Soil denitrification dynamics in urban impacted riparian zones throughout Tampa, FL

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Abstract Denitrification is the anaerobic, microbial transformation of nitrate (NO_3^-) into inert, atmospheric nitrogen (N) under ideal conditions. It is a critical process in the management of anthropogenic NO_3^-, and has been shown to respond to elevated N concentrations within the soil of urban riparian zones. We investigated the relationship between land use / land cover (LULC) classifications on soil denitrification and associated biogeochemistry within coastal, sub-tropical riparian zones. Soil samples were collected from low-order streams throughout Tampa, FL at distances of 0 m, 5 m, and 10 m from the streambank. Results from factorial analysis indicate that LULC classification ($p = 0.005$, $F = 4.406$) was significant in predicting denitrification enzyme activity (DEA) potential, with high density residential sites showing the greatest average DEA potential at 2.439 mg N kg^{-1} h^{-1}. Variables showing significant difference based on LULC classifications were pH and soil carbon to N ratio, and showing that these factors likely had the most influence over riparian zone soil DEA potential based on LULC classification. These findings suggest that urban riparian zones are responding to elevated N loads when they are present; however, high residential areas showed lower carbon to nitrogen ratios than other sites, suggesting that some of the most urbanized areas could be improved to act as better NO_3^- sinks.

Keywords Denitrification, urban, riparian, forest, Florida

Introduction

Nitrate (NO_3^-) is a highly prevalent and chemically mobile water contaminant with negative impacts on human and environmental health (Spalding and Exner 1993, Townsend et al. 2003). Many uncertainties exist in the dynamics of landscape biogeochemistry and management of NO_3^- as the sources, transformations, transport, and fate of NO_3^- can be difficult to determine (VanBreemen et al. 2002, Puckett 2004) – especially given the amount work and resources needed to

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conduct studies on relatively small-scales (White and Reddy 1999, Kaushal et al. 2011). However, urban areas have generally been shown to be sources for nitrogen deposition and loading due to anthropogenic activity (Groffman et al. 2003, Xian et al., 2007, Fang et al. 2011). Correspondingly, control of NO_3^- has been emphasized in urban riparian zones, where soil biogeochemistry has been shown to support elevated denitrification (Groffman and Crawford 2003).

Denitrification is carried out by facultative, anaerobic microbes, when NO_3^- is reduced into dinitrogen (N_2) gas. The regulating factors of soil denitrification have been well studied. Nitrate, organic carbon, anoxic conditions, and a community of denitrifying microbes are required; however, pH, soil temperature, redox potential, and hydrogeological factors (e.g., water residence time) are also highly influential (Boyer et al. 2006, Rivett et al. 2008, De and Toor 2016). Nitrate is highly mobile within soil and water, but like many other non-point contaminants, prevention of its movement has been demonstrated via forested riparian strips adjacent to areas with intensive land use practices (Wenger 1999). Vegetation within riparian zones can influence the regulating factors of denitrification by providing a carbon source for microbial denitrifiers and altering soil hydrology to decelerate the transport of nitrate to open bodies of water or ground networks (Wenger 1999, Groffman and Crawford 2003).

Geographically, studies on nitrogen cycling and denitrification in urban riparian zones throughout the United States have been largely focused on eastern seaboard and a few western locations of the United States (Groffman and Crawford 2003, Puckett 2004, Grimm et al. 2005, Waters et al. 2014). These studies have primarily emphasized the impacts of reduced hydrologic complexity and the greater prevalence of impermeable surface area on ecosystem functionality, but others have since shown alterations that increase stream residence time can intensify the performance of ecosystem services (e.g., soil denitrification) within riparian zones (Kaushal et al. 2008; Newcomer et al. 2012).

Within the United States, Florida showed the second most coastal population growth from 1980-2003 and was estimated to have become the third most populous state in 2014 (Crosset et al. 2004, US Census Bureau 2015). Rapid, urban population growth and land use change is usually associated with increased impervious surface area (Lu and Weng 2006, Song et al. 2016). The hydrological result is usually a reduction to belowground water tables, residence times, and the base flow of stream water bodies (Shuster et al. 2005). For example, the high urban population density in Pinellas and Hillsborough counties (FL) has promoted run-off with high nutrient loads, resulting in the eutrophication of Tampa Bay (Wang et al. 1999); with similar conditions existing throughout the state (Barile 2004, Lapointe et al. 2015). Greater impervious surface area and population density has been shown to spatially correlate with increased deposition of NO_3^- in Tampa, likely due to residential land use practices like septic tanks, lawn fertilizers, pet waste, and landfills that can produce nitrogen leachates (Xian et al. 2007, Kaushal et al. 2011, Carey et al. 2012, Yang and Toor 2016, Lusk et al. 2017).

