observed that water-soluble carbon may promote denitrification in stream sediment (Hill and Sanmugadas 1985, Cooke and White 1987, Goodale et al. 2005). The carbon to nitrogen ratio of stream sediments, an indicator of C quality, has also been correlated to denitrification (Garcia-Ruiz et al. 1998). However, these results are not equivocal with many studies demonstrating little effect of carbon quality and quantity on denitrification rates (Herrman et al. 2008, Craig et al. 2008).

There is much spatial and temporal heterogeneity in stream denitrification potential (Kemp and Dodds 2002, Wall et al. 2005, Pina-Ochoa and Alvarez-Cobelas 2006, Bohlke et al. 2009). Temporally, there can be seasonal variation in NO_3^- loads and carbon inputs, as well as flood-related drying/rewetting and freeze/thaw related changes in channel geomorphic complexity and microbial community (Bohlke et al. 2009, Groffman et al. 2009). Spatially, local stream morphology and differing sediment characteristics can lead to variation in denitrification rates (Bohlke et al. 2009).

Denitrification is important in agricultural stream ecosystems as it represents permanent nitrate removal from the system and can prevent nutrient saturation and subsequent eutrophication in downstream areas. However, denitrification is a challenging process to measure, as the end product is N_2 gas, which comprises 79% of the earth's atmosphere. Denitrification enzyme activity (DEA) assays using the acetylene (C_2H_2)-block method (Smith and Tiedje 1979), which inhibits the conversion of N_2O to N_2 gas, have been developed as a way to quantify the denitrification potential of stream sediments and riparian soils. Because N_2O gas has a low atmospheric concentration and highly sensitive detectors are available, N_2O concentration can be measured at various time points to derive a denitrification rate (Christensen et al. 1989, Groffman et al. 2006). The acetylene-block method on a sealed sediment/soil core has found widespread use as it is relatively simple, easy to perform, and allows for processing large numbers of samples to fully capture spatial and temporal variation in denitrification rates (Groffman et al. 2006). Denitrification enzyme activity assays can be performed with ambient stream water or water amended with nitrate and glucose when examining relative activity of the microbial communities under ideal non-limiting conditions. However, a weakness of this assay method is the possibility of incomplete inhibition of N_2O reduction as well as inhibition of nitrification occurring during these experiments, leading to concern over whether the measured denitrification potential rate reflects the true rate of denitrification (Groffman et al. 2006).

Nevertheless, the acetylene inhibition method is a commonly accepted method and can be useful in comparing results of this study to other agricultural streams and various riparian settings. Though there are limitations to the DEA methods (e.g., actual denitrification rates are not derived in-situ), it is a useful method in areas where high nitrate concentrations may be expected (e.g., agricultural areas) and for determining environmental drivers of denitrification. In this study, DEA assays were determined to be a useful method of comparing stream sediments to floodplain sediments and riparian soils to gain an idea of the denitrification potential of a stream along lateral (stream-floodplain-riparian zone) and longitudinal (various stream features along the reach) gradients as well a means of providing direct comparison of streams to riparian zones.

The interface between streams and riparian zones is dynamic. Previous studies have examined either the stream or the riparian zone alone as important for biogeochemical cycles, but a comprehensive, holistic view of the biogeochemical functioning as a landscape transitions from riparian zone to floodplain to stream is needed. This study aims to examine nitrogen removal via denitrification along the stream-floodplain-riparian interface and identify specific drivers of denitrification in North Carolina Piedmont streams as a case study to help guide future restoration approaches.

1.2 Objectives

The objectives of this study are to: (1) quantify denitrification enzyme activity along a gradient from stream to riparian zone in four different stream types in North Carolina and compare differences in denitrification potential between sites and seasons (2) identify the driving environmental factors that promote/predict denitrification at these sites and (3) compare results from this study to other denitrification enzyme activity studies in various ecosystem types.

1.3 Research Questions

This study aims to investigate spatial and temporal variation in denitrification potential along a stream-floodplain-riparian continuum. Specifically, the following research questions will be addressed:

1. What are the differences in DEA among four site types and seasons?

2. Do differences exist in DEA among in-stream features (e.g. pool, riffle, run, point bar) and what is the role of geomorphic complexity in promoting in-stream denitrification?
3. Does distance from the agricultural field edge influence DEA?
4. What are the environmental drivers of denitrification at our North Carolina Piedmont streams, which have lower nitrate concentrations than other Midwestern agricultural streams and are restored via NCD (rather than a two-stage ditch approach)?
5. Does NCD have the added benefit of promoting denitrification?

1.4 Hypotheses

The hypotheses of this study are structured around four main research areas and are as follows:

1. *Denitrification potential along the stream-floodplain-riparian continuum*

   Null hypothesis: There will be no differences in DEA between riparian zone soil, floodplain sediments, and in-stream sediments.

   Alternative hypothesis: DEA will be higher in floodplain sediments and riparian zone soils than in the stream channel sediments at all sites because floodplain and riparian areas may consist of sediments and soils high in organic matter due to the presence of vegetation, while stream sediment often contains sand and rock fragments and lower percent organic matter.
2. *DEA in restored versus unrestored streams*

   Null hypothesis: DEA will not be different in restored versus unrestored agricultural streams.

Alternative hypothesis: Restored agricultural streams will have higher DEA than unrestored agricultural streams. Stream restoration may promote denitrification via installation of cross vane structures, which lead to riffle-pool-run sequences, leading to coupled in-stream nitrification-denitrification as N passes through oxic riffles and anoxic pools with high retention times.

3. *Drivers of denitrification in North Carolina Piedmont streams*

   Null hypotheses: DEA will not be significantly different between various stream feature types. Differences in nitrate concentrations and percent organic matter will not lead to differences in DEA.

   Alternative hypotheses: In terms of in-stream environmental drivers of denitrification, nitrate concentration of stream water, organic matter content of sediments, and geomorphology will drive differences in DEA. In-stream DEA will be highest in pools, due to anoxia, presence of organic matter, and higher water residence times.

4. *Drivers of denitrification in North Carolina Piedmont floodplains and riparian zones*

   Null hypothesis: DEA will not be significantly different throughout the floodplain and riparian zone based on organic content and nitrate concentration.

   Alternative hypothesis: Organic content and contact with nitrate may also drive DEA in the floodplain and riparian zone. Riparian DEA will be highest closest to the field edge due to high concentrations of nitrate in runoff that has not had increased contact time with the riparian zones.

CHAPTER 2: MATERIALS AND METHODS

2.1 Site Descriptions

Study sites consisted of four low-order stream types (restored agricultural, unrestored agricultural, unrestored agricultural plus forested buffer, forested reference) in the Piedmont region of North Carolina. These streams are located in the upper Yadkin River Basin in Surry County, North Carolina (Figure 1). The upper Yadkin River Basin is 2,069,760 acres and it contains 108 local watersheds (NCEEP 2009).

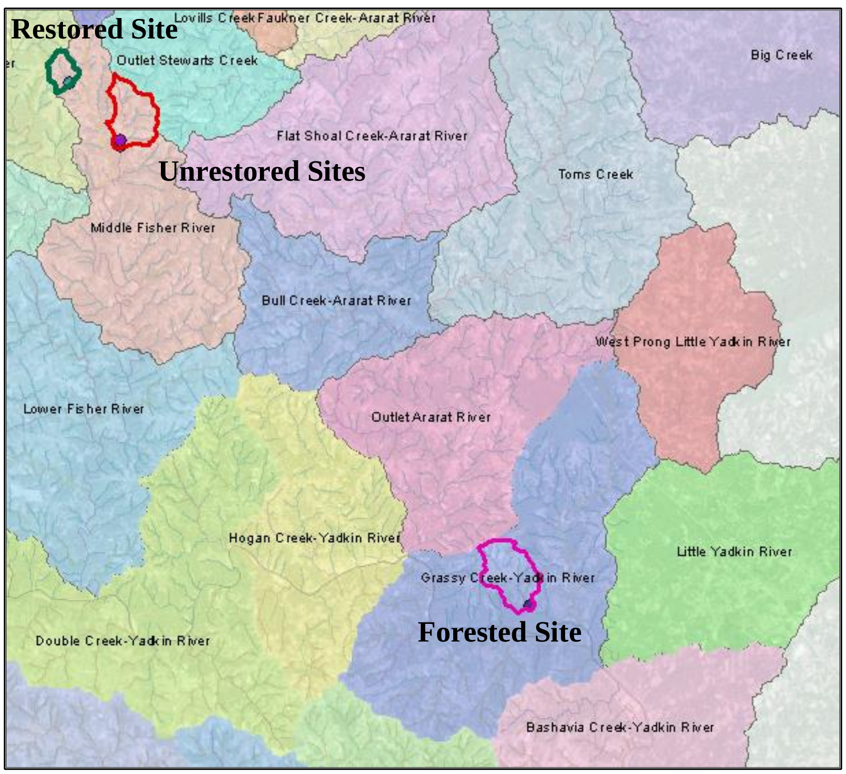


Figure 1: Map of field site drainage areas and surrounding watersheds in the Upper Yadkin River Basin. The restored and unrestored field sites are located in the Middle Fisher River Watershed and the forested site is located in the Grassy Creek-Horne Creek Watershed.

The restored and unrestored sites are located in the same watershed, the Middle Fisher River Watershed (Figure 1), which is 17,920 acres and is comprised of 1.3% impervious cover, 39.6% agricultural area, and 51% forest and wetlands (NCEEP 2009, Figure 2a). The restored site is located on a first-order tributary of Cook's Creek (Figure 3a). The restored site has a drainage area of 352 acres (15,339,770 square feet) (Figure 3a).

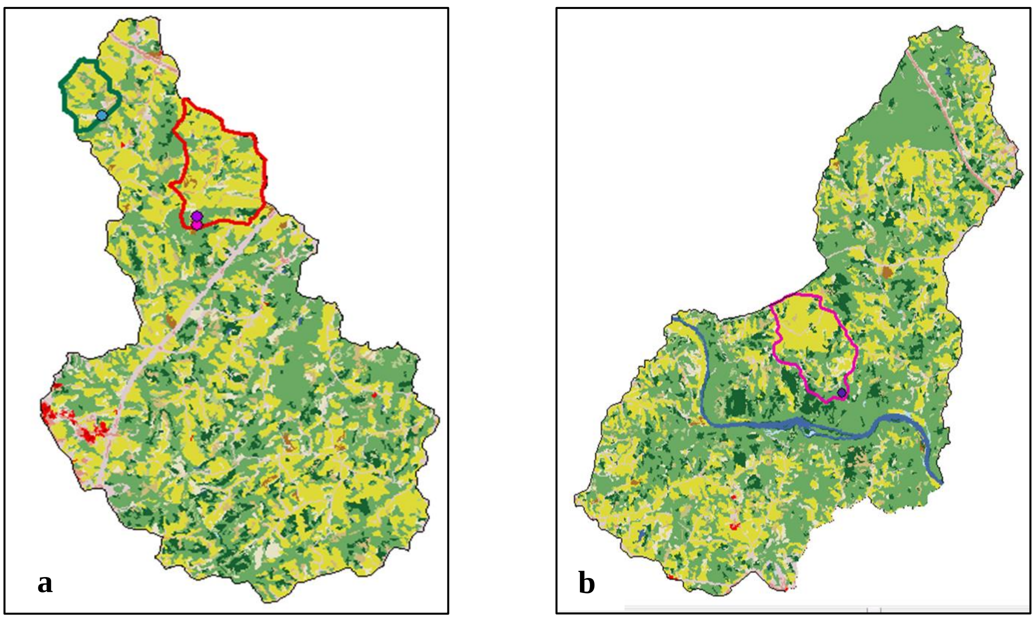
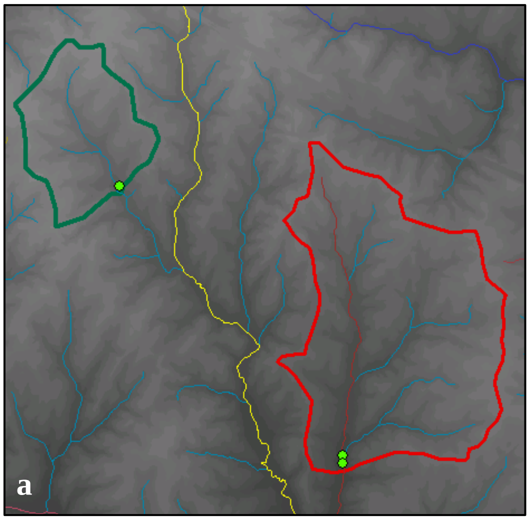


Figure 2: Watershed land use for: a) Middle Fisher River Watershed with sites represented by circles (light blue, restored, within the green drainage area and purple, unrestored, and pink, unrestored + buffer within the red drainage area) and b) Grassy Creek-Horne Creek Watershed with the forested site represented by the dark blue circle within the pink watershed. Land use key: Yellow indicates crop/pasture, light green indicates deciduous forest and wetlands, dark green indicates evergreen forest, and red indicates development.



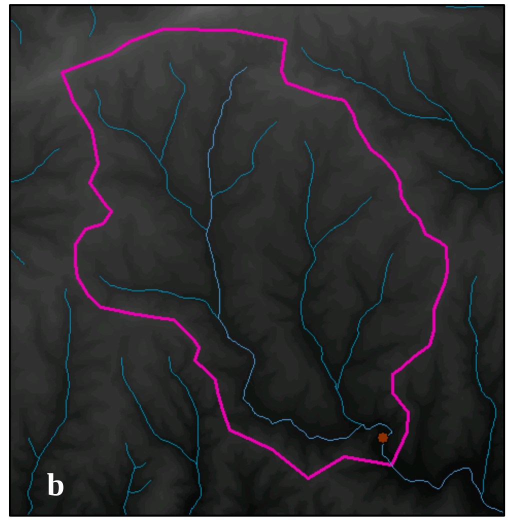


Figure 3: Drainage areas for each of the field sites. a) The restored site drainage area is to the left, outlined in green. The unrestored site drainage area is outlined in bright red and contains Jackson Creek (burgundy). Cook's Creek (yellow) runs between both drainage areas before joining Jackson Creek. b) The forested site drainage area is marked in pink.

The unrestored sites are located on a first-order reach of Jackson Creek and these sites have a drainage area of 1,094 acres (47,674,340 square feet) (Figure 3a). Jackson Creek joins Cook's Creek downstream of the restored and unrestored field sites (Figure 3a) before Cook's creek joins the Fisher River.

The forested reference site is located in Pilot Mountain State Park on Horne Creek, which is located in the Grassy Creek-Horne Creek Watershed. The Grassy Creek-Horne Creek Watershed is 24,640 acres and contains 0.6% impervious cover, 30.8% agricultural area, and 61.2% forest and wetlands (NCEEP 2009, Figure 2b). The Grassy Creek-Horne Creek Watershed is a Wildlife Resources Commission priority area for aquatic habitat (NCEEP 2009). The drainage area of the forested reference site is 1,242 acres (54,119,219 square feet) (Figure 3b).

The restored field site was reconstructed in 2012 using a Natural Channel Design approach, consisting of boulder cross vane structures creating riffle-pool-run sequences, floodplain reconnection, and riparian re-vegetation (Figures 4a and b). The riparian zone was planted with native herbaceous vegetation, including grasses and sedges, and the stream runs through a deciduous buffer strip upstream of the restored reach (Figures 5a and 6a). The agricultural fields are planted with a rotation of soybeans, tobacco, and corn.

