

Effects of Riparian Buffer Vegetation and Width: A 12-Year Longitudinal Study

S. E. King, D. L. Osmond,* J. Smith, M. R. Burchell, M. Dukes, R. O. Evans, S. Knies, and S. Kunickis

Abstract

Agricultural contributions of nitrogen are a serious concern for many water resources and have spurred the implementation of riparian buffer zones to reduce groundwater nitrate (NO_3). The optimum design for buffers is subject to debate, and there are few long-term studies. The objective of this project was to determine the effectiveness over time (12 yr) of buffer types (trees, switchgrass, fescue, native, and a control) and buffer widths (8 and 15 m) by measuring groundwater NO_3 -N and dissolved organic carbon (DOC) trends. At the intermediate groundwater depth (1.5–2.1 m), NO_3 -N reduction effectiveness was 2.5 times greater (46 vs. 16%) for the wider buffer, and, regardless of width, buffer effectiveness increased $0.62\% \text{ yr}^{-1}$. Buffer vegetative type was never statistically significant. In the deep-groundwater depth (2.1–3.5 m), there was no change in NO_3 -N removal over time, although the statistical interaction of width and vegetative type indicated a wide range of removal rates (19–82%). The DOC concentrations were analyzed at the field/buffer and buffer/stream sampling locations. Depending on location position and groundwater sampling depth, DOC concentrations ranged from 1.6 to 2.8 mg L^{-1} at Year 0 and increased at a rate of 0.13 to 0.18 $\text{mg L}^{-1} \text{ yr}^{-1}$ but always remained low ($\leq 5.0 \text{ mg L}^{-1}$). Greater DOC concentrations in the intermediate-depth groundwater did not increase NO_3 -N removal; redox measurements indicated intermittent reduced soil conditions may have been limiting. This study suggests that riparian buffer width, not vegetation, is more important for NO_3 -N removal in the middle coastal plain of North Carolina for a newly established buffer.

Core Ideas

- Unique, long-term replicated riparian buffer study in the southeastern United States.
- Long-term riparian buffer study from establishment through Year 12.
- Effects of five different riparian-buffer vegetation types of nitrate reduction.

NUTRIENT IMPAIRMENT of waters remains a concern throughout the United States (Dubrovsky et al., 2010). In particular, discharged nitrogen (N) can result in algae blooms and fish kills (Paerl and Otten, 2013). To reduce agricultural nonpoint source N contributions, riparian buffers are promoted as a conservation practice because they have repeatedly been shown to improve water quality through the removal of groundwater nitrate (NO_3 -N) (Gilliam, 1994; Haycock and Pinay, 1993; Hill, 1996; Hubbard et al., 1998; Lowrance et al., 1984; Mayer et al., 2007; Osborne and Kovacic, 1993; Peterjohn and Correll, 1984; Simmons et al., 1992; Smith et al., 2006). Variation in reported NO_3 removal efficiencies has been attributed to vegetation, width, hydrology, carbon availability, and buffer age.

There is no consensus as to the best riparian buffer vegetation for groundwater NO_3 removal. Some researchers observed generally greater NO_3 removal in forested buffers (Haycock and Pinay, 1993; Hefting and de Klein, 1998; Osborne and Kovacic, 1993), whereas others observed greater removal by grass (Lowrance et al., 1995; Schnabel et al., 1996). Still other researchers have suggested that vegetation type has no significant effect (Addy et al., 1999; Dukes et al., 2002; Haycock and Pinay, 1993; Hubbard et al., 1998; King, 2005; Lowrance et al., 2000; Mayer et al., 2007; Osborne and Kovacic, 1993; Ricks, 2002).

There is also debate regarding the buffer width required for maximum effectiveness because studies have shown mixed results in attempting to identify an ideal width (Mayer et al., 2007; Wenger 1999). Nevertheless, wider buffers have historically been considered superior in reducing groundwater NO_3 -N concentrations (Fennessy and Cronk, 1997; Messer et al., 2011; Petersen et al., 1992; Smith et al., 2006), despite the fact that several studies have shown that, under more ideal conditions, very high NO_3 -N reduction rates can be obtained in relatively narrow buffers (Jacobs and Gilliam, 1985; Johnson et al., 2012; Jordan et al., 1993; Osborne and Kovacic, 1993; Wafer 2004). Meta-data analysis of riparian buffer width by Mayer et al.