Relative to previous studies on riparian zone denitrification in urban areas, coastal Florida may support wider distribution of denitrification throughout the landscape due to extremely high water tables, high annual precipitation, and relatively warm climate temperatures; however, these favorable conditions may be countered by the dominance of well-drained soils and the heavily altered urban hydrology (Ovalles and Collins 1986, Brockmeyer et al. 1997, Burns et al. 2012). Due to these climate factors and the typically low water tables, we thought there may be little impact on riparian soil denitrification based on proximity to the steam. We also wanted to investigate if land use intensity could be used as a spatial predictor for riparian soil denitrification based on adjacent land classifications. Previous studies had compared rural sites to urban sites (Lowrance 1998, Wenger 1999, Watson et al. 2010), but few had explored the gradient from relatively undisturbed forest sites to areas that had high anthropogenic alteration (Groffman and Crawford 2003). Based on this, we decided to use forests as a proxy for relatively low intensity land use. Urban areas in Florida are also heavily used for residential purposes, so residential land was used in estimating anthropogenic impacts on urban areas. When viewing the composition of a given area based on the perspective of land use, the disciplines of geography and land management have tended to refer to these spatial, vector type classifications as 'land use / land cover' (i.e., LULC).

Our objectives for this study were to (i) evaluate if soil denitrification potential (DEA) is influenced by distance from the stream bank and land use intensity (using adjacent LULC class as a proxy for land use intensity), (ii) identify biogeochemical variables that are limiting and influential to DEA based on LULC, and (iii) determine if soil NO_3^- is potentially controlled within the sampled soils by denitrification.

Materials and Methods

Site selection and field methods. A watershed scale approach was taken towards understanding the geospatial patterns influencing denitrification throughout Tampa, FL and surrounding metropolitan areas (Figure 1). Most sites were located within a composite of the United States Department of Agriculture – Forest Service (USDA-FS) and United States Geological Survey (USGS) basin boundary for the Hillsborough River. The study was performed to characterize the upper limits of denitrification in relatively well-drained, upland areas that interface with riparian wetlands, and to provide a complementary research perspective to the more poorly-drained areas throughout the landscape for an inter-agency study in conjunction with the Environmental Protection Agency (EPA), the United States Geological Survey (USGS), the United States Department of Agriculture – Forest Service (USDA-FS), the government of Hillsborough County, the city government of Tampa, and the University of Florida (UF). Site selection was given preference towards low-order streams along 'upland' soil orders (i.e. spodosols and ultisols), with moderately well drained to excessively drained water retention capacity based on USDA-Natural Resources Conservation Service (USDA-NRCS) soil surveys.

Using LULC as a criterion for land use intensity, site selection ranged from relatively mature forests (i.e., remnant forests), younger, secondary forests (i.e., emergent forest), light intensity anthropogenic use (i.e., light density residential), and high intensity anthropogenic use (i.e., high density residential) (Figure 1). Forested sites were selected to represent relatively natural conditions and were determined to be either remnant (present >50 years) or emergent (present <50 years) based on historical aerial imagery. Sites were selected in high density residential (>5 housing units per acre) and light density residential (<2 housing units per acre) areas based on photo-interpreted Florida Land Use and Cover Classification System (FLUCCS) criteria from the Southwest Florida Water Management District.

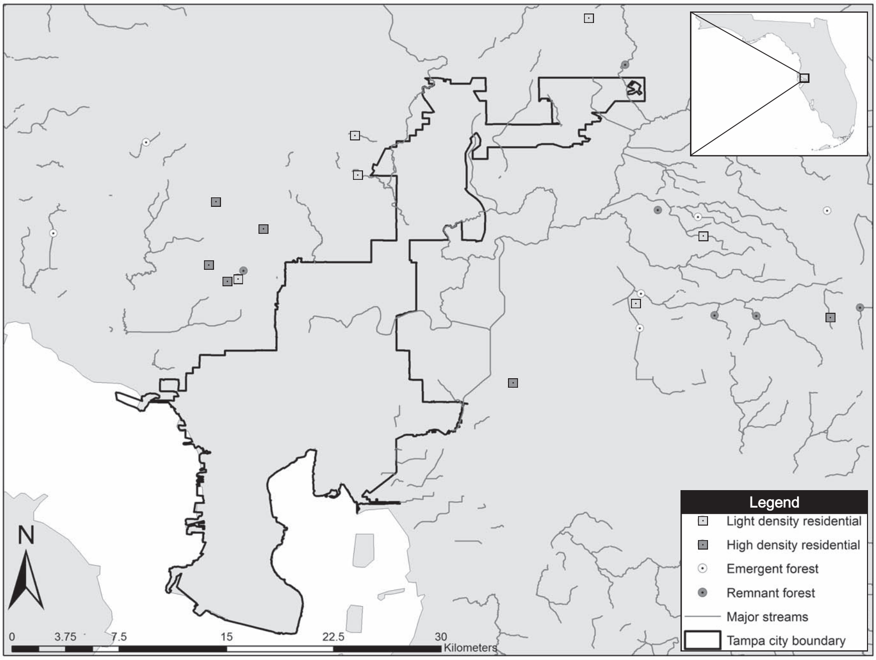

Figure 1. Map of the Tampa metropolitan area and study site locations based on LULC category.