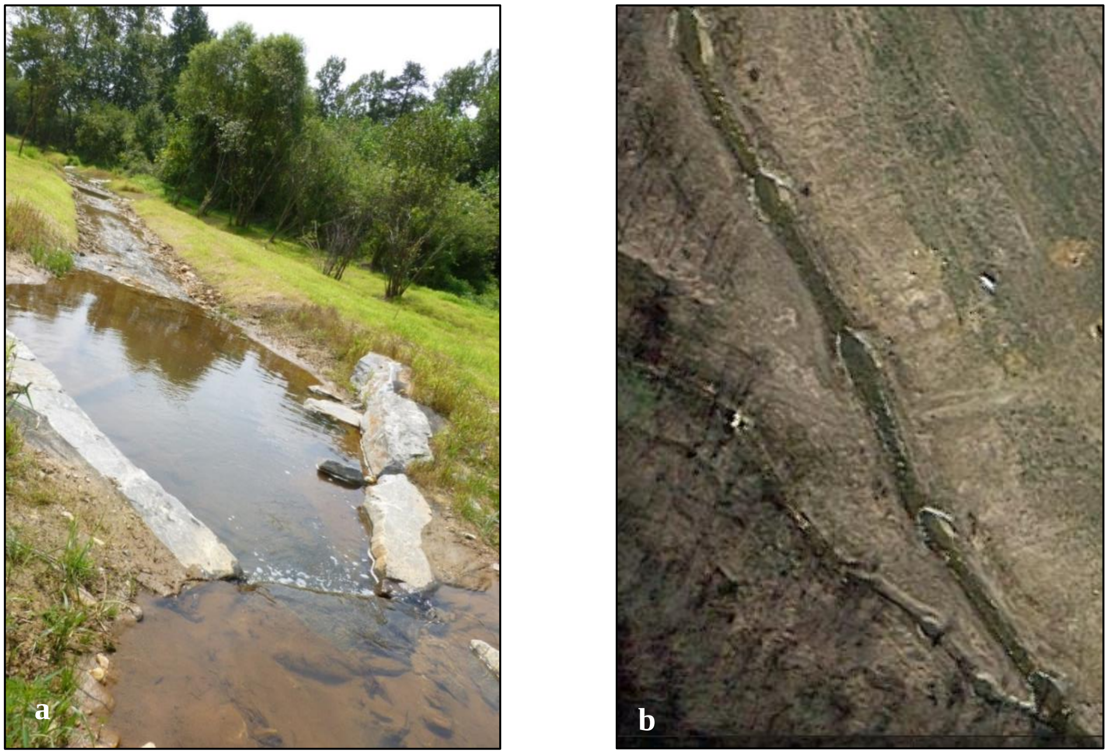


Figure 4: Natural channel design stream restoration approach. a) A boulder cross vane structure in the restored stream channel, creating a riffle, step, pool sequence. b) Aerial view of cross vane structures in the restored stream channel.

The unrestored stream site has two distinct reaches — the upstream site (referred to as unrestored) has a four meter herbaceous buffer, consisting of sedges and grasses (Figures 5b and 6b), while the downstream site (referred to as unrestored + buffer) has a 17 meter forested buffer consisting of oak trees and other deciduous vegetation (Figures 5c and 6c). There is a culvert between the two reaches. The upstream channel is surrounded by agricultural fields on both sides and is experiencing considerable erosion and consequent bank slumping, leading to small, temporary benches of sediment next to the active stream channel. Numerous overland flow paths have been observed in the agricultural fields and riparian zone after storm events in the upstream portion of the reach. Due to steep topography, the downstream stream channel is deeply incised on the left bank as the stream traverses the toe of the hillslope. The downstream channel does not resemble a traditional straightened agricultural "ditch" as much as the upstream site, as it is not located as close to the agricultural fields and develops large natural point/gravel bars and meander bends. The unrestored agricultural fields are planted with a rotation of soybeans, tobacco, and corn.

The forested reference site has an extensive forested riparian area on one side of the channel consisting primarily of oak, magnolia, and beech trees with high connectivity between the stream and adjacent floodplain (Figures 5d and 6d). The other side of the channel is bordered by an access road into the park and is deeply incised, with minimal stream-floodplain connectivity. See Table 1 for summary descriptions of each site.

Table 1: Field site summary descriptions.

Site ID	Land Use	Stream	Riparian area
Restored	Agriculture	Restored; NCD with cross vane structures, riffle/pools, floodplain regrading	27 m; herbaceous
Unrestored	Agriculture	Unmanaged ditch	4 m; herbaceous
Unrestored + Buffer	Agriculture	Incised channel but high complexity/meanders	17 m; forested
Forested	Forest + Agriculture	Floodplain connection on inner meander; high bed complexity; incised & widened	20 + m; forested on one side

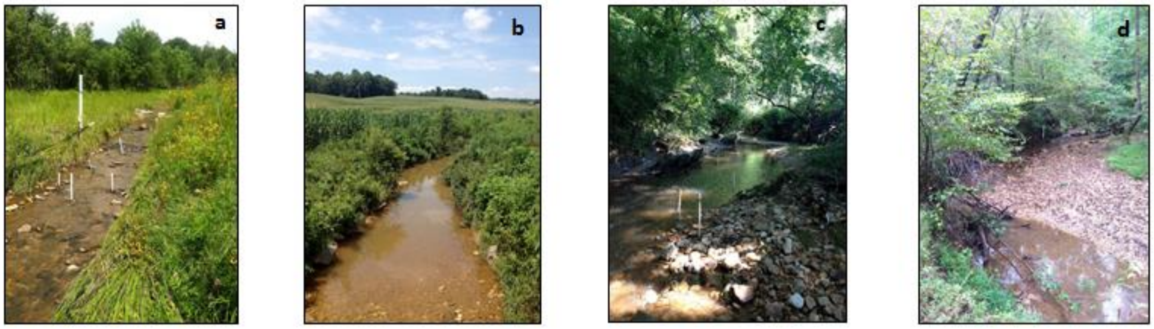


Figure 5: Field sites: a) Restored, agricultural stream, 27 m vegetated buffer b) Unrestored, agricultural stream, 4 m vegetated buffer c) Unrestored + buffer, 17 m forested buffer d) Forested, reference stream 20 m riparian study zone

Figure 6: Aerial views of field sites: a) Restored agricultural stream b) Unrestored agricultural stream c) Unrestored agricultural stream + buffer d) Forested reference stream

2.2 Sampling Approach

Benthic stream sediment and riparian soil samples were collected for denitrification enzyme activity, determination of organic content, and soil textural analysis. Benthic sediment samples were taken in representative stream geomorphic features, including pools (i.e., areas of greater depth and retention compared to the surrounding channel), riffles (i.e., rocky areas of shallow, turbulent flow), runs (i.e., smooth flowing sections of the stream), point bars (i.e., intermittently submerged coarse inorganic sediments (Groffman et al. 2005)) , and above cross vane structures (where present). Soil samples were taken in the floodplain/riparian area along a transect from the stream to the edge of the agricultural field or toe of the hill slope. Sediment and soil samples were taken during the growing season (July-September 2013) and dormant season (January-February 2014). During the growing season, samples were taken at various depths (0-5 cm, 5-15 cm, and 15-25 cm, where able) and in the floodplain next to various stream features.

Sediment cores were collected from 0-5 cm in the growing and dormant seasons using clear PVC tubes with a diameter of 5 cm. The top 5 cm of soil has been shown to have the greatest amount of biological activity (Groffman et al. 1992, Garcia-Ruiz et al. 1998). In streams, five cores were taken per geomorphic feature and homogenized. In the riparian zone, five cores were also taken at each riparian distance sampling point along a transect from stream to field or toe of hillslope. When it was too difficult to core with a PVC tube, an auger was used to collect soil to various depths. Loss on ignition was used to determine the percent organic matter using ~25 g soil subsamples dried for 72 hours at 60° C, weighed and subsequently burned at 500°C for four hours, and re-weighed post combustion. Percent organic matter was derived via the difference in dry versus ashed weight.

2.3 Denitrification Enzyme Activity

Denitrification enzyme activity (DEA) anoxic bottle activity studies were performed using the acetylene-block method (Smith and Tiejede 1979), in which ~25 g of field-moist sediment or soil was added to 50 mL of deionized water amended with 20 mg/L carbon in the form of glucose ($C_6H_{12}O_6$) and 10 mg/L nitrogen in the form of sodium nitrate ($NaNO_3$) to create a 75-mL slurry in a 125 mL glass bottle. Chloramphenicol, an antibiotic, was added to a minimum concentration of 0.3 mM to inhibit synthesis of new enzymes during the experiment. Triplicate bottles were run of homogenized sediment from each stream feature. Bottles were capped and the headspace was alternately evacuated and purged with helium to create anoxic conditions. Acetylene gas (made via calcium carbide and water) was added to attain a concentration of 10 percent by volume. Acetylene gas (C_2H_2) inhibits the activity of nitrous oxide reductase (responsible for reducing nitrous oxide to dinitrogen gas), preventing denitrification from going to completion so that N_2O can be measured to approximate the total rate of denitrification. Slurries were vigorously shaken and incubated for 4 to 5 hours, with 5 samples taken every 45 minutes to 1 hour over the course of the incubation via a 500-uL gastight syringe. When possible, gas samples were analyzed immediately on a Shimadzu Gas Chromatograph-2014 equipped with an electron capture detector. Replicate bottles were sampled and 5 mL was injected into 3-mL pre-evacuated glass vials; 5 mL of the bottle headspace was replaced with a 10% acetylene-helium mix. Concentrations were corrected for solubility of N_2O via the Bunsen coefficient adjusted to room temperature. DEA was expressed as ng N/g dry mass (DM)/hour.

2.4 Physical Site Parameters

Cross sections were surveyed at multiple riparian transects at each site to show changes in topography along the transition from the active stream channel, floodplain, and riparian zone to the agricultural field. The floodplain area was marked as the “hydrologic floodplain,” the area from the edge of the stream bank to bankfull. Bankfull was determined in the field by changes in topography as well as bankfull indicators such as vegetative disturbance, leaf litter lines, scour marks, and near-channel ponding after storm events. The riparian area was denoted as the vegetated area adjacent to the floodplain. The floodplain is a zone of deposition and frequent inundation and thus contains hydrophilic vegetation. The riparian zone contains some hydrophilic vegetation but predominately consists of upland plants, particularly in the forested riparian zones.

Stream water samples were collected in triplicate and analyzed for nitrate (NO_3^-), phosphate (PO_4^{3-}), ammonium (NH_4^+), dissolved organic carbon (DOC) and dissolved nitrogen (DN) at the time of each DEA assay. Water samples were stored on ice, returned to the laboratory and filtered within 24 hours of collection using 0.7 m Whatman GF/F filters. Concentrations of nitrate, ammonium, and orthophosphate were determined using a Lachat QuickChem 8500 Flow Injection Analysis System (Hach Inc., Loveland, Colorado). Nitrate concentrations were determined using cadmium reduction (QuikChem Method 10-107-04-1-A; detection limit 0.016 mg NO_3-N L-1) and orthophosphate concentrations were determined using the ascorbic acid method (QuikChem Method 10-115-01-1-A; detection limit = 0.001 mg PO_4-P L-1) (APHA, 2005). Ammonium was analyzed colorimetrically using the phenol method (QuickChem Method 10-107-06-1-C; detection limit 0.02 mg NH_3-N/L). Stream samples were also run for dissolved organic carbon and dissolved nitrogen on a Shimadzu Total Organic Carbon Analyzer (Shimadzu Inc., Kyoto, Japan) via high-temperature combustion (detection limit = 0.08 mg L-1). Dissolved organic nitrogen (DON) was determined as the difference between total dissolved nitrogen and inorganic nitrogen species (NO_3^- and NH_4^+). Soil pH measurements were obtained using a Sonde XLM pH probe on a 1:1 slurry of soil and deionized water (Kalra, 1995). Soil texture analysis was conducted using Natural Resources Conservation Service (NRCS) hand texture methods (R. Burt and Soil Survey Staff 2014). Temperature and weather patterns were noted on each sampling date.

Floodplain sediment and riparian soil nutrients (NO_3^- and NH_4^+) were derived via 2.0 M KCl-extraction during the growing season.

Watersheds were delineated in ArcGIS (Esri, Redlands, CA) using digital elevation models and 4 and 20 foot contour maps for Surry County, NC from the Enterprise Geographic Information Systems (GIS) services of the North Carolina Department of Transportation (NCDOT) and the hydrography maps available from the United States Department of Agriculture-Natural Resources Conservation Service (USDA-NRCS) Geospatial Data Gateway. Watershed land use maps were developed using the watershed boundary maps and the national land cover data set provided via the USDA-NRCS Geospatial Data Gateway for Surry County, NC.

2.5 Statistical Analyses

Normality of data was tested using the Shapiro-Wilk test and transformations were performed on non-normal data distributions to adhere to the assumptions of analysis of variance (ANOVA). The cube root of in-stream DEA and logarithm of riparian DEA was used in individual ANOVAs. When all in-stream, floodplain, and riparian data were analyzed together, a cube root distribution was used. A two-way ANOVA was run on all stream, floodplain, and riparian samples ($n = 262$), to determine statistical differences in denitrification rates between season (growing versus dormant), location (stream, riparian, or floodplain) and the combined effect of season by location. Additionally, two-way ANOVAs were run on each subset of riparian ($n = 72$), in-stream, active channel ($n = 76$), and floodplain ($n = 90$) samples to determine the effect of field site (restored, unrestored, unrestored + buffer, and forested) and season (growing versus dormant). A one-way ANOVA was conducted on soil texture and in-stream DEA and on riparian

DEA. Tukey's Honestly Significant Difference and Student's T-tests were used for post-hoc analysis for all ANOVAs. Reported statistics were determined to be significant at the $\alpha = 0.05$ level.

To explain variability in data and grouping of site characteristics, principal components analysis (PCA) was used to investigate clustering of various in-stream site variables, including water chemistry (nitrate (NO_3^-), phosphate (PO_4^{3-}), ammonium (NH_4^+), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), dissolved organic nitrogen (DON)), water temperature, width and depth of geomorphic features, percent organic matter of the sediment, and rainfall within the preceding 48 hours of collection. Point bar DEA values were excluded from the in-stream PCA as water depth and surface water chemistry was not applicable. Principal components analysis was also used to investigate the clustering of various riparian variables, including soil moisture, percent organic matter, groundwater chemistry, and nutrient leaching.

Following the PCA, a series of simple linear regressions were performed on in-stream DEA and water chemistry parameters, temperature, and sediment percent organic matter. A similar series of linear regressions were conducted between riparian and floodplain DEA and soil moisture, groundwater chemistry, percent soil organic matter, and soil pH. Multiple linear regression models were constructed for riparian and floodplain DEA using stepwise regression with a p-value threshold stopping rule of 0.25 and standard least squares. The absence of multicollinearity in the multiple regression model was checked using variance inflation factors. Distribution of the residuals was checked to assure normality. Statistical analyses were performed using JMP, Version 10 (SAS Institute Inc., Cary, NC).

CHAPTER 3: RESULTS

3.1 Denitrification Along a Stream-Floodplain-Riparian Continuum

In both the growing and the dormant seasons, DEA in floodplain sediment and riparian soil was significantly higher than stream sediment ($p < 0.001$, Figure 7). However, DEA in the floodplain was not significantly different than in the riparian area. Overall, DEA was significantly greater in the dormant season than the growing season across all locations ($p < 0.001$, Figure 7), though considerable variability was present at each field site.