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*Corresponding author (deanna_osmond@ncsu.edu).

S.E. King, Michael Baker Engineering, 8000 Regency Parkway – Suite 600, Cary, NC 27518; D.L. Osmond and S. Knies, NC State Univ., Dep. of Soil Science, Box 7619, Raleigh, NC 27695; J. Smith, NC State Univ., Dep. of Statistics, Box 8203, Raleigh, NC 27695; M.R. Burchell and R.O. Evans, NC State Univ., Dep. of Biological and Agricultural Engineering, Box 7625, Raleigh, NC 27695; M. Dukes, Univ. of Florida, Dep. of Biological and Agricultural Engineering, Box 110570, Gainesville, FL 32611; S. Kunickis, USDA, Office of Pest Management, 1400 Independence Avenue, Rm. 3871, Washington, DC 20250-0002. Assigned to Associate Editor John Lory.

Abbreviations: DOC, dissolved organic carbon.

(2007) also suggests that a relatively narrow buffer can be highly effective in reducing groundwater NO_3^- .

The concentration and source of dissolved organic carbon (DOC) can be important factors in buffer effectiveness because low concentrations can inhibit NO_3^- -N reduction by limiting energy sources available to the denitrifying microorganisms (Bradley et al., 1992; Greenan et al., 2006; Lowrance and Smittle, 1988; Hill et al., 2000; Obenhuber and Lowrance, 1991; Schnabel et al., 1996; Starr and Gillham, 1993). Concentrations of DOC from 7 to $\geq 10 \text{ mg C L}^{-1}$ have been demonstrated to promote high rates of NO_3^- -N reduction through denitrification, whereas concentrations $\leq 4 \text{ mg L}^{-1}$ have shown relatively low reduction rates (Obenhuber and Lowrance, 1991; Starr and Gillham, 1993; Wu et al., 2012). Additionally, the molecular weight of the DOC has been demonstrated to be an important factor in NO_3^- -N reduction (Beauchamp et al., 1980; Knies, 2009; Pavel et al., 1996; Pintar and Lobnik, 2005; Wu et al., 2012), with lower-weight forms of DOC, such as the labile organic acids released into the soil rhizosphere by plant roots, being more conducive.

Hydrogeologic settings of buffers can affect the processing and removal of NO_3^- . Some results suggest that a shallow, horizontal groundwater flow path above an impermeable layer is needed to ensure denitrification by increasing groundwater residence time in the upper soil horizons (Hill, 1996; Lowrance et al., 2000; Wafer, 2004). Groffman and Tiedje (1989) observed much lower denitrification rates in a well drained sandy soil as compared with poorly drained clay loam.

Few experiments have measured the long-term performance of riparian buffers relative to NO_3^- -N removal (Newbold et al., 2010). To address riparian buffer NO_3^- -N removal effectiveness over time and to elucidate the role of vegetation, width, and DOC concentrations on buffer effectiveness, we present a longitudinal analysis of a replicated riparian buffer study from its inception through Year 12. Five vegetation types and two buffer widths were compared over 12 yr for changes in groundwater NO_3^- -N removal and DOC concentrations in the middle coastal plain of North Carolina.

Materials and Methods

Site Description

This study was conducted at the Center for Environmental Farming Systems located in the middle coastal plain near Goldsboro, NC (Fig. 1). Within the farm, four buffers established in 1998 were studied. Each buffer was located adjacent to the farm's extensive channelized stream network, which empties into the Neuse River located south of the farm (Dukes et al., 2003). Channelized stream depths (0.9–3.0 m) were incised such that the ditched streams were not hydrologically connected to their floodplain at the same frequency as natural streams. This system is very representative of the middle coastal plain landscape in the southeastern region of the United States.