Residential areas with high housing density could potentially have extremely high (>6 units per acre to super-dense city centers); but for the purposes of this study, high density residential sites were generally adjacent to housing developments with small property parcels or apartment complexes with less than three floors.

The twenty-four sites (six per LULC category) were sampled in March 2013. Preliminary sampling of riparian zone soil showed relatively little denitrification activity below 0-10 cm relative to soil near the surface, likely due to low levels of soil organic matter below this depth (Roberts, unpublished data). Therefore, sampling priority was placed on distance from the stream. Sites consisted of transects set perpendicular to the stream, and samples were taken at distances of 0 m, 5 m, and 10 m from the stream bank. The elevation of the 5 m and 10 m sampling points along the transect were assessed relative the 0 m sample point at the stream bank with a clinometer and surveying rod. Surface soil from 0-5 cm was collected at points 5 m parallel to each side of the main transect with a 1.9 cm wide soil probe. Three homogenized samples, one for each distance from the stream bank (i.e., 0 m, 5 m, and 10 m), were taken for each site. Each subsample was taken with the probe in close proximity (~0.5 m radius) to the sampling point (i.e., 0 m, 5 m, and 10 m, respectively) to produce ~250 mL of soil per bag for each distance along the transect. Each sample was homogenized by stirring within the bag *in situ* to compensate for small-scale heterogeneity often encountered when collecting soil for soil denitrification potential (Lowrance 1992, Gold et al. 1998, Florinsky et al. 2004). Tree stem basal area for each site was observed from each soil sampling point at 5 m from the stream bank with a 10 factor wedge prism. Vegetative structure and canopy dominance varied by site, but prevalent species were laurel oak (*Quercus laurifolia* Michx.), water oak (*Quercus nigra* L.), live oak (*Quercus virginiana* Mill.), red maple (*Acer rubrum* L.), sugarberry (*Celtis laevigata* Willdenow), and slash pine (*Pinus elliottii* Engelm.). Average annual precipitation in the area has been shown to be approximately 117.6 cm (NOAA 2014). Precipitation patterns vary by season, with summer considered the rainy season and winter the dry season. There is fairly consistent rainfall from June to September (i.e., on average, 70.7 cm total from June-September) but much less throughout the rest of the year (46.9 cm total from October-May) (NOAA 2014). Sampling occurred in March 2013 (i.e., towards the end of the dry season). Given the spatial context of this study (i.e., riparian zones in well-drained areas), this study emphasized the role of soil denitrification for a relatively wet system in the landscape (i.e., due to high water tables) under relatively dry seasonal conditions.

Analytical methods. Soil samples were transported on ice to the UF Wetland Biogeochemistry Laboratory (WBL) in Gainesville, FL. Samples were stored in a refrigerator at 4°C ±1°C until preparation or analysis. Moisture content was determined by placing samples in a drying oven at 70°C for 72 h (McInnes et al. 1994, Reddy et al. 2013). Soil samples were passed through a 2 mm sieve to break down organic matter and soil aggregates. Soil organic matter content was determined by loss on ignition (LOI) method by combusting soil at 550°C for 4 h (Nelson and Sommers 1996). Inorganic N content (NO_3^- and NH_4^+) within soil samples was determined with colorimetric analysis at the UF Analytical Research Laboratory in accordance with EPA methods 353.2 (USEPA 1993a) and 350.1 (USEPA 1993b). Soil pH was determined in a 1:2 soil:water suspension after equilibration for 30 min (Reddy et al. 2013). Total carbon and total nitrogen analyses were conducted with an elemental analyzer (Thermo FlashEA® 1112, CE ElantechInc., USA).

Denitrification enzyme assay. Denitrification enzyme activity (DEA) was measured within two weeks of sampling based on the method described by Smith and Tiedje (1979) and further refined by White and Reddy (2003). Briefly, two sets were prepared with each field moist soil sample (5 g, dry weight equivalent) in a 60 ml serum bottle with 5 ml sterile distilled deionized water. Chloramphenicol was added to inhibit de novo enzyme synthesis. One of the two sets was also amended with nitrate (0.40 KNO_3^--N g L^{-1}) to determine the potential of denitrifying enzyme activity under non-limiting N conditions and the other was amended with glucose (0.72 $C_6H_{12}O_6$-C g L^{-1}) and NO_3^- (0.40 KNO_3^--N g L^{-1}) to determine the presence of carbon limiting denitrification. Serum bottles were closed with butyl stoppers and sealed with aluminum crimps before being purged with nitrogen gas to create anaerobic conditions. Approximately 10% of the headspace gas was replaced with acetylene to block the reductive transformation of N_2O to N_2. Serum bottles were incubated in dark with gentle shaking for 1 h to ensure adequate diffusion and exposure to acetylene. The gas in the serum bottle headspace was sampled and analyzed for nitrous oxide (N_2O) with a gas chromatograph (GC-14A, Shimadzu Scientific Instruments, Columbia, MD) fitted with a porapak Q column and an electron capture detector (ECD) with the rate of injection intervals at $\sim$1 hr^{-1} over the course of 4-6 hrs (White and Reddy, 2003).