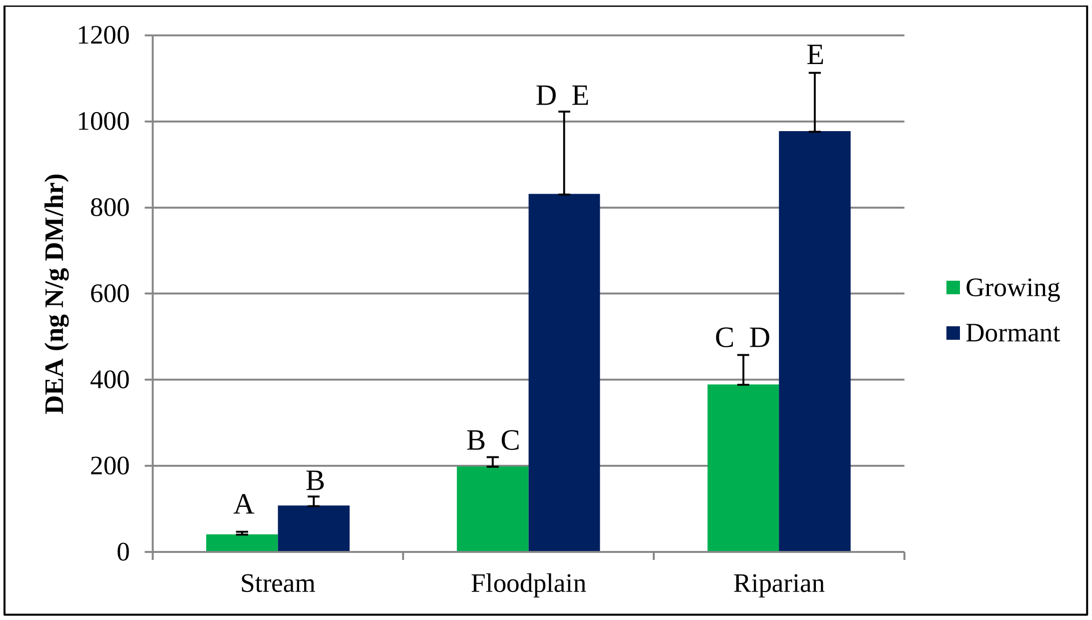


Figure 7: Comparison between stream (n = 100; growing = 49, dormant = 51), floodplain (n = 90; growing = 66, dormant = 24), and riparian (n = 72; growing = 36, dormant = 36) denitrification potential by season (total n = 262). Location, season, and the interaction term of location*season were significant. Bars not connected by the same letter are significantly different ($p < 0.001$).

DEA, percent soil moisture, and percent organic matter were measured along a transect from stream to field edge at each field site during the growing and the dormant seasons (Figures 8-11). At the restored site, DEA was highest along the field edge in the growing season (Figure 8b), which corresponds to the area with the highest percent organic matter (Figure 8d). During the dormant season, DEA was highest at four points along the transect: in the floodplain, near bankfull, five meters into the riparian area, and at the field edge. The floodplain, bankfull, and five meter locations all had relatively high soil moisture (over 30%) compared to the other points along the cross section (Figure 8c). The field edge again had the highest percent organic matter along the cross section during the dormant season (Figure 8d).

At the unrestored site, DEA was highest in the middle of the herbaceous buffer (5 m from stream channel) during both the growing season and dormant seasons. During both seasons, high DEA occurred at the confluence of high soil moisture (20-30%) and organic content (5.5%) (Figure 9 c and d).

During the growing season at the unrestored + buffer site, DEA was highest 5 m from the stream edge at the beginning of the riparian zone. This location had the highest organic matter and soil moisture (Figure 10 c and d). During the dormant season, DEA was highest 10 m into the riparian zone, where soil moisture and percent organic matter were highest (Figure 10 c and d). At both the unrestored and unrestored + buffer sites, percent organic matter and percent soil moisture followed the same trends along the transect, suggesting correlation between percent organic matter and soil moisture at the unrestored sites, which was also observed in a linear regression ($p < 0.001$, $R^2 = 0.38$).

At the forested site, DEA was the highest 15 m into the riparian area and at bankfull during the growing season. During the dormant season, DEA was lowest in the stream and highest 15 m in the riparian zone and in the floodplain. The 15 m location had high soil organic content and soil moisture in both the growing and dormant seasons (Figure 11 c and d). Site-wide, soil moisture was higher during the dormant season but percent organic matter in the soil was higher during the growing season. Soil moisture and percent organic matter were significantly linearly correlated during both seasons at the forested site ($p < 0.001$, $R^2 = 0.75$).

The forested site also has a secondary channel that cuts across the meander bend and is only accessed during high flow conditions. Alluvium deposits in this channel suggest frequent connectivity with the stream channel. DEA of the secondary channel (31 ± 3 ng N/g dry mass/hour during the growing season and 29 ± 8 ng N/g dry mass/hour during the dormant season) was more similar to values measured in the active channel than the riparian area. This secondary channel is sandy and does not contain water during normal baseflow conditions.

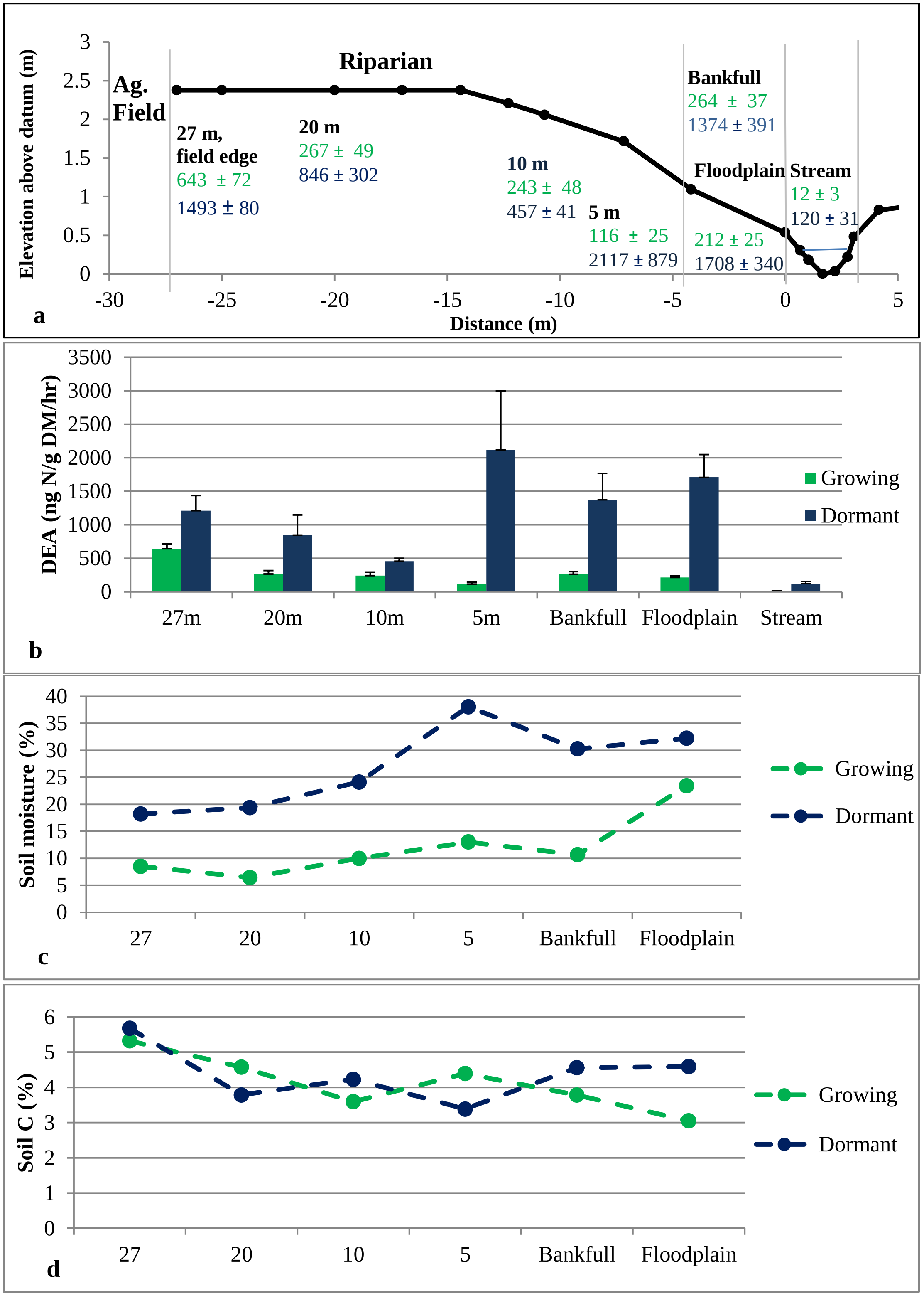


Figure 8: Restored site DEA along a transect (a and b) from stream to field edge during the growing (green) and dormant (blue) seasons and corresponding percent soil moisture (c) and percent organic matter (d). DEA values are mean ± standard error, n = 3.

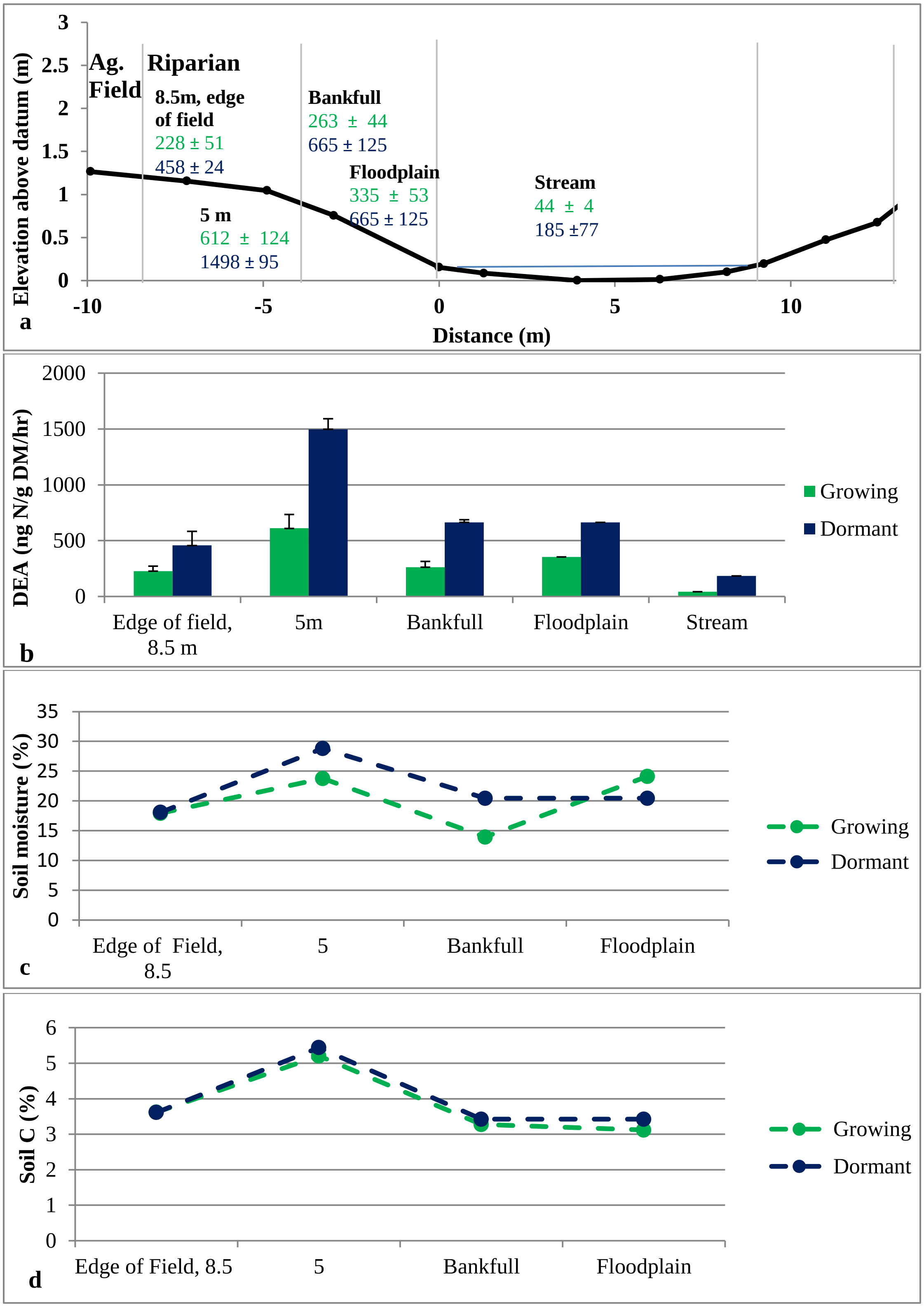


Figure 9: Unrestored site DEA along a transect (a and b) from stream to field edge during the growing (green) and dormant (blue) seasons and corresponding percent soil moisture (c) and percent organic matter (d) DEA values are mean ± standard error, n = 3.

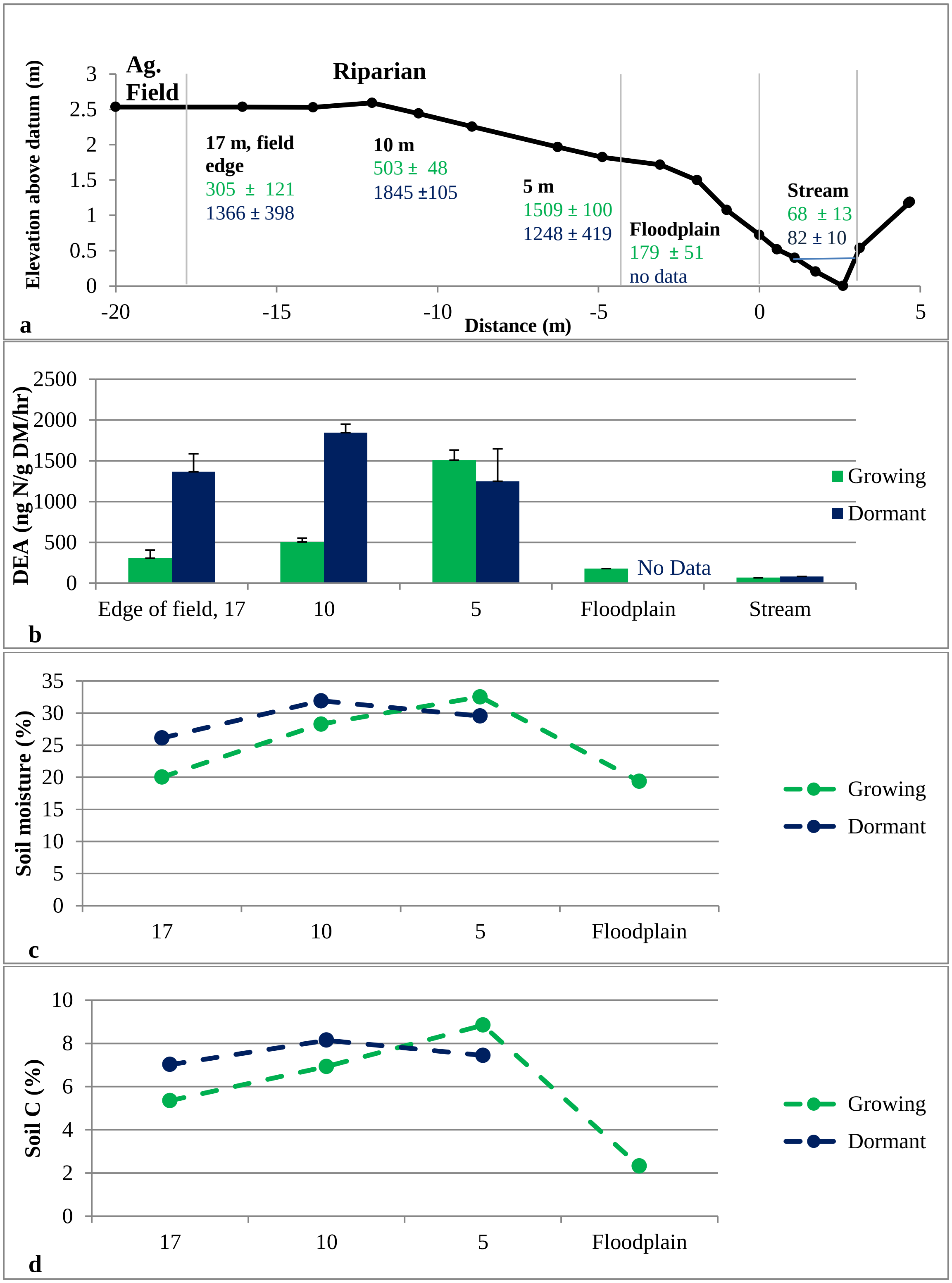


Figure 10: Unrestored + buffer site DEA along a transect (a and b) from stream to field edge during the growing (green) and dormant (blue) seasons and corresponding percent soil moisture (c) and percent organic matter (d). DEA values are mean ± standard error, n = 3.