Each buffer (R1, R2N, R4W, and R5N) was divided into two widths (8 and 15 m) as measured from the stream edge to the field edge. Buffer widths were selected based on prevailing practices in North Carolina. In nutrient-impaired watersheds, buffer width has been set at 15 m by the Department of Environmental

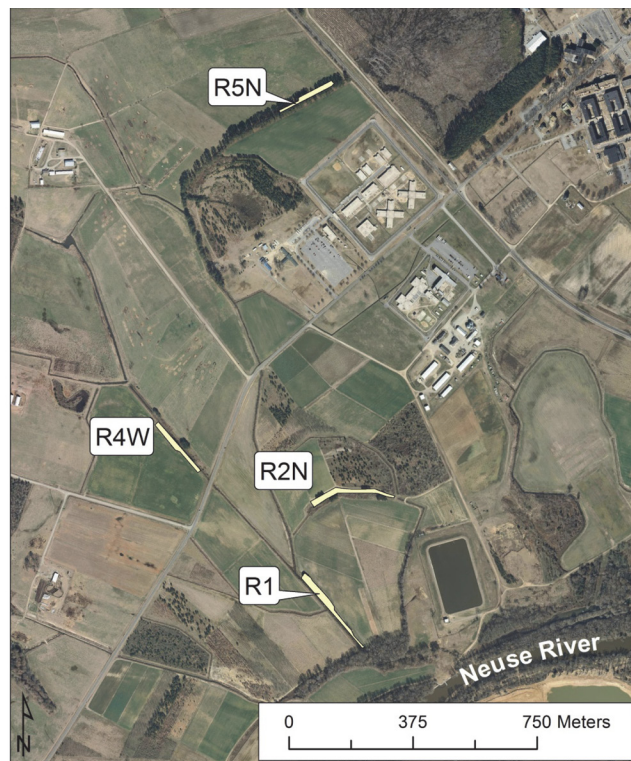


Fig. 1. Locations of the four buffer replications at the Center for Environmental Farming Systems, Goldsboro, NC.

Quality. Cost-share in North Carolina allows filter strips to range from 6 to 8 m.

Each width was subsequently subdivided into five sections, each 24 m long and revegetated with different species: trees (mostly loblolly pine [*Pinus taeda* L.] but with some mixed hardwoods, including American sycamore [*Platanus occidentalis* L.] and green ash [*Fraxinus pennsylvanica* Marsh.], switchgrass (*Panicum virgatum* L. 'Alamo'), fescue grass (*Festuca arundinacea* 'Kentucky 31'), native vegetation, and a control. The native vegetation strips were allowed to revegetate naturally into whatever early successional species arose, mostly grasses, vines (trumpet creeper [*Campsis radicans* (L.) Seem. ex Bureau], black raspberry [*Rubus* sp.], Japanese honeysuckle [*Lonicera japonica* Thunb.]), and weeds (*Solidago* spp.), although a scattering of trees (mostly *P. taeda* and black cherry [*Prunus serotina* Ehrh.]) began to establish themselves over time. The control strips were the equivalent of the agricultural field behind each buffer; therefore, the management of the fields and controls varied from year to year over the experimental period (Table 1).

Before the inception of the experiment in 1997, all experimental land was in conventional corn and soybean production. A buffer and a cropping systems experiment (land behind the buffers) was established the same year. The majority of fields behind buffers R1 and R2 were predominantly planted to cash crops (grain legume, cereal, and vegetables) using no-till and cover crops, whereas R4W and R5N were transformed into beef cow (*Bos taurus*) pasture (predominantly ryegrass [*Lolium perenne* L.] and/or sudangrass [*Sorghum bicolor* L.]) (R4W) and organic dairy cow pasture (predominantly ryegrass or clover [*Trifolium* sp.]) (R5N) (Table 1). Beginning in 2007, the field adjacent to R1 was established in fescue grass for organic hay production;

turkey litter was applied at a rate of 212 kg N ha⁻¹, with no additional N being applied after that.