Data analysis. Modeling for DEA was performed as a factorial analysis using the lm() function in R (R Development Core Team 2013). The main effects tested were LULC, distance from the streambank, and the presence or absences of glucose as an amendment for DEA microcosms. Additionally, the measured soil and site factors (i.e., pH, NO_3^-, NH_4^+, moisture, total nitrogen, total carbon, carbon to nitrogen ratio, soil organic matter, stem basal area) were compared across LULC and distance from the stream using MANOVA. Significant factors from the factorial analysis and the MANOVA were tested via univariate ANOVA and Tukey's range test. All variables were also compared by distance (i.e, 0 m, 5 m, and 10 m) with ANOVA. Student *t*-tests and Pearson's correlation coefficient were selectively utilized to compare significant factors based on LULC, while paired t-test analyses were used for comparing these variables based on distance within a given LULC. Residual plots were used to assess where the underlying assumptions of the analyses were met, and all conclusions were made with an α=0.05 of type 1 error. All statistical analyses were performed with the SAS statistical program and R programming (SAS Institute 2008, R Development Core Team 2013).

Results

Comprehensive findings for all sites. Average DEA potential was 0.58 mg N kg^{-1} h^{-1}, with the maximum rate recorded at 2.86 mg N kg^{-1} h^{-1} on a high density residential site (Figure 2). Regression analyses for all categorical variables (i.e., LULC, distance from stream, and use of glucose during assay extraction) showed that LULC was a significant predictor for DEA (p = 0.41, Table 1). Based on

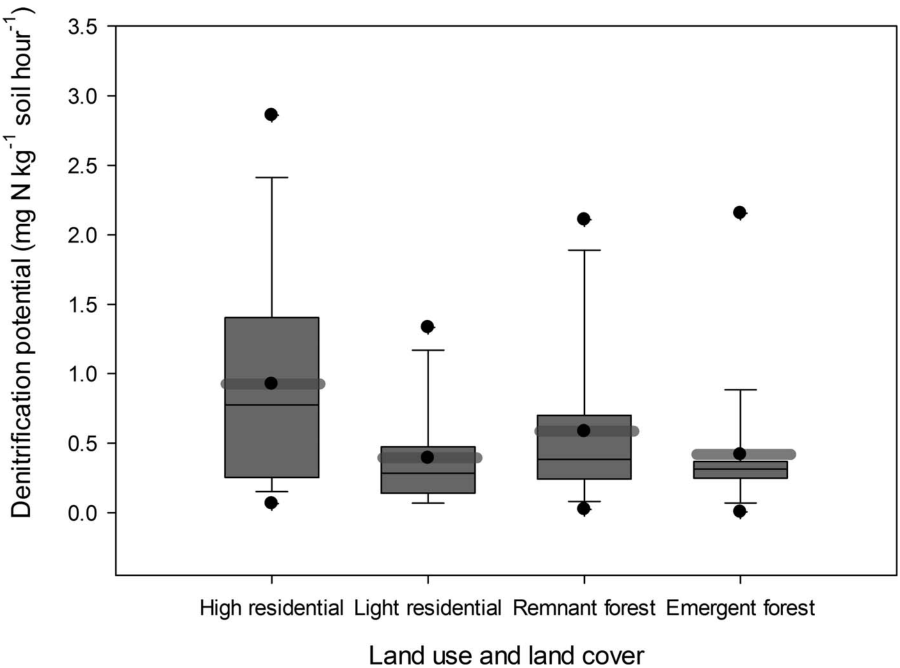

Figure 2. Box-plot distributions of DEA samples amended with NO_3^- and glucose based on LULC with standard error, outliers, and means (indicated with horizontal lines with rounded sides). All distances (0 m, 5 m, and 10 m) are combined (n=18).

LULC, high density residential showed significantly greater DEA potential than light density residential and emergent forest LULC classifications based on Tukey's range test (Table 2). Although the glucose inclusion was not significant for modelling DEA potential, direct comparison showed soils amended with glucose and NO_3^- were marginally greater than samples only amended with NO_3^- ($p = 0.050$, $t = 2.01$), with significance at 5 m ($p = 0.023$, $t = 2.44$; Figure 3). Average DEA potential of samples amended with glucose and NO_3^- were also significantly greater at 5 m than 10 m ($p = 0.028$, $t = 2.357$; Figure 3), and in particular, high residential sites showed a 130.2% increased average DEA potential from 5 m to 10 m ($p = 0.04$, $t = 2.74$; Figure 3).

Table 1. Model for DEA potential based on land use / land class, distance, and presence of glucose as an amendment.

Factor	df	Sum of Squares	Mean Square	F Value	p Value
Land use / land cover	3	36.35	12.116	4.406	0.005**
Distance	1	2.77	2.772	1.008	0.317
Glucose amendment	1	5.64	5.643	2.052	0.154
Residuals	136	374.01	2.750		

** Statistically significant variable based on general linear model at $p < 0.01$.

Table 2. ANOVA for DEA potential based on land use / land cover classification.