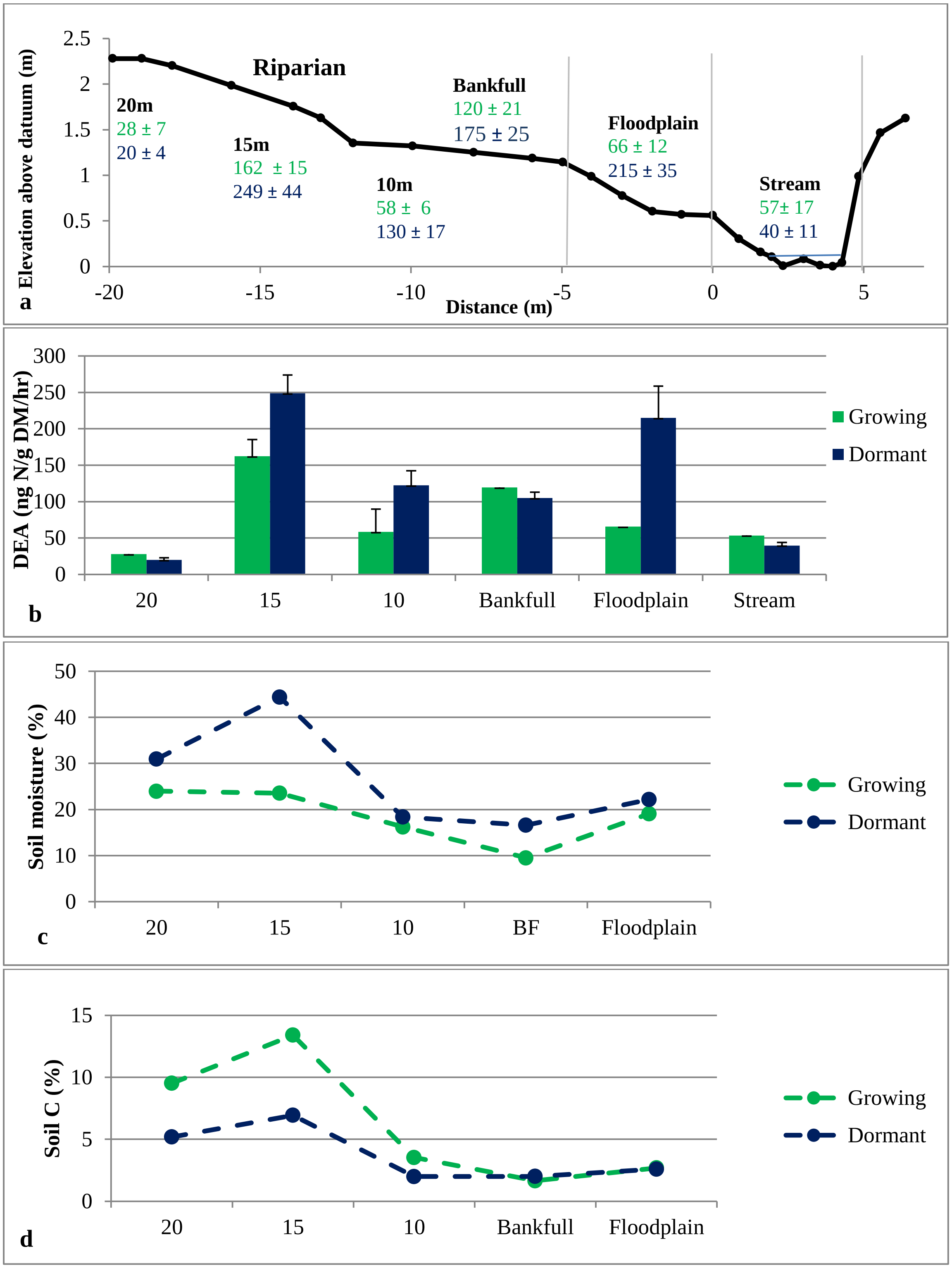


Figure 11: Forested site DEA along a transect (a and b) from stream to field edge during the growing (green) and dormant (blue) seasons and corresponding percent soil moisture (c) and percent organic matter (d). DEA values are mean ± standard error, n = 3.

3.2 Denitrification in Riparian and Floodplain Soils

Denitrification rates were higher in the dormant season than the growing season ($p < 0.001$) across all sites except the forested site (Figure 12). Soil moisture was greater during the dormant season than the growing season at all sites ($p < 0.001$). Soil and water temperatures were lower in the dormant season than the growing season. In both the growing and dormant seasons, the riparian locations had significantly lower DEA in the forested compared to the restored and unrestored sites ($p < 0.001$). Additionally, DEA significantly decreased with depth in the riparian zone at the three sites based on depth measurements taken at 0-5, 5-15, and 15-25 cm during the growing season in the mid-riparian area at all four sites (Appendix A, Figure 23, $p = 0.0252$).

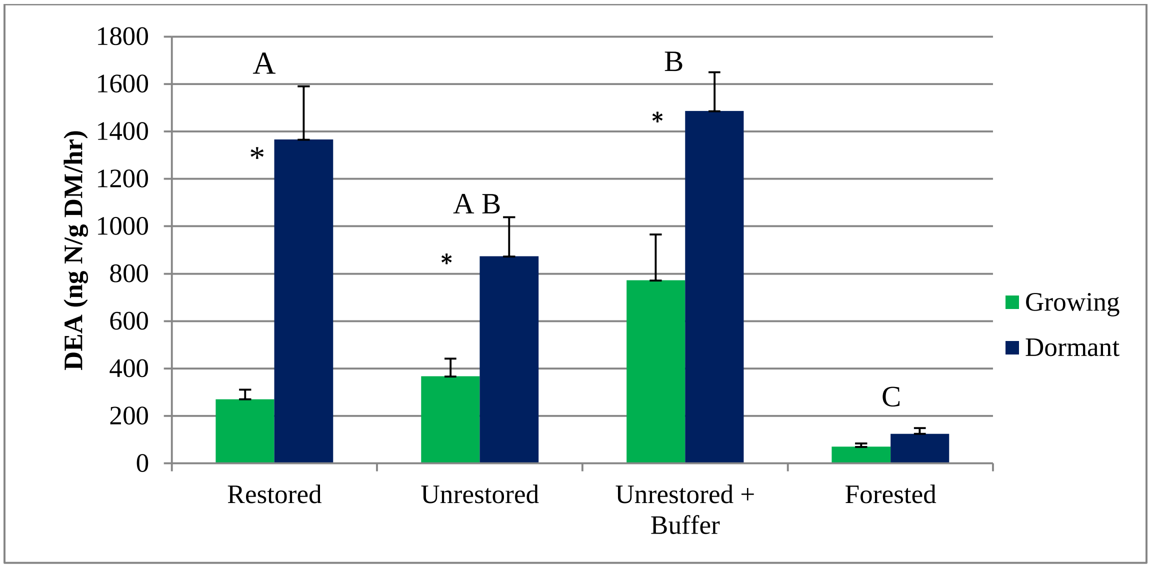


Figure 12: Riparian denitrification potential at four sites during the growing and dormant seasons (n = 72, n = 36 each season; restored site n = 12 each season, unrestored site and forested sites n = 9 each season, unrestored + buffer n = 6 each season). Both site and season were significant ($p < 0.001$), but the interaction effect of site*season was not significant. Different letters indicate significant differences between sites. Asterisks indicate there are significant differences between seasons within the same site.

Overall, when DEA from all sites was lumped together, floodplain DEA was higher in the dormant season than the growing season at all sites (Figure 13). The greatest difference in DEA occurred at the restored site. The floodplain at the restored site had greater percent moisture and percent organic matter during the dormant season than the growing season (Figure 8 c and d).

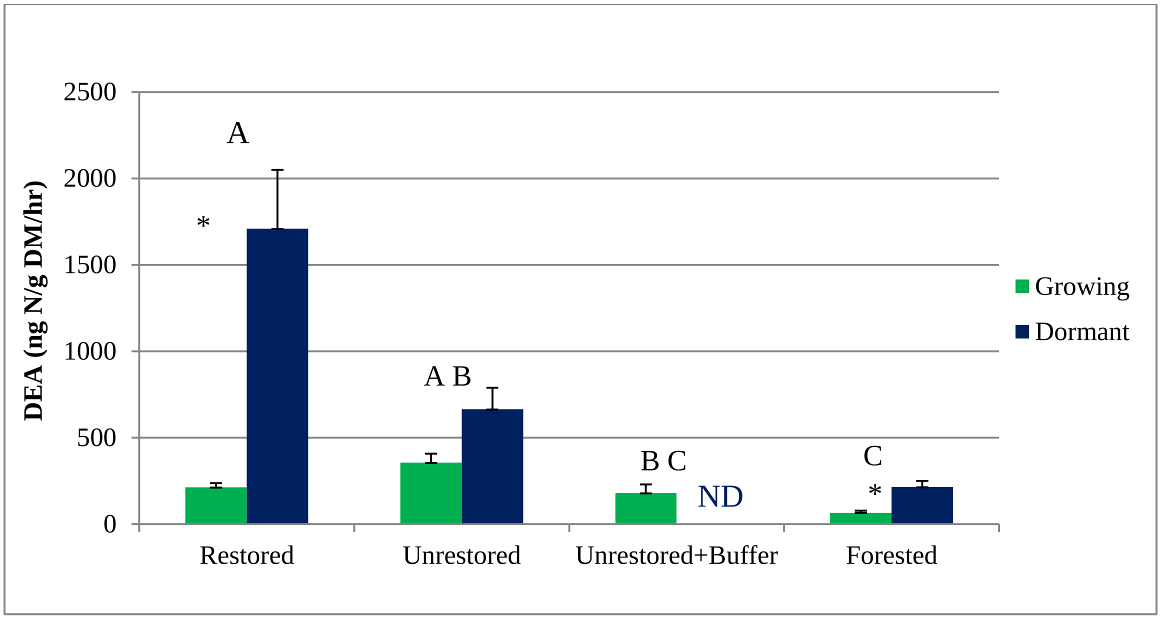


Figure 13: Floodplain DEA during the growing and dormant seasons. Site and season were significant ($p < 0.001$) but the interaction effect of site*season was not significant. Sites connected by the same letter are not significantly different. More samples were collected in the growing season (n = 66, restored = 21, unrestored = 15, unrestored+buffer = 12, forested = 18) than the dormant season (n = 24, restored = 9, unrestored = 3, unrestored + buffer = no data (ND), forested = 12) for a total n of 90. Asterisks indicate there are significant differences between seasons within the same site.

Principal components analysis (PCA) was conducted on all riparian and floodplain data (inputs: soil moisture, percent organic matter, soil temperature, soil pH, groundwater chemistry) and showed apparent site (component 1) and seasonal (component 2) effects (Table 2). Component 1 explained 35% of the variance and loaded highly on site, DEA, and nitrogen species. Component 2 explained 24% of the variance and seemed to correspond to season, as it loaded highly on season, groundwater temperature, and air temperature. See Appendix B, Figure 25 for graphical riparian and floodplain PCA representation.

Table 2: PCA loadings and correlation coefficients of environmental parameters on the first two components (PC1 and PC2). Bold indicates five highest-loading parameters on each component.

Riparian Environmental Parameter	PC1 (site function)		PC2 (season function)	
	Loading	Correlation coefficient	Loading	Correlation coefficient
Site	**0.34**	0.78	**0.28**	0.52
DEA	**0.37**	0.85	0.11	0.21
Soil C (%)	0.17	0.39	0.27	0.50
pH	0.13	0.29	-0.02	-0.03
Moisture	0.25	0.58	-0.04	-0.07
Texture	-0.18	-0.42	-0.24	-0.45
Season	0.21	0.50	**-0.45**	-0.85
Temperature	-0.15	-0.34	**0.48**	0.91
Groundwater temperature	-0.24	-0.57	**0.42**	0.80
GW NO_3^-	**0.39**	0.91	0.03	0.05
GW NH_4^+	**0.33**	0.76	-0.02	-0.04
GW PO_4^{3-}	-0.01	-0.01	**-0.36**	-0.67
GW DOC	0.19	0.43	0.01	0.02
GW TDN	**0.41**	0.94	0.07	0.14
GW DON	0.13	0.30	0.19	0.36

In addition to site and season being drivers of variance in the data set, PCA also highlighted the importance of environmental controls, namely soil moisture, temperature and organic matter. There was a significant correlation between DEA and soil organic matter (Figure 14, $p < 0.001$ $R^2 = 0.14$). There was also a significant correlation between soil moisture and floodplain and riparian DEA (Figure 15, $p < 0.001$, $R^2 = 0.18$). Soil moisture was highest in the dormant season, likely due to increased precipitation totals and reduced evapotranspiration. Dormant season precipitation totals were 160 mm in December and 224 mm in January whereas precipitation totals in growing season when floodplain sediments and riparian soils were sampled were 120 mm in August and 47 mm in September. There was a significant correlation between soil pH and DEA (Figure 16, $R^2 = 0.03$, $p = 0.0106$). Additionally, there was a significant correlation between DEA and soil NO_3^-, though data from soil nutrient leaching experiments was only available during the growing season (Figure 17, $R^2 = 0.086$, $p = 0.0269$).