As a consequence of different land management behind the buffer replications and in the control, the amount of added N varied from 0 to more than 200 kg N ha⁻¹ yr⁻¹ (Table 1), with an average application of approximately 100 kg N ha⁻¹ yr⁻¹. Under pasture management, ryegrass was added as a fall forage crop, whereas ryegrass on cropped acres was used as a nonfertilized cover crop. Ordinarily, the control strips were managed exactly as the adjacent crop fields. Due to miscommunication, however, the control strips in R1 and R2N were not maintained beginning in 2007; thus, data collected from control plots in R1 and R2N were excluded for January 2007 through 2010.

The soil series, as mapped by Barnhill et al. (1974), are as follows: R1, Lumbee sandy loam (fine-loamy over sandy or sandy-skeletal, siliceous, thermic Typic Endoaquults) found in the buffer, with Wickham sandy loam (fine loamy, mixed, thermic Typic Hapludults) in the adjacent field; R2N, Nahunta very fine sandy loam (fine silty, siliceous, thermic Aeric Paleaquults) in buffer, with Wickham loamy sand in the adjacent field; R4W, Lumbee sandy loam in the buffer, with Wagram loamy sand (loamy, kaolinitic, thermic Arenic Kandiodults) in the adjacent field; R5N, Weston loamy sand (coarse-loamy, siliceous, thermic Typic Endoaquults) in the eastern portion of the buffer and field, and Kalmia sandy loam (fine-loamy over sandy or sandy-skeletal, siliceous, thermic Typic Hapludults) in the western portion with some Rains sandy loam (fine-loamy, siliceous, thermic Typic Paleaquults) at the west end.

Field sampling of the soil during buffer installation was conducted to confirm the USDA soil descriptions (Kunickis, 2000).

This detailed analysis revealed similar soil series as published in the soil survey; however, there was significant soil variability relative to texture within replications and even within the same plot. Because these are floodplain soils, it is not surprising that soils exhibited such heterogeneity. Both soil survey and analysis by Kunickis (2000) indicated that these soils have low (1–2%) organic matter content in the top 20 cm.

Groundwater Monitoring

Each of the 40 buffer strips (five vegetation types, two widths, and four replications) had two well nests installed. One was located along the field edge of the buffer strip (~1 m into the buffer), and the other was located along the ditch edge of the buffer strip (~0.5 m from the stream top of the bank). Each well nest consisted of three wells within the shallow groundwater zone, which was above the aquitard that separated the surficial and deep groundwater. The well nests consisted of one shallow (0.6–1.0 m), one intermediate (1.5–2.0 m), and one deep (2.1–3.5 m) well, as measured from ground surface to the top of the well screen. All wells were made of polyvinyl chloride and had a 0.6-m screened section below the bottom of the well.

The initial research determined that the shallow wells were frequently dry and were therefore abandoned after 2 yr of monitoring (Dukes et al., 2002). The “intermediate” wells were installed at a depth sampling from the upper portion of the local shallow water table aquifer, effectively functioning as the shallow wells had been intended. The deep wells were correctly installed at a depth to allow sampling from the lower portion of the surficial aquifer.

Table 1. Crop and nitrogen fertilization rates of the control buffer treatment and the fields behind the riparian buffer replications.