Land Use / Land Cover Class	Mean DEA (mg N kg^{-1} h^{-1})	Standard Error	r	Tukey HSD
High density residential	2.439	2.345	36	a
Light density residential	1.124	0.976	35	b
Remnant forest	1.622	1.434	35	ab
Emergent forest	1.305	1.583	36	b

Significant differences were detected between LULC categories based on MANOVA for pH ($p = 0.015$, $F = 4.416$ at 5 m; $p = 0.041$, $F = 3.345$ at 10 m), carbon to nitrogen ratio ($p = 0.018$, $F = 4.251$ at 5 m; $p = 0.001$, $F = 7.666$ at 10 m), and total nitrogen ($p = 0.049$, $F = 3.142$ at 10 m) (Table 3). However, only pH ($p =$ 0.034 at 10 m, Table 4) and carbon to nitrogen ratio ($p = 0.001$ at 10 m, Table 4) were significant under an ANOVA (Table 4). High density residential sites showed significantly higher average pH relative to emergent forest sites, and high density residential showed a lower carbon to nitrogen ratio relative to both 'forested' classifications based on Tukey's range test (Table 4).

The ANOVA comparison of variables (n=24) between distance (0 m, 5 m, and 10 m) showed few differences. However, moisture content ($p = <0.0001$, $F =$ 36.465) and pH ($p = 0.0043$, $F = 5.909$) were significantly greater at 0 m (Table 3).

Inferences on significant variables based on LULC. Average DEA potential for high density residential sites was significantly higher (p = 0.04,

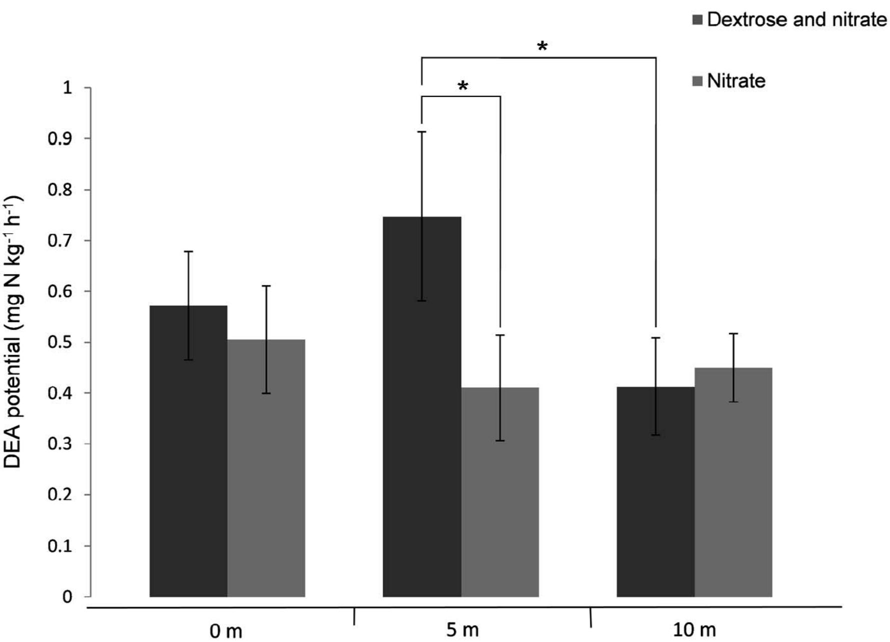

Figure 3. Average DEA based on distance comparing sample amendments (+N +C vs. +N) with standard error (n=24). * indicates significant difference at p < 0.05.

Table 3. Variables sampled based on distance from stream and LULC averages are presented (n = 6) for each combination of categories along with the total average (n = 24). Values for associated standard error (SE) are in parentheses.