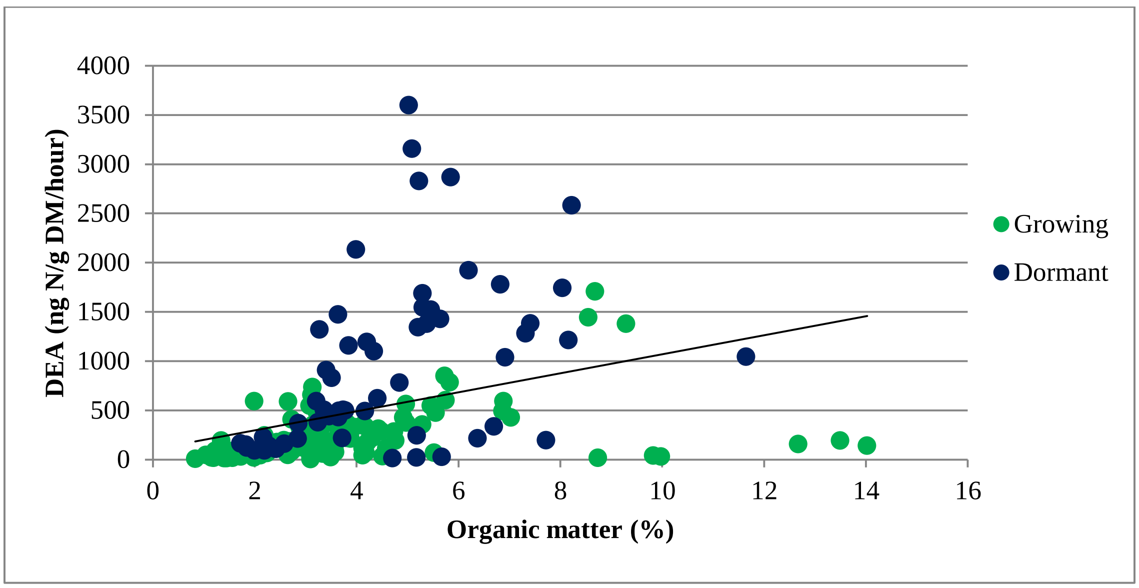


Figure 14: Floodplain sediment and riparian soil DEA versus percent organic matter, all sites, both seasons, n = 162 ($p < 0.001$, $R^2 = 0.14$).

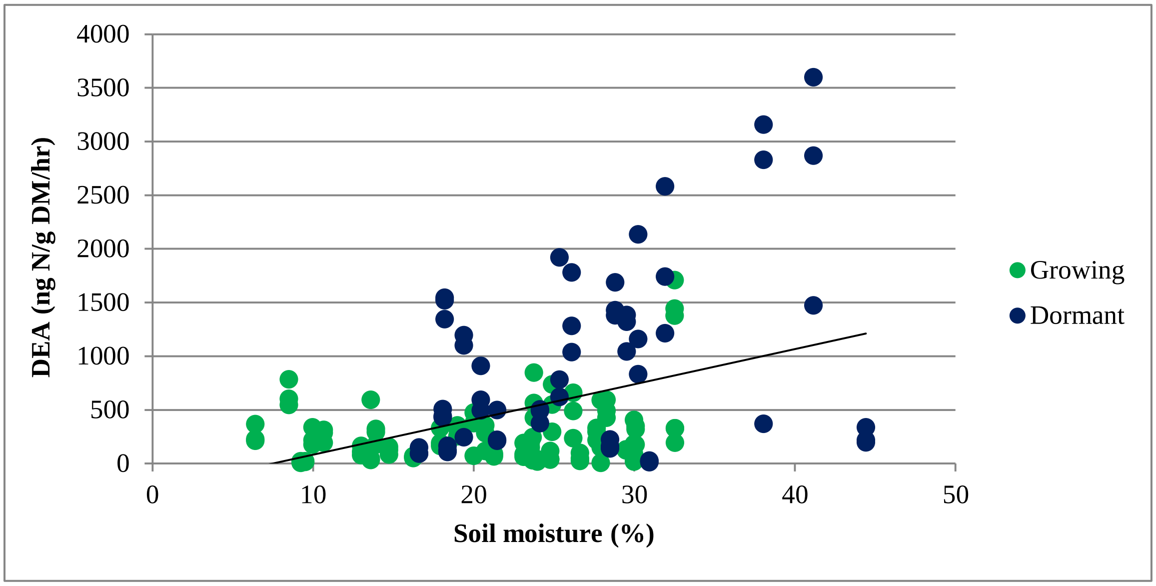


Figure 15: Floodplain sediment and riparian soil DEA versus percent soil moisture, all sites, both seasons, n = 162 ($p < 0.001$, $R^2 = 0.18$).

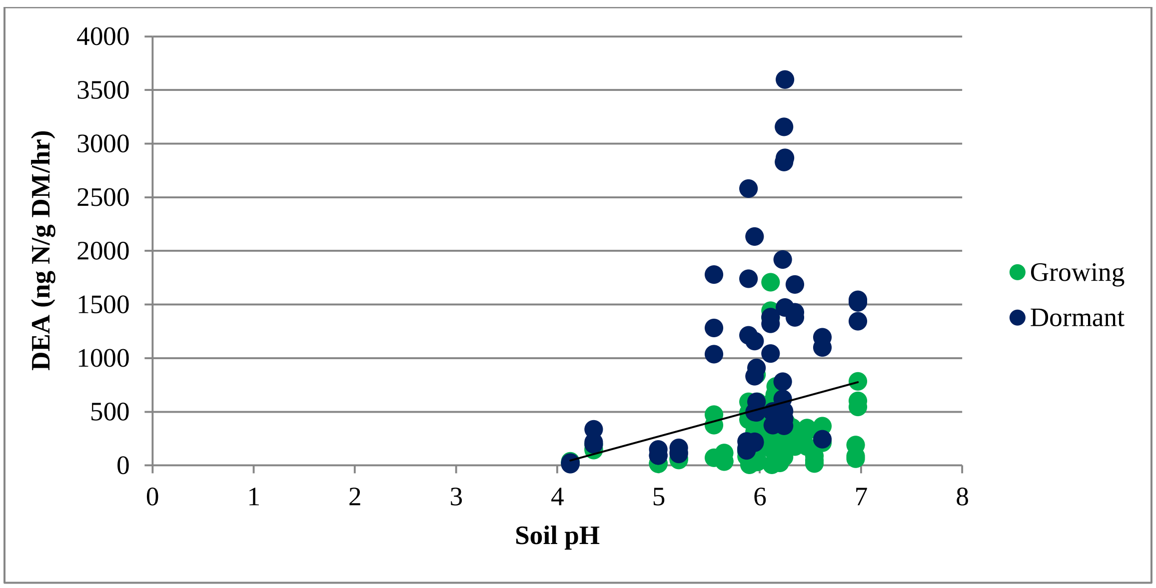


Figure 16: Floodplain and riparian soil DEA versus pH, all sites, both seasons, n = 162 ($R^2 = 0.03$, $p = 0.0106$).

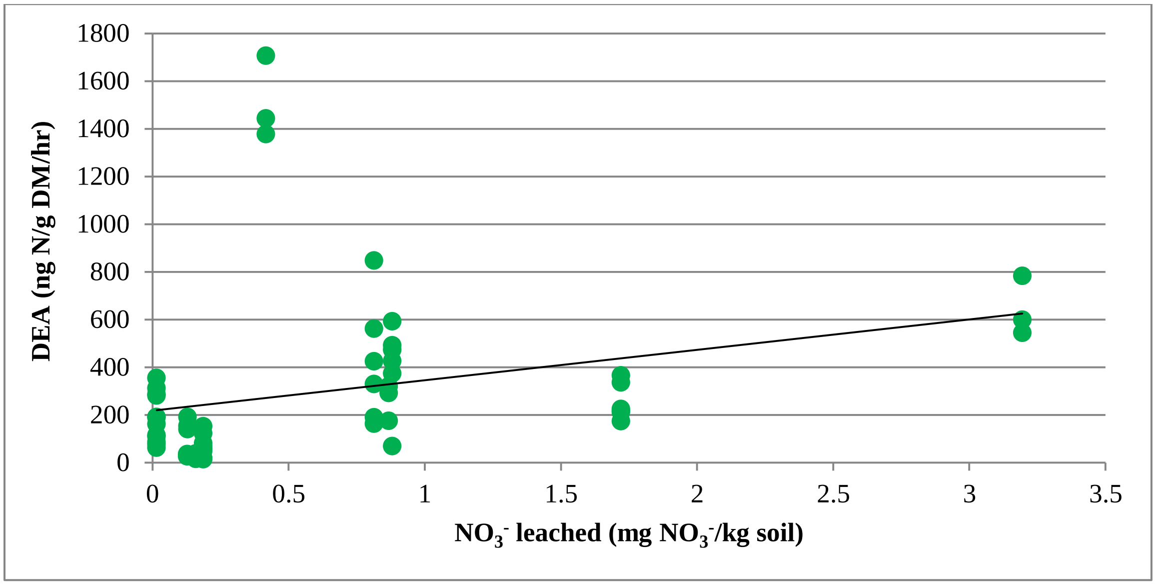


Figure 17: Riparian and floodplain DEA versus NO_3^- leached from soil, growing season only, n = 57 (R^2 = 0.086, p = 0.0269).

A multiple linear regression model was constructed between DEA and floodplain and riparian environmental variables, wherein soil moisture, organic matter, and pH were important predictor variables for DEA (DEA = -2698 + 110*(% organic matter) + 358*(pH) +26*(% moisture); Adj- R^2 of 0.36, $p < 0.001$).

3.3 In-Stream Denitrification

Overall, DEA was significantly lower in stream sediments during the growing season than during the dormant season, particularly in the agricultural sites. However, several geomorphic features (the pool, riffle, and run) of the forested site had greater DEA in the growing season. In both the forested site and unrestored + buffer site, which were similar in channel form and riparian vegetation, DEA was not significantly different between seasons. Overall, percent organic matter in the stream sediment was greater during the growing season, though the overall correlation between percent organic matter and DEA was weak (p = 0.0020, R^2 = 0.12).

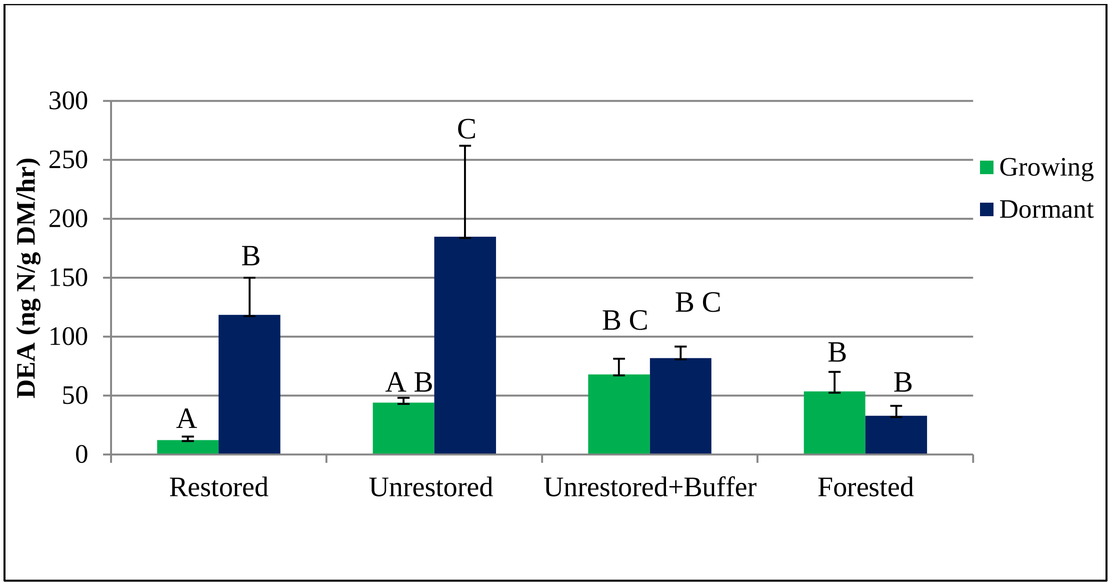


Figure 18: In-stream DEA by site during the growing (n = 49) and dormant (n = 51) seasons. Bars connected by the same letter are not significantly different. Site, season, and the interaction term of site*season were all significant ($p < 0.001$, $p = 0.014$ and $p = 0.0021$, respectively).

During the growing season, DEA at the restored site was significantly lower than at the forested site. The unrestored site was not significantly different than the restored or unrestored + buffer and forested sites. During the dormant season, the restored site had significantly lower DEA than the unrestored site, but similar DEA to the unrestored + buffer and forested sites (Figure 18).

DEA of in-stream features was compared via sites in each season. In the growing season, sediment from pools had the highest DEA in the unrestored, unrestored + buffer, and forested sites. Conversely, the point bar and riffle had the greatest DEA at the restored site. In the dormant season, the pool at the unrestored site had the highest DEA, followed by the run at the restored site (Figure 19). Pools in this reach were characterized by sandy clay sediment and high percent organic matter (over 5%) in comparison to lower organic matter (under 1.5%) of other sediment samples collected in the dormant season.

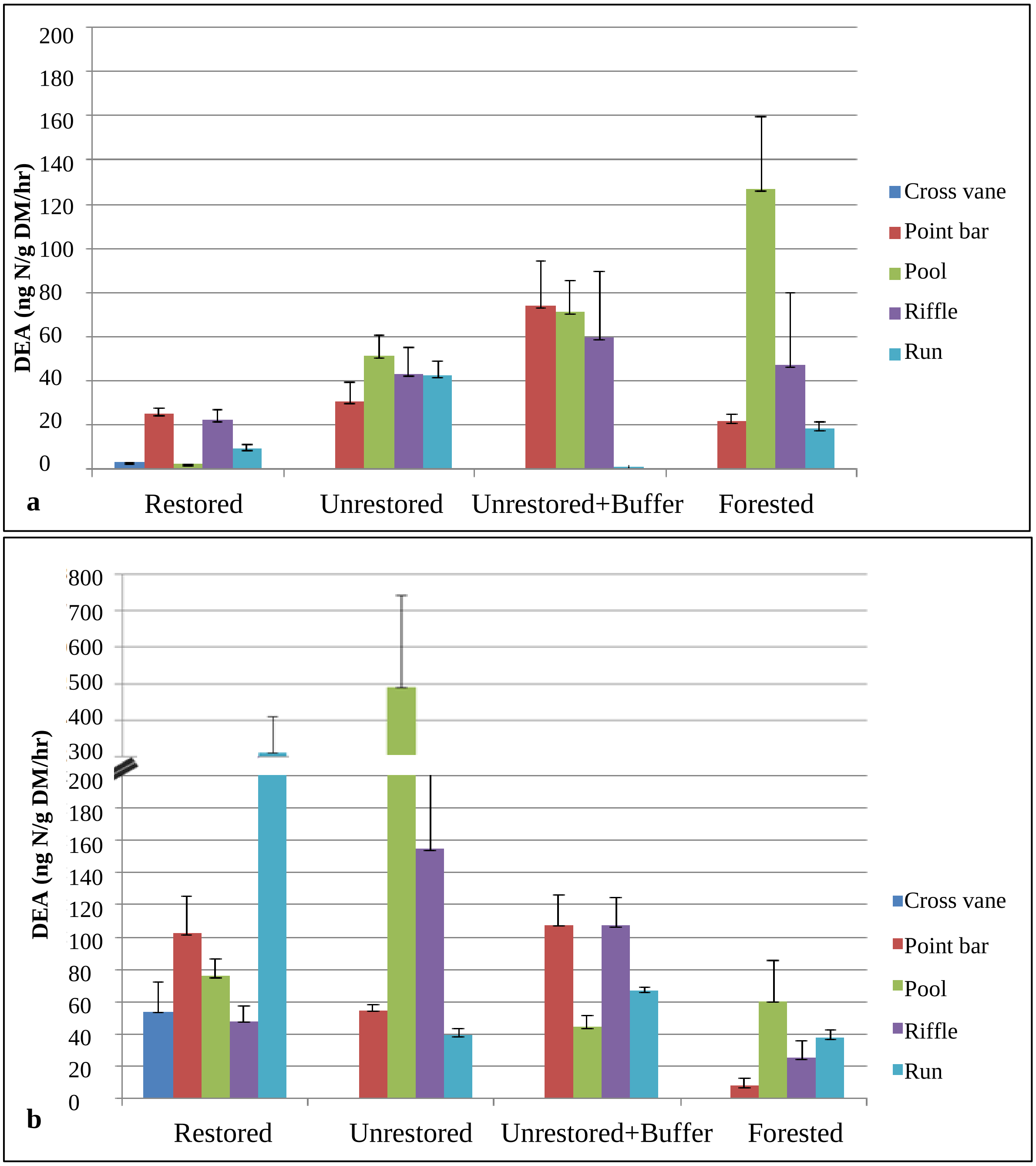


Figure 19: DEA during the (a) growing season and (b) dormant season in various geomorphic features (n = 100 total, n = 49 growing and n = 51 dormant). Each bar represents average of n = 3 ± standard error except run in unrestored + buffer site growing season, where n = 1.