Year	Replications							
	R1		R2N		R4W		R5N	
	Crop	N fertilization	Crop	N fertilization	Crop	N fertilization	Crop	N fertilization
		kg ha ⁻¹		kg ha ⁻¹		kg ha ⁻¹		kg ha ⁻¹
1998	no-till corn/no-till ryegrass cover	168	no-till corn/no-till ryegrass cover	168	managed pasture	168	fescue/ryegrass/clover	168
1999	no-till corn/no-till ryegrass cover	168	no-till corn/no-till ryegrass cover	168	managed pasture	168	fescue/ryegrass/clover	168
2000	soybean	0	no-till corn	176	managed pasture	NA†	fescue/ryegrass/clover	NA
2001	sweet potato	0	no-till cotton	160	no-till ryegrass	10	fescue/ryegrass/clover	NA
2002	cabbage	NA	no-till corn	250	NA	NA	fescue/ryegrass/clover	NA
2003	soybeans	0	strip-till peanuts/no-till ryegrass cover	0	millet/ryegrass	259	fescue/ryegrass/clover	174
2004	fallow	0	no-till corn/no-till ryegrass cover	176	sudangrass/ryegrass	157	clover	0
2005	sudangrass	134	no-till corn/no-till ryegrass cover	176	sudangrass/ryegrass	157	clover	0
2006	wheat, cowpea/soybean	0	no-till sorghum	NA	NA	NA	NA	NA
2007	fescue hay	212	no-till wheat/no-till soybeans	131	sudangrass/ryegrass	190	clover	0
2008	sudangrass/fescue	0	no-till sorghum/no-till ryegrass	117	sudangrass/ryegrass	190	clover	0
2009	sudangrass/fescue	0	no-till corn	250	sudangrass/fescue	120	clover	0
2010	sudangrass/fescue	0	no-till wheat/no-till soybeans	117	sudangrass/fescue	120	clover	0

† No data available due to incomplete farm records.

The wells were sampled starting July 1998, and the last sampling date evaluated for this analysis was August 2010. The wells were sampled a total of 82 times over the 146-mo period and were generally sampled monthly, except for two breaks of about 18 mo each (2002–2003 and 2005–2006). The data collection and site evaluations were conducted by five different researchers over the 12 yr (Dukes et al., 2002; King, 2005; Knies, 2009; Kunickis, 2000; Ricks 2002).

The depth to water table was measured for each well using an electronic water level meter before sampling. Samples were then collected using a peristaltic pump after purging three well volumes. All samples were kept on ice until reaching the laboratory, where samples were filtered using 0.45- μ m filters, acidified to pH 2 with a 5% H_2SO_4 solution, and stored at 4°C in a cold room until analyzed within 4 wk of sampling.

The samples were originally analyzed for $\text{NO}_3\text{-N}$, ammonium ($\text{NH}_4\text{-N}$), phosphate ($\text{PO}_4\text{-P}$), chloride (Cl), and DOC by the Environmental and Agricultural Testing Service in the Soil Science Department at NC State University. The laboratory used a QuikChem 8000 Automated Ion Analyzer (Lachate Instruments) to measure $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and $\text{PO}_4\text{-P}$ and a digital chloridometer (Haack-Buchler) for Cl. An automated total organic carbon analyzer was used to measure DOC (Shimadzu Scientific Instruments). The analysis of $\text{NH}_4\text{-N}$ was only periodically performed after the first year due to consistently low values at or below the detection limit; similarly, $\text{PO}_4\text{-P}$ concentrations were also very low, representing only background levels (data not shown). Thus, neither constituent is reported.

The subsurface groundwater flow paths for each buffer were determined using relative water table elevations calculated from monthly depth to water measurements along with the previously gathered survey data for all of the wells. Groundwater contours were created, and results indicated that subsurface flow was generally perpendicular toward the stream in each of the buffers, although the hydraulic gradients were often very low (Dukes et al., 2002; King, 2005; Knies, 2009). Groundwater elevations were fairly constant over time, with no dramatic seasonal variations. It was also previously determined that an aquitard was present at a depth of approximately 4 m, so little deep seepage occurred at this site (Dukes et al., 2002).

A comparison of $\text{NO}_3\text{-N}$ to Cl concentration ratios were made for the field/buffer and buffer/stream wells to determine if simple dilution caused by an upwelling of uncontaminated water from a deeper aquifer was causing any of the observed decreases in $\text{NO}_3\text{-N}$ concentration. Although such an upwelling seemed unlikely due to the presence of an aquitard, these ratio comparisons were conducted by several of the researchers involved in this long-term experiment, and each researcher independently concluded that dilution did not appear to be a substantial influencing factor (Dukes et al., 2002; King, 2005; Knies, 2009). Many other researchers (Altman and Parizek, 1995; Jacobs and Gilliam, 1985; Messer