Dist.	Variable	High Density Residential	Light Density Residential	Emergent Forest	Remnant Forest	Total Average
0 m	pH	7.07 (0.32)	6.35 (0.28)	6 (0.26)	6.04 (0.31)	6.37 (0.17)
	Soil NO_3^-, mg N kg^{-1}	3.59 (2.38)	1.96 (0.97)	3.71 (3.07)	3.47 (2.1)	3.18 (1.06)
	Soil NH_4^+, mg N kg_{-1}	7.79 (2.35)	6.02 (1.19)	7.62 (1.26)	8.29 (1.68)	7.43 (0.81)
	Soil moisture, g kg^{-1}	262.53 (22.27)	216.07 (11.08)	234.75 (10.65)	249.53 (25.4)	240.7 (9.37)
	Total nitrogen, g kg^{-1}	1.33 (0.6)	0.67 (0.2)	1.13 (0.37)	1.23 (0.41)	1.09 (0.2)
	Total carbon, g kg^{-1}	24.3 (10.54)	12.28 (3.56)	17.99 (5.25)	23.08 (9.05)	19.41 (3.69)
	C:N ratio	17.4 (1.44)	18.81 (1.76)	16.4 (0.63)	17.65 (0.73)	17.57 (0.6)
	Soil organic matter, g kg^{-1}	41.6 (18.33)	25.91 (6.97)	39.11 (11.49)	42.76 (14.68)	37.35 (6.46)
5 m	pH*	6.32[a] (0.27)	5.4[ab] (0.43)	4.5[b] (0.18)	5.69[ab] (0.47)	5.48 (0.22)
	Soil NO_3^-, mg N kg^{-1}	10.89 (4.83)	4.17 (1.98)	1.36 (0.64)	6.25 (2.55)	5.67 (1.54)
	Soil NH_4^+, mg N kg^{-1}	7.85 (1.77)	9.89 (2.62)	10.33 (2.08)	7.33 (1.07)	8.85 (0.95)
	Soil moisture, g kg^{-1}	119.16 (31.45)	125.67 (48.87)	115.98 (29.34)	93.82 (16.56)	113.66 (1.59)
	Total nitrogen, g kg^{-1}	1.65 (0.5)	1.28 (0.42)	1.28 (0.17)	1.38 (0.17)	1.38 (0.17)
	Total carbon, g kg^{-1}	25.17 (7.41)	25. 68 (10.27)	27.15 (3.95)	24.64 (4.01)	25.66 (3.24)
	C:N ratio*	15.95[b] (0.8)	18.92[ab] (1.19)	21.1[a] (1.28)	18.86[ab] (0.73)	18.71 (0.61)
	Soil organic matter, g kg^{-1}	54.21 (13.6)	109.57 (52.15)	53.01 (6.45)	50 (7.76)	66.72 (13.79)
	Stem basal area, m^2 $100^{2\ -1}$	1.44 (0.42)	3.89 (0.42)	2.69 (0.51)	3.7 (0.51)	2.93 (0.47)
10 m	pH*	6.38[a] (0.28)	5.75[ab] (0.47)	4.78[b] (0.16)	5.65[ab] (0.43)	5.64 (0.2)
	Soil NO_3^-, mg N kg^{-1}	8.91 (2.45)	7.35 (3.47)	1.68 (0.93)	6.02 (2.14)	5.99 (1.26)
	Soil NH_4^+, mg N kg^{-1}	7.95 (0.75)	6.55 (1.15)	11.14 (2.95)	7.75 (1.08)	8.35 (0.87)
	Soil moisture, g kg^{-1}	107.96 (22.9)	96.24 (23.04)	119.66 (29.7)	107.13 (16.25)	108.25 (11.09)
	Total nitrogen, g kg^{-1}	2.03 (0.32)	1.13 (0.2)	1.17 (0.19)	1.63 (0.28)	1.49 (0.14)
	Total carbon, g kg^{-1}	29.81 (5.16)	20.46 (20.46)	23.87 (2.65)	31.34 (6.87)	26.37 (2.45)
	C:N ratio**	14.58[b] (0.5)	17.97[ab] (1.04)	21.3[a] (1.14)	18.69[a] (1.13)	18.14 (0.68)
	Soil organic matter, g kg^{-1}	60 (0.95)	55.22 (21.27)	47.93 (5.46)	100.6 (45.87)	67.41 (11.71)

* Statistically significant variable at $p < 0.05$.
** Statistically significant variable at $p < 0.01$.
[a, b, ab] Statistical comparison between variables based on Tukey HSD

Table 4. ANOVA of significantly different variables based on land classification

Factor	Land Class	Mean	Standard Error	r	Min	Max		Tukey HSD
pH (5 m)	emergent forest	6.32	0.44	6	3.71	4.98		b
	remnant forest	5.69	1.16	6	4.42	7.17		ab
	high density residential	6.32	0.66	6	5.55	7.29		a
	light density residential	5.40	1.05	6	4.51	7.37		ab
							Pr(>F)	0.0154
C:N ratio (5 m)	emergent forest	21.10	3.13	6	16.83	26.16		a
	remnant forest	18.86	1.78	6	15.60	20.32		ab
	high density residential	15.95	1.96	6	14.12	18.64		b
	light density residential	18.92	2.90	6	16.23	22.99		ab
							Pr(>F)	0.0178
pH (10 m)	emergent forest	4.78	0.40	6	4.20	5.42		b
	remnant forest	5.65	1.06	6	4.64	7.22		ab
	high density residential	6.38	0.56	6	5.80	7.44		a
	light density residential	5.75	1.16	6	4.44	7.93		ab
							Pr(>F)	0.0344
C:N ratio (10 m)	emergent forest	21.31	2.79	6	17.97	25.08		a
	remnant forest	18.69	2.77	6	14.80	22.30		a
	high density residential	14.58	1.23	6	12.73	15.94		b
	light density residential	17.97	2.54	6	13.94	21.12		ab
							Pr(>F)	0.0012

+130.2%) at 5 m than 10 m, but average NO_3^- did not correspondingly decrease (Table 3). Relative to light density residential sites, high density residential sites showed a lower carbon to nitrogen ratio ($p = 0.034$, -18.62% at 5 m; $p = 0.011$, -18.86% at 10 m) and stem basal area ($p = 0.0218$, $t = -3.8244$; Table 2), but greater average total nitrogen ($p = 0.023$, +79.65% at 10 m) (Table 2).