Based on a series of DEA assays measured at 0-5 cm, 5-15 and 15-25 cm during the growing season, DEA significantly decreased with depth (Appendix A, Figure 22, $p < 0.001$). DEA was highest in the top 0-5 cm of sediment, which has been demonstrated to have the greatest microbial activity in the soil profile (Groffman et al. 1992).

The PCA conducted on all of the in-stream data (inputs: water chemistry parameters, temperature, site, season, stream feature dimensions, soil texture, percent organic matter of sediments, pH, 24 and 48-hour rainfall totals) demonstrated that chemical and physical properties clustered uniquely between sites and seasons, indicating chemical and physical properties were driving differences in between-site differences. Component one explained 28% of the variance in the data and best represented site differences in water chemistry, as site, nitrogen species, pH, and dissolved organic carbon loaded the highest (Table 3). Twenty one percent of the variance in the data was explained by component 2, which best represented physical environmental properties, as temperature, recent rainfall (rainfall within 48 hours preceding collection), feature width, season, and phosphate loaded highest (Table 3). Environmental variables clustered by site and season but not by feature. See Appendix B, Figure 24 for graphical representations of in-stream PCA results.

Table 3: PCA loadings and correlation coefficients of environmental parameters for the first two principal components (PC1 and PC2). The five highest loadings on each component are bolded in each column.

In-Stream Environmental Parameter	PC 1 (site water chemistry function)		PC 2 (physical environmental variables)	
	Loadings	Correlation coefficient	Loadings	Correlation coefficient
Site	**0.38**	0.85	0.11	0.21
DEA	0.12	0.26	-0.04	-0.07
Sediment C (%)	0.02	0.04	0.24	0.47
NO_3^-	0.25	0.56	-0.17	-0.34
PO_4^{3-}	-0.07	-0.15	**0.40**	0.79
NH_3^+	**0.35**	0.78	0.01	0.03
DOC	0.17	0.38	0.14	0.28
TDN	**0.40**	0.90	-0.08	-0.16
DON	**0.40**	0.90	-0.02	-0.04
pH	**0.39**	0.88	0.15	0.30
Feature depth	0.12	0.27	0.21	0.42
Feature width	0.05	0.11	**0.31**	0.61
Feature code	-0.10	-0.22	-0.21	-0.42
Season code	0.17	0.38	**-0.34**	-0.67
Water temperature	-0.16	-0.37	**0.37**	0.74
Precipitation-24 hr	0.10	0.23	0.24	0.47
Precipitation-48 hr	-0.10	-0.22	**0.39**	0.77
Soil texture	-0.24	-0.55	-0.21	-0.42

Table 4: In-stream water chemistry parameters at the time of DEA sediment collection, dormant season. Bold indicates higher nitrate concentrations were found in the restored and unrestored sites, driving higher potential denitrification rates.

Site, In-Stream Season, Dormant	**DEA (ng N/g dry mass/hr) (Mean ± SE)**	**NO_3^-**	**PO_4^{3-}**	**NH_4^+**	**DOC**	**DON**	**pH**
Restored	122 ± 32	**1.05**	nd	0.03	1.15	0.00	6.69
Unrestored	185 ± 77	**1.20**	0.02	0.03	8.04	0.74	7.06
Unrestored + Buffer	82 ± 10	0.87	0.03	0.07	8.65	1.46	7.54
Forested	40 ± 11	0.68	0.02	0.02	4.33	0.00	6.79

Table 5: In-stream water chemistry parameters at time of DEA sediment collection, growing season.

Site, In-Stream Growing	**DEA (ng N/g dry soil/hr) (Mean ± SE)**	**NO_3^-**	**PO_4^{3-}**	**NH_4^+**	**DOC**	**DON**	**pH**
Restored	12 ± 3	0.76	0.02	0.02	11.07	0.17	6.66
Unrestored	46 ± 15	0.95	0.02	0.03	11.41	0.74	7.06
Unrestored + Buffer	43 ± 4	0.94	0.03	0.03	7.69	0.00	7.19
Forested	53 ± 16	0.47	0.04	0.02	3.33	0.00	6.78

Regressions were run between in-stream DEA and various environmental parameters (e.g., water chemistry, pH) and a significant positive correlation was found between percent organic matter and DEA ($p = 0.002$, Figure 20). Additionally finer soil textures had significantly higher DEA than coarse soil textures ($p = 0.0038$, Figure 21).

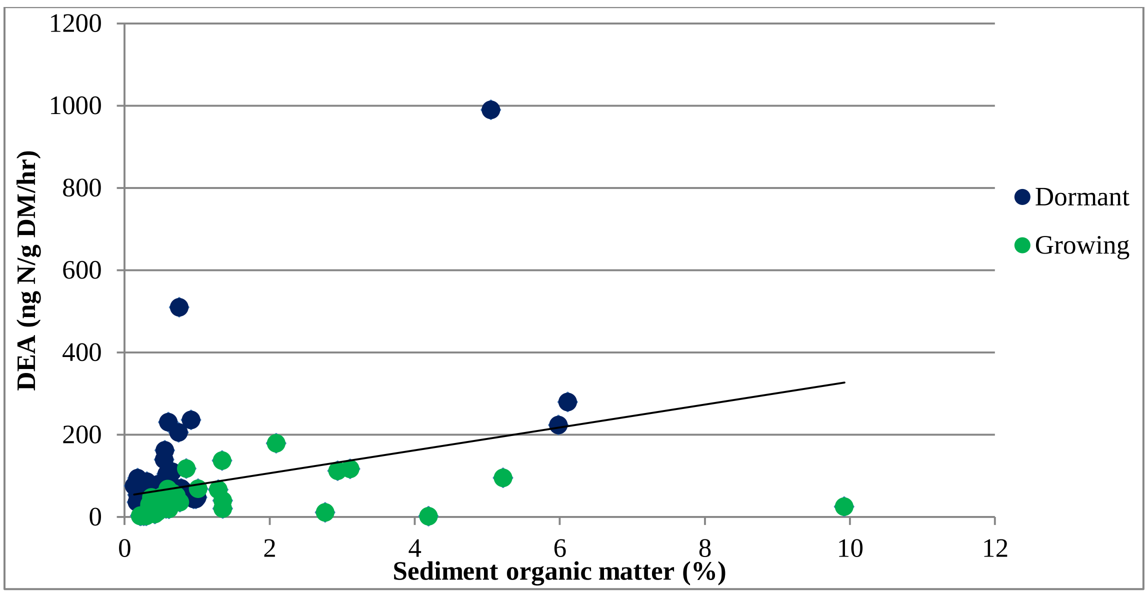


Figure 20: In-stream wetted-channel sediment organic matter versus DEA, growing and dormant seasons ($n = 76$), ($p = 0.002$, $R^2 = 0.12$).

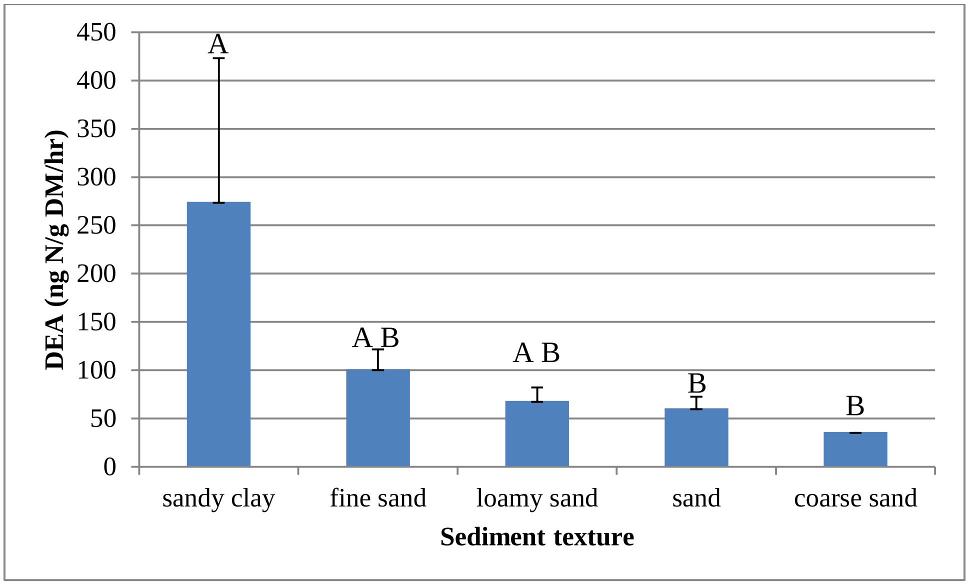


Figure 21: In-stream DEA of various sediment textures during the growing and dormant seasons (n = 100 (includes point bars) sandy clay = 6, fine sand = 6, loamy sand = 19, sand = 51, coarse sand = 18). Bars not connected by the same letter are significantly different (p = 0.0038). Sandy clay denitrification rates are significantly higher than coarse sand denitrification rates.

CHAPTER 4: DISCUSSION

4.1 Riparian/Floodplain versus In-Stream Denitrification

DEA was higher in floodplain sediments and riparian soils than in-stream sediments during both the growing and dormant seasons. Riparian zones have been recognized as hot spots of denitrification (McClain et al. 2003, Vidon et al. 2010) as they often exhibit conditions conducive to denitrification including high organic matter content, low oxygen, and high subsurface water residence times. In this study, stream sediments had significantly lower organic carbon than riparian soils ($p < 0.001$). Additionally, many stream sediments were dominated by coarse sand, particularly at the restored site. Generally, floodplain sediments and riparian soils had finer textures (sandy loams and clays), enabling more surface area to be available for microbial colonization.

Physical evidence observed at the unrestored and restored sites indicated these soils may have high retention times to contribute to significant denitrification. At the unrestored site, various concentrated flowpaths were observed in both the agricultural fields and the riparian zones, which remained saturated after storm events longer than surrounding areas on the landscape. At the restored site, considerable near-stream ponding was observed in the floodplain the day after a storm, likely due to expansion of clay soils and high connectivity to the stream. This increases contact time of subsurface flow, overland runoff, and stream water with near-stream, anoxic soils.

Overall, in-stream DEA in this study fell within the range of other published stream restoration studies in urban areas but on the lower end of values from agricultural streams. In a study of Midwestern agricultural streams, Arango et al. (2007) found DEA that ranged from 10 to 4,770 ng N/g dry mass/hour in Illinois and 120 to 11,060 ng N/g dry mass/hour in Michigan. Organic matter was a large driver of higher DEA. Wang et al. (2011) measured DEA in sediments from eight central North Carolina streams with varying degrees of urbanization, and DEA ranged from 41 to 561 ng N/g dry mass/hour (mean: 195 ng N/g dry mass/hour). Groffman et al. (2005) measured DEA of < 250 ng N/g dry mass/hour in pools and riffles in a series of restored and unrestored urban streams in Maryland. Groffman et al. (2005) also found organic debris dams and gravel bars containing high percent organic matter had significantly higher DEA than surrounding stream features. Similarly, the high DEA observed in this study in pools at the unrestored site during the dormant season can be attributed to higher percent organic matter of sediments compared to the surrounding stream features (over 5% and < 1%, respectively).

Riparian and floodplain DEA was also comparable to other measured values. Dandie et al. (2011) reported DEA of 3,750-7,000 ng N/g dry mass/hour in riparian zones next to agricultural fields. An analysis of denitrification potential of various land use types (e.g. wetlands, ditches) in agricultural watersheds in the Mississippi, conducted by Ullah and Faulkner (2006) reported DEA of 160 to 1180 ng N/g dry mass/hour. Gift et al. (2010) measured DEA of approximately 150 to 900 ng N/g dry mass/hour in the top 0-10 cm of soil in the riparian zone of reference, restored, and unrestored urban streams. Orr et al. (2007) reported floodplain DEA of 0 to 15 ng N/g dry mass/hour in a Midwestern agricultural floodplain.

4.2 Restoration Effect on DEA

During the growing season, there were no significant differences between DEA in the restored versus unrestored streams. However, in the dormant season, the unrestored stream had greater DEA than the restored site, driven by high denitrification rates in pools with fine sediment texture and higher organic matter. During the dormant season, the unrestored site sediments had greater organic matter content and higher DOC. However, during the growing season, organic matter and DOC were similar in both the restored and unrestored sites. Nitrate values in the restored and unrestored streams were also comparable between seasons.

Though the pools at the unrestored site had higher DEA than other features in all streams in this study, these “pools” did not have the same structure as a typical pool formed via a log/debris impoundment in a forested area. Thus, retention times in these slightly deeper, more retentive sections of the channels deemed pools may be much lower than retention times in a pool formed via a log jam, indicating that water may not remain in contact with stream sediment in these areas long enough for substantial denitrification to occur.

The unrestored agricultural stream has reduced hydrologic connectivity between the stream, floodplain, and riparian zone. Severe bank slumping may limit floodplain access during storm events and decrease hyporheic exchange, preventing nitrate in stream water from coming in contact with biologically active sediments and nitrate in groundwater from coming into contact with dissolved organic carbon from the stream channel. Channelization and incision contributes to a lower water table causing groundwater to bypass biologically-active near-surface riparian soils and floodplain sediments. Additionally, a small buffer strip limits NO_3^- filtering capacity via denitrification and vegetative uptake. Bank erosion may also clog in-stream pore spaces over time, limiting diffusion of nitrate into anoxic microsites in the streambed.

At the restored site, the floodplain had significantly higher DEA during the dormant season than growing season. During wet periods, floodplain clay soils swell and enable extended contact between subsurface and ponded surface water. Additionally, the riparian area had high DEA. The buffer at this site was 17 m wide (versus 4 m at the unrestored site), leading to greater potential for denitrification and plant uptake.

In-stream, low DEA was observed directly above the cross vane restoration structure as well as in pools below the cross vane. This could be due to the nature of these pools, which are constructed to create turbulent conditions that move sediment through the structure (e.g., cross vane) rather than allow sedimentation of fine-grained sediments. As a result, coarse sands, which do not have high surface area to promote higher microbial populations, are primarily found in pools. During the growing season, the restored stream had significantly lower rates than the unrestored, unrestored + buffer, and forested sites, indicating that restoration did not serve to facilitate increased DEA as hoped. However, during the dormant season, DEA in the restored site stream sediments was not significantly different than the unrestored + buffer and forested sites, though it was significantly lower than at the unrestored site. At this point, it cannot be said with certainty that this particular NCD stream restoration had the added benefit of promoting in-stream nutrient removal.

However, it must be noted that this restoration project is still relatively young, as the restoration was completed in 2012 and DEA soil and sediment sampling started in the growing season of 2013, when the site was a year old. The vegetation may still be undergoing succession and soils may have not had enough time for adequate development and accumulation of carbon in the upper layer of the profile. Additionally, stream features may perform differently over time (closer to reference stream), as sediments become finer and organic carbon builds up. During the course of sampling, some in-stream cross vane structures failed and plunge pools filled up with large cobbles, converting to riffle-like areas. Over time, water quality improvement may occur as the restoration project ages.

The unrestored + buffer site did not have significantly different in-stream DEA than the unrestored and forested sites in both the growing and dormant seasons. However, unrestored + buffer DEA was significantly higher than the restored stream in the growing season. Geomorphic complexity was greater at the unrestored + buffer site than at the unrestored site and the pools at the unrestored + buffer site were better-defined and often formed behind organic debris dams. Though floodplain DEA at this site was comparable to the unrestored site, the stream at this site is better able to access the floodplain than the unrestored site, as the meandering nature of the stream and herbaceous/deciduous mix reduces channelization and incision.

The forested site was the only site that did not have an adjacent agricultural field, therefore, in-stream ammonium and nitrate likely comes from up-stream sources, as there is significant agricultural activity and pasture lands in the headwaters of the drainage area. Therefore, at this particular reference site, the floodplain may be more important than the riparian area in terms of denitrification, as the floodplain may be accessed during storm events to remove N contained in surface water.

4.3. Drivers of DEA in North Carolina Piedmont Streams

We hypothesized that in-stream DEA would be dictated by stream geomorphology, and that DEA would be highest in pools across all sites. However, statistical analysis indicated site and season were more important determinants of DEA than similar geomorphic features across streams. Site and seasonality dictated spatial variability in both surface water chemistry (e.g., dissolved N and C) and physical environmental parameters (e.g., sediment percent organic matter, water depth, temperature, precipitation totals, soil texture). When DEA across all sites was averaged, in-stream DEA was greater in the dormant season than growing season. These results agree with previous studies by Roley et al. (2012), who found in-stream denitrification rates were greater in the winter and late spring than the summer and fall and Christensen et al. (1990) who found denitrification rates were highest in winter and decreased in the spring.

One possible factor in these higher winter rates is higher precipitation during the dormant months of this study, allowing greater transport of dissolved solutes to streams. Other studies have shown seasonal variation in NO_3^- loads and carbon inputs as well as flood-related drying/rewetting and freeze/thaw related changes in channel geomorphic complexity and microbial community (Bohlke et al. 2009, Groffman et al. 2009). The agricultural restored and unrestored sites may receive greater pulses of nitrate entering the stream during the dormant season due to higher precipitation totals leading to saturated soils, reduced infiltration, and greater nitrate export off of the agricultural fields in storm water runoff. This observation is consistent with the higher in-stream nitrate concentrations measured in the dormant season at the time of sediment sampling at the restored and unrestored sites. These pulses of nitrate entering streams in turn led to an increase in the denitrifying community activity at the restored and unrestored sites. However, there was only a significant linear correlation between nitrate and DEA at the restored site ($p = 0.0028$, $R^2 = 0.3$), not across all sites, indicating that other environmental parameters were responsible for explaining variability in DEA.

Across all sites, there was a positive correlation between sediment organic matter and DEA ($p = 0.002$, $R^2 = 0.12$). At the unrestored site, percent organic matter of in-stream sediment was greater in the dormant season than growing season, leading to a corresponding increase in DEA. The pools at the unrestored site which had particularly high DEA during the dormant season had a combination of high percent organic matter and fine clay sediment texture.

Finer textured sediments in the streambed, particularly those containing clay, had significantly greater denitrification rates than sand or coarse sand sediments ($p = 0.0038$). The influence of soil texture on denitrification was observed in other studies, including Roley et al. (2012), where denitrification rates were higher in fine organic matter than sand, Harrison et al. (2012) where higher denitrification rates were observed in fine-grained pools than coarse-grained riffles and Opdyke et al. (2007), where fine benthic sediments with high organic matter exhibited greater denitrification rates than coarse sediments with low organic matter. Additionally, Weigelhofer et al. (2013) showed potential denitrification rates decreased significantly with increasing grain size and Cooke et al. (1987) observed denitrification potential was five times greater in silty sediment than sandy sediment. Finer soil presents greater surface area for colonization by microbial communities (Inwood et al. 2007). Additionally, the smaller pore size of finer soil may promote conditions of anoxia necessary for denitrification to occur as it allows for less diffusion of oxygen into the streambed (Groffman and Tiejde 1989). However, ability of nitrate to diffuse into streambed sediments to contact active microbial communities responsible for denitrification is also important. Cooke et al. (1987) showed that peaks in DEA were found at the limit of the nitrate diffusion front.

Denitrification rates in geomorphic features did not exhibit the same pattern at each site — all pools, for example, did not function as hot spots and have the highest rates at each site. This is likely due to differences in the nature of each site. Pools in the forested site were generally formed behind fallen trees, logs, or debris packs which had high accumulation of organic matter. Pools in the forested site were also deeper on average than all other sites. Pools at the unrestored + buffer site were formed in a similar manner to pools at the forested site. Pools at the unrestored site did not have much structure, and were defined as deeper areas in the channel where visible transient retention was occurring. Pools in the restored site were formed as a result of scour from the drop from the cross vane structure to the streambed. Thus, pools at the restored site have much less organic matter and coarser-textured sediments than those found in a forested location. Weigelhofer et al. (2013) found that following restoration, stream sediment grain size increased followed by a decrease in denitrification rates. Since the restored site has soils with high clay content in the riparian area and the unrestored site contains finer-texture stream sediments by comparison (e.g. sandy clay), it is possible fine sediments were excavated from the stream channel and a similar accumulation of coarser stream bed sediments as that observed by Weigelhofer et al. (2013) occurred following restoration, accounting for the reduced rates of denitrification at the restored site. Following storm events at the restored site, pools beneath cross vanes often became filled in with gravel and cobble and took on the characteristics of a riffle, eliminating any retention capabilities previously demonstrated by a pool in the same location. Additionally when represented graphically (Appendix B, Figure 24c), PCA did not show any environmental parameters clustering by feature, indicating that all geomorphic features do not behave like each other if they are in different streams.

4.4 Drivers of DEA in North Carolina Piedmont Floodplains and Riparian Zones

The dormant season had greater precipitation totals than the growing season, causing greater percent soil moisture and promotion of anoxic conditions, leading to increased presence of anaerobic microbial communities. Similarly to in-stream findings, soil texture also influenced riparian denitrification rates. The unrestored + buffer site had the greatest riparian DEA, and the soils were fine-textured (loam and fine sandy loam). Additionally, the unrestored + buffer site also had the greatest amount of organic matter in soils. The restored site also had high riparian DEA during the dormant season, and soils were clay-based and were able to swell and hold water in the pore spaces, facilitating anoxia and a higher water table. The high clay content of the riparian zone in the restored site may lead to saturation of pores and increased overland flow, leading to greater transport of nitrate to streams under wet conditions. However, the clay also increases surface retention time in the floodplain during flood events as evidenced by surface ponding, leading to greater contact of water with denitrifying microbial communities on the sediment. As floodplain clay soils become saturated, they quickly become anoxic and post-storm ponding of nutrient-rich water on top of these anoxic soils presents potential hot spots for denitrification to occur. The forested site had the lowest riparian DEA in both seasons and it had sandier soils. Sandy soils in the forested site facilitate fast drainage and deep water tables, so denitrifying microbial communities are not large due to lack of exposure to shallow subsurface nitrate-containing water.

Physiochemical factors such as soil moisture and percent organic carbon content of sediments seemed to drive denitrification. Though it seems floodplains, which have high connectivity to streams and shallower water tables, would have conditions of anoxia, often areas of the riparian zone had greater soil moisture than floodplains due to finer soil texture and topographic complexity (e.g., areas of depression, secondary channels, concentrated overland flowpaths). In the growing season, the unrestored + buffer site had the highest average denitrification rates. Riparian soils from this site had the greatest percent organic matter during the growing season. In the dormant season, the unrestored + buffer site and restored site also had the highest mean riparian denitrification rates, and had the highest percent organic matter and soil moisture. This indicates a combination of soil texture, organic matter, and anoxic conditions created by soil moisture may promote greater microbial activity and result in greater denitrification rates. Similarly, Groffman et al. (1989) found that soil texture and drainage accounted for 86% of the variability in annual denitrification N loss, and that as percent sand increases denitrification rate decreases. Soil texture and drainage control wetness which in turns controls oxygen availability. Additionally, plant uptake of nitrate in groundwater will be higher in the growing season, decreasing the concentration of nitrate available for denitrification. Roley et al. (2012) reported the presence of floodplain vegetation appeared to reduce soil nitrate availability.

Additionally, the forested site did not have nutrients running off an agricultural field to stimulate microbial community response. Rather, the forested stream, which runs through an agricultural catchment, is a source of nutrients to the floodplain. Thus, in-stream nutrient concentrations are higher than we would observe in a typical forested reference site, leading to greater potential denitrification values. However, this site is representative of the impacted nature of North Carolina Piedmont streams and illustrates the fact that catchment-level land use (e.g. agricultural activities and livestock operations) can still impact streams that are not located directly next to farms due to nutrient export in surface water. Though the reference site is not pristine it provides a representative forested stream type for examination that reflects water quality impacts of land use disturbance in a North Carolina Piedmont watershed. Conversely, the restored and unrestored site riparian zones supported larger microbial communities in response to exposure to nitrate.

4.5 Management Implications

This study demonstrates the importance of the existence of a wide riparian buffer and floodplain zone, as the riparian zone and floodplain possess the greatest capacity for denitrification and provide organic matter to participate in oxidation-reduction reactions and allow for vegetative uptake of ammonium and nitrate. Mayer et al. (2007) conducted a meta-analysis of nitrate removal and riparian buffers and asserted that wider buffers (>50 m) are more effective at removing a greater proportion of the nitrate load. It is important to have high stream-floodplain-riparian connectivity so that water from the channel can access the floodplain and riparian zone during storm events, when a high proportion of nitrate from fertilizers is likely to be transported to the stream via overland runoff and shallow subsurface flow. It is also important to design restoration projects to facilitate increased contact time between stream water and the floodplain to increase the nutrient retention and transformation capacity of floodplains. Replanting of riparian buffers also aids in reduction of sedimentation, which has implications for denitrification as sediments from fields can clog pores of streambed materials, reducing hyporheic exchange (Weigelhofer et al. 2013).

Seasonal differences in DEA exist. In this study, DEA was highest in the dormant season, when precipitation was highest. This has implications for timing of fertilizer application and importance of crop rotation. Soybeans, nitrogen fixers, should be alternated with nitrate-demanding crops so that fertility of the soil can be restored and fertilizer application can be minimized. Additionally, fertilizer should not be applied during times of high precipitation or in areas that produce a high amount of runoff and overland flow. Land use management and proper citing of best management practices, such as storm water retention ponds and constructed wetlands, is important in watersheds in the Piedmont region of North Carolina**,** as watershed land use can adversely impact all streams within the watershed.

Stream restoration is often used in conjunction with other best management practices so it is important to design stream restoration projects in a way that will be conducive not only to restoring the physical structure of the stream but to promoting nutrient removal. In unrestored streams, there is often increased downstream transport of nutrients due to restricted riparian and hyporheic exchange. Stream restoration should improve riparian exchange, locally increase hyporheic exchange (around restoration structures) and improve in-stream retention of nutrients, bringing streams to closer to an ideal pristine state of intensive riparian exchange and small downstream transport (Weigelhofer et al. 2013). Restoration may increase streambed heterogeneity and promote stream-floodplain connectivity to intensify contact between the stream water, riparian zone, and biogeochemically-active channel surfaces which may bind or remove nitrogen (Welti et al. 2012). Floodplain restoration has also been shown to increase substrate availability, leading to more efficient nitrogen and carbon cycling in the near-stream area (Welti et al. 2012). If space allows, restoration design may include re-meandering the stream to further increase stream-floodplain connectivity, instead of the particular design approach investigated in this study, which placed structures within a straightened channel. Another area of concern is that stream sediment in agricultural areas may be loaded with nutrient-rich soil from the catchment and have high nitrate demand and potential for ammonium mobilization, which may limit the effects of stream restoration in agricultural streams (Weigelhofer et al. 2013). Thus, a catchment-level approach to stream restoration may be necessary to also mitigate sources of internal stream eutrophication.

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APPENDIX A: DEA BY DEPTH

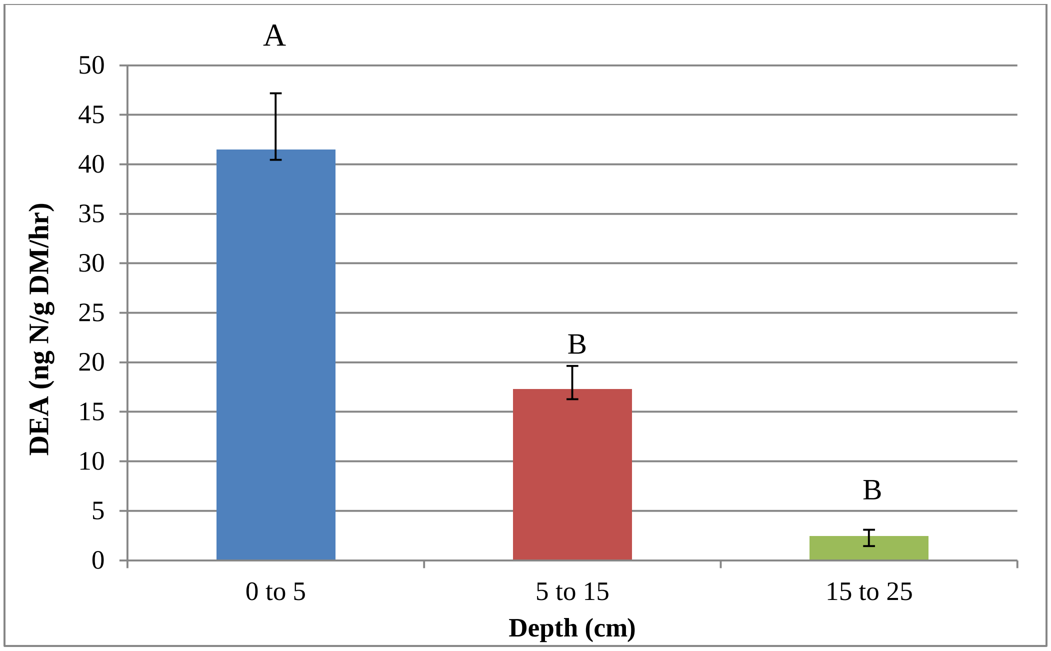


Figure 22: In-stream DEA by depth during the growing season (n = 49 at 0 to 5 cm, n = 40 at 5 to 15 cm and n = 15 at 15 to 25 cm), (one-way ANOVA, $p < 0.001$).