Average soil NO_3^- concentrations in remnant forests were over three times that of emergent forests at distances of 5 m and 10 m, but would have only shown marginal significant difference at an $\alpha = 0.1$ (Table 3). The average pH of emergent forest samples showed significant difference from the other LULC classifications based on Tukey's honest significance test, and average pH levels were 4.5 and 4.78 at distances of 5 m and 10 m, respectively (Table 3; Table 4). Low pH on emergent forest sites also correlated with a decrease in DEA potential ($r^2 = 0.224$, $p = 0.0038$).

Discussion

Land use / land cover. Similar to results observed by Groffman and Crawford (2003), urban conditions showed little impairment on soil denitrification potential relative to other sampled LULC classifications, while the high density residential sites in this study showed significantly elevated DEA potential relative to other LULC categories (Table 1; Table 2). The DEA potentials on soil collected at the end of the subtropical dry season were relatively low compared those yielded by Groffman and Crawford (2003), ranging from 0.23 to 7.59 mg N kg^{-1} h^{-1} in

Baltimore during June, but fit well in comparison to other studies sampling DEA potential from riparian zone soil (Table 5).

The average DEA potential was significantly greater for high density residential sites relative to emergent forest and light density residential classifications (Table 2). High density residential sites also showed other significant differences, like a relatively neutral pH level and lower carbon to nitrogen ratios – this likely best explains what drove higher average DEA potential for high density residential sites relative to emergent forest and light density residential land classifications (Table 4).

Xian et al. (2007) noted that impervious surface area and population density could predict total nitrogen ($r^2 = 0.72$), total Kjehldahl nitrogen ($r^2 = 0.70$), and nitrate/nitrite ($r^2 = 0.64$) loading in Tampa. Given that high density residential areas also tend to have greater impervious surface and population densities, the LULC classifications may be a potential alternate to population density and traffic density for the spatial prediction of nitrogen deposition and soil nitrogen concentrations. This inference is supported by our data indicating that greater DEA potential and lower carbon to nitrogen ratios were also found on high residential sites (Tables 2 - 4).

Stream proximity and biogeochemical implications. Average nitrate levels did not significantly change (i.e., at α=0.5) through the riparian zones, but there was a decrease of 43.7% from 5 m to 0 m ($p = 0.0835$, $t = -1.809$; Table 3), indicating nitrate removal from soil denitrification, vegetative uptake, movement into the hyphorheic zone, removal from the site via inundation and stream transport, or some combination of these events. Removal of the soluble biogeochemical drivers of soil denitrification (i.e., carbon and nitrogen) can be influenced by proximity to the stream via saturation and periods of inundation, so microbial denitrification cannot be exclusively attributed for this decreased N or NO_3^- (Hill 1996, Wenger 1999; Table 3).

Our biogeochemical findings largely support what researchers and managers often already know: areas suspected of high nitrogen concentrations are usually accompanied by corresponding enzymatic response by soil denitrifiers. Opportunities for *in situ* treatment of NO_3^- may often be undercapitalized – potentially evidenced by the increased DEA potential for samples amended with carbon at 5 m, and significantly lower carbon to nitrogen ratios for high density residential sites relative to the forested land classes (Figure 2, Table 4). Forest carbon pools have been shown to become less labile and more passive with increasingly urban conditions, but for this study, there exists too many uncertainties regarding carbon pool composition and soil microbial communities to attribute the increase in soil DEA potential at 5 m to carbon quality (McDonnell et al. 1997). Management implications between light density residential and high density residential showed that significant differences in stem basal area existed ($p = 0.0218$, $t = -3.824$, Table 3); however, there was still a carbon to nitrogen ratio and DEA potential gradient that loosely followed land use intensity (i.e., high density residential sites to light residential to the forested land types) (Table 2, Table 4). The greater stem basal area for light density residential sites was likely an effect of residential housing

Table 5. Comparison of average soil DEA potential rates from this project (listed as Roberts et al., 2018) and similar studies (all soil samples are riparian, from the top 0-10 cm, and amended with nitrate, dextrose, and acetylene unless otherwise noted).