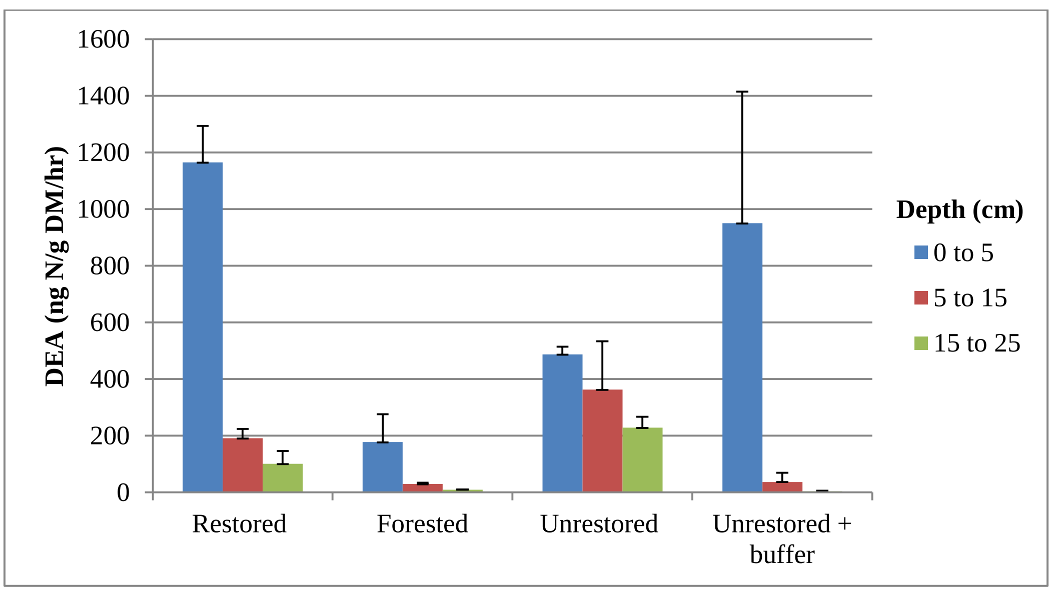
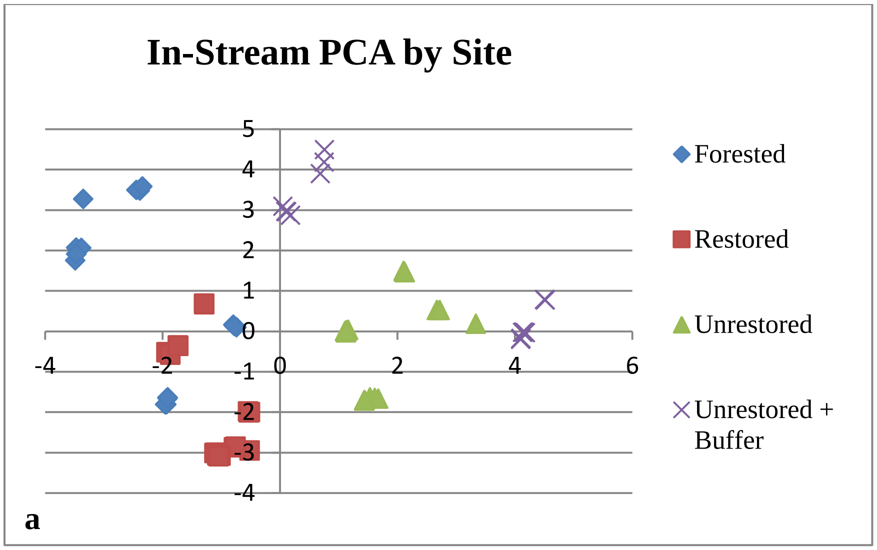
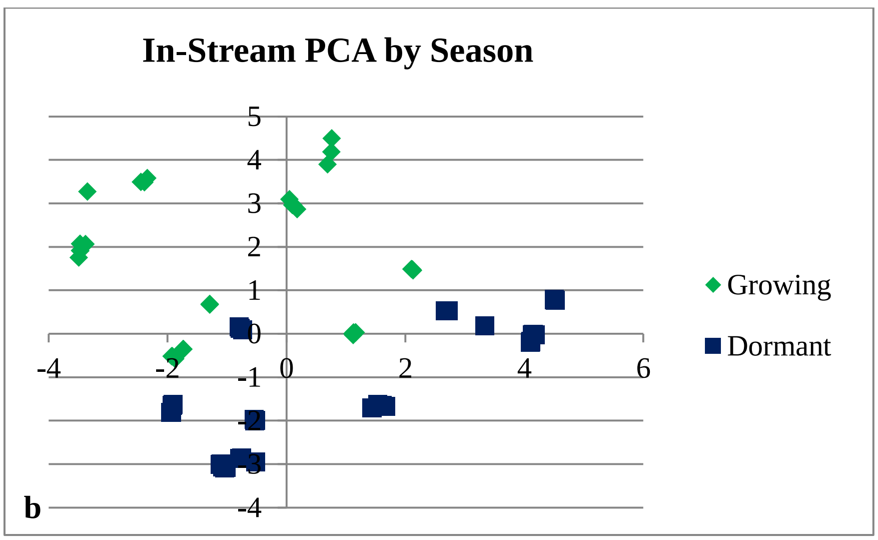


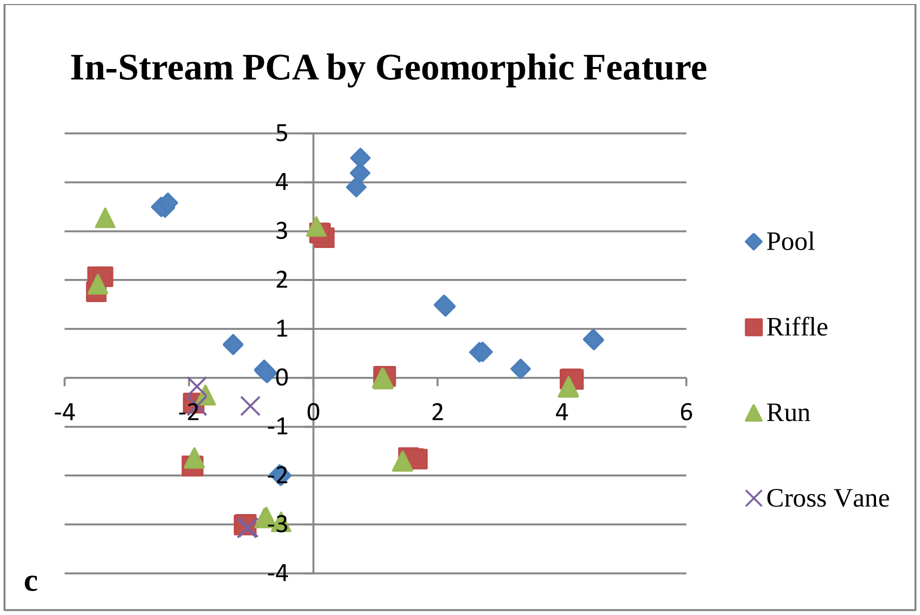
Figure 23: Riparian DEA by depth during the growing season, taken approximately 5 m into the riparian zone at each site (n = 3 for each depth at each site). Overall, the top 0-5 cm had significantly higher DEA than 15 to 25, while 5 to 15 cm was not significantly different than 0-5 cm or 15 to 25 cm (one-way ANOVA, $p = 0.252$).

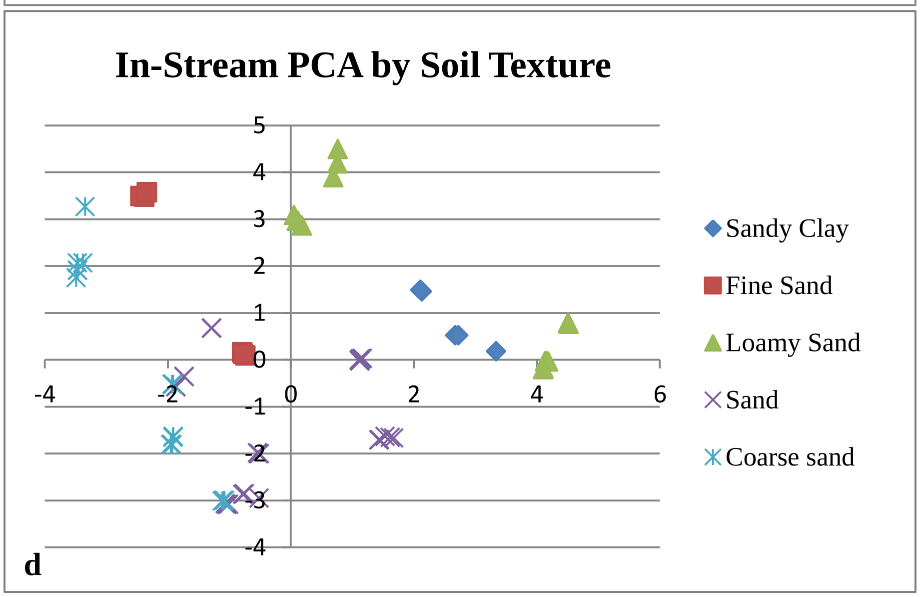
APPENDIX B: PRINCIPAL COMPONENTS ANALYSIS

In-Stream



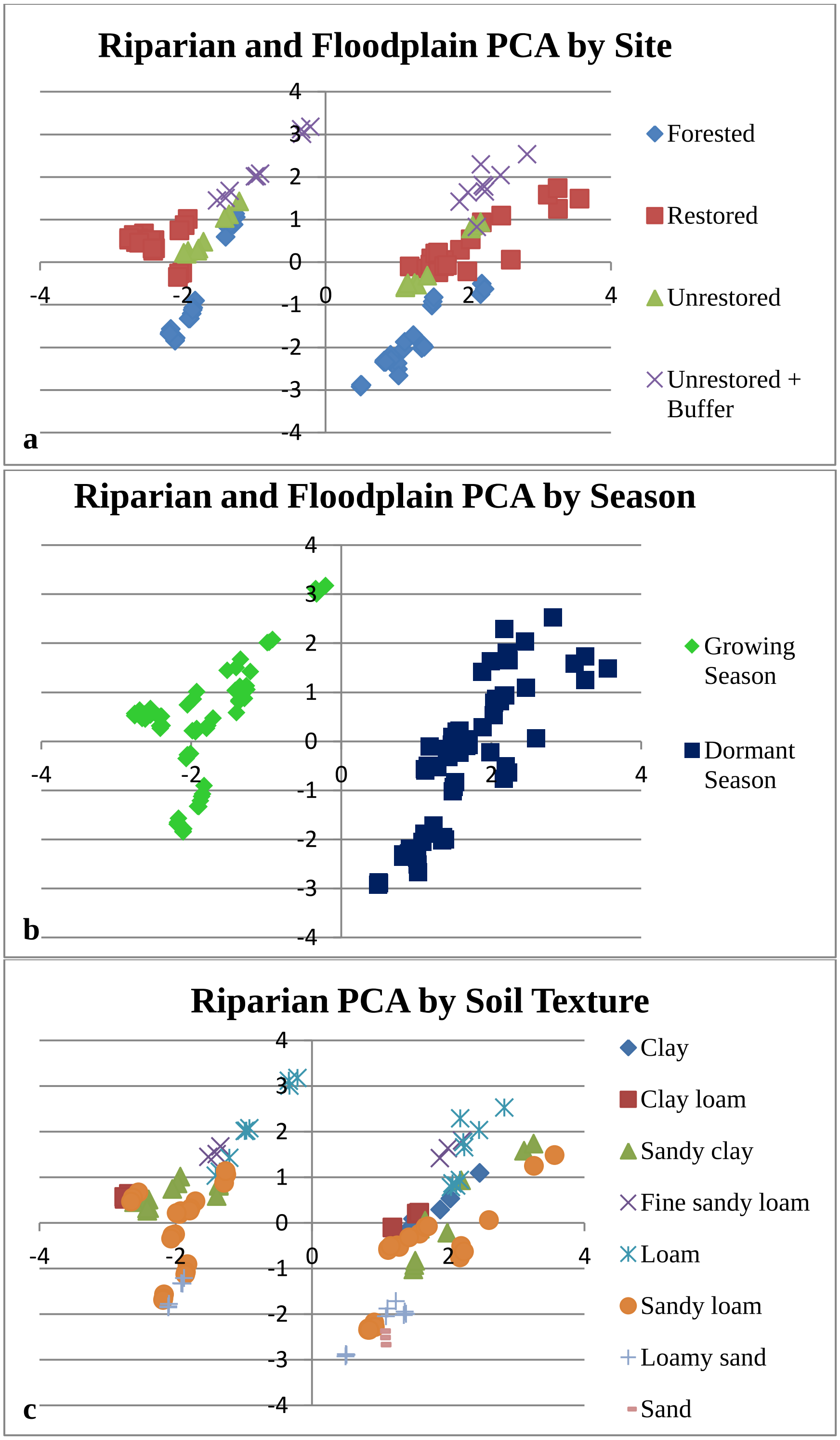






Figures 24 a-d: In-stream PCA results by site, season, geomorphic feature, and soil texture. Clustering was apparent between site, season, and soil texture. Clustering was less-defined between various stream features, indicating that stream morphological features did not behave the same in terms of DEA at each site.

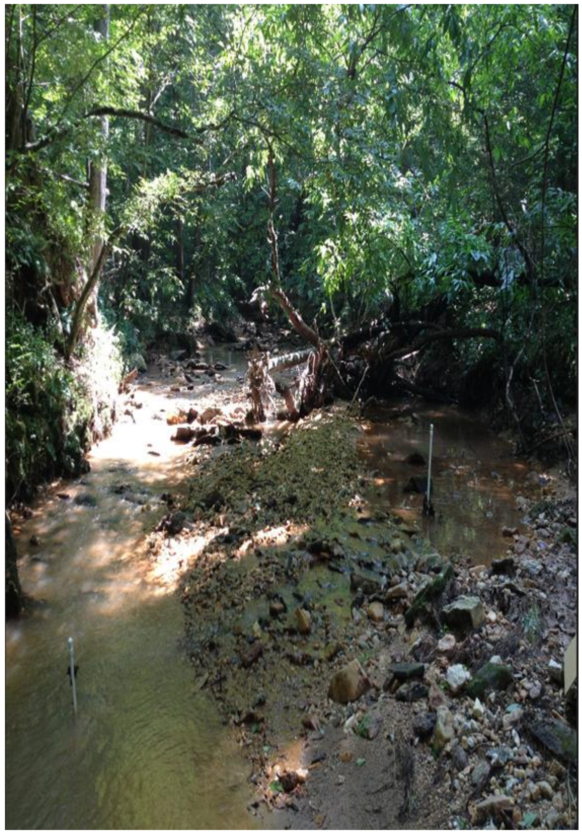
Riparian and Floodplain



Figures 25 a-c**:** Riparian PCA results by site, season, and soil texture. Clustering was apparent between seasons and sites.

APPENDIX C: SITE PICTURES

Forested Reference Site, Horne Creek



Restored Site, Tributary of Cook's Creek

Unrestored Site, Jackson Creek, Upstream

Unrestored Site + Buffer, Jackson Creek, Downstream