Publication	**Location**	**Time of Sampling**	**Land Classification**	**Substrate**	**Distance from Stream**	**Average DEA Rate as mg N kg^{-1} h^{-1} (SE - When Applicable)**
Bettez and Groffman 2012†	Baltimore County, MD, USA	September 2011	rural and urban	herbaceous	varied	0.21 - 0.53
			rural and urban	forest	varied	0.07 - 0.7
			urban	herbaceous/forest	3 - 5 m	0.27 (0.228)
Groffman et al. 2001‡	Talamanca, Costa Rica	June 1995 and February 1996	rural	forest	varied	2.2 (4.9)
			rural	converted forest	varied	5.1 (2.1)
			rural	forest	varied	28.6 (21.9)
			rural	converted forest	varied	5.8 (2.1)
Groffman and Crawford 2003	Baltimore, MD, USA	June 1998	urban	herbaceous and forested	5 m	2.63 (1.02)
			rural	herbaceous and forested	5 m	3.59 (0.5)
Hill and Cardaci 2004§	Boyne River, ON, Canada	N/A	rural	mixed forest	varied	1.49 (0.13)
Lowrance 1992	Tifton, GA, USA	year-round average 1988-1989	rural - agricultural	forest	5 - 55 m	0.0025
		April 1989	rural - agricultural	forest	10 m	0.0007
		August 1990	rural - agricultural	forest	10 m	0.004
Newcomer et al. 2012¶	Baltimore County, MD, USA	June 2006	urban	N/A	~1 m	0.0301 (0.0088)
			rural	forest	~1 m	0.002 (0.001)
Roberts et al. 2018 (this publication)	Hillsborough County,FL, USA	March 2013	urban / rural interface (all sites)	herbaceous/forest	0 m	0.57 (0.11)
			urban / rural interface (all sites)	herbaceous/forest	5 m	0.75 (0.17)
			urban / rural interface (all sites)	herbaceous/forest	10 m	0.41 (0.10)

Table 5. Continued.

Publication	Location	Time of Sampling	Land Classification	Substrate	Distance from Stream	Average DEA Rate as mg N kg^{-1} h^{-1} (SE - When Applicable)
urban - high residential	herbaceous/forest	5 m	1.45 (0.47)			
White and Reddy 1999[#]	SFWMD Water Conservation Area 2A, FL, USA	August 1996 and March 1997	rural	wetlands	N/A	0.0013-2.4659

[†] 0-5 cm soil depth
[‡] 0-15 cm soil depth
[§] Not amended with dextrose (C)
[¶] Not amended with nitrate (NO_3^-) because of saturation, soil depth sampled ~0.5 m below baseflow
[#] Non-riparian

development practices, as vegetation near streams is often left intact relative to nearby property (Searns 1995).

Emergent forest sites showed low soil pH levels, which also correlated with decreased concentrations of NO_3^- ($p = 0.0025$). The transformation of ammonium (NH_4^+) to NO_3^- is known to be inhibited under acidic soil conditions, and this appears to be the case for emergent forest sites based on ratios of soil NO_3^- to NH_4^+ at 5 m and 10 m (Anthonisen et al. 1976, Simek and Cooper 2002; Table 3). Acidic conditions can also negatively impact denitrification, as Müller et al. (1980) showed soils with a pH < 4.5 were only able to reduce 3 to 10% of added NO_3^- into nitrous oxide (N_2O) under anoxic conditions. Greater DEA potential and pH were correlated for emergent forests at 0 m, which may indicate the stream alters soil pH. Stream transport of NO_3^- may also exert some influence on riparian zone denitrification under acidic soil conditions and when NO_3^- is limited throughout the rest of the riparian zone (Warwick and Hill 1988, Royer et al. 2004). It is unclear what the driver of low-pH conditions in emergent forest riparian zones might be, but other studies have shown connections between clearcutting and old-field weathering on pH (Johnson et al. 1991). Markewitz et al. (1998) showed fields converted to pine forests on ultisols decreased in pH-value by 1 unit in the upper 0 to 15 cm of soil over 34 years; the decrease in pH was largely attributed to biomass accumulation, soil respiration, and the development of soil organic matter.

Conclusion and future work. Similar to Groffman and Crawford (2003), we showed that riparian zones in urban areas could respond to elevated nitrogen loads with increased denitrification, and that urban riparian soil denitrification appears to be impacted by LULC (i.e., a proxy for land use intensity). Carbon to nitrogen ratio and pH were the main drivers linked to denitrification (i.e., via DEA) potential when differentiating sites based on land classification. The more urbanized sites (i.e., high density residential) could likely be the focus of future management and potential stream restoration since these areas showed relatively low carbon to nitrogen ratios. The soil DEA microcosms responded significantly at 5 m when amended with a carbon source (particularly from soil collected from high density residential sites) – suggesting carbon limitations relative to the existing presence of denitrifying enzymes in the soil at an influential portion of the riparian zone. However, this trend would greatly benefit from accompanying insight on carbon quality or microbial biomass.

As sampling in this study was oriented towards the expected upper-bounds of riparian DEA potential on well-drained sites during the subtropical the dry season, it would be complemented by perspectives on more poorly drained and coastal areas throughout the landscape. Larger streams and rivers may extend the influence of the water table throughout the surrounding terrestrial landscapes (i.e., throughout floodplains) and buffer against from aerobic conditions during intervals of low precipitation that can lead to decreased or ceased enzyme production by microbial denitrifiers (Naiman and Décamps 1997). Future work in the study area, and other regions lacking perspective on this topic, would also benefit from a time-series perspective. This would be particularly useful for determining the responses of soil microbial communities to different moisture conditions over short-term (i.e., intervals of high or low precipitation over days or weeks) and long-term sampling durations (i.e., seasonal variation) throughout the landscape.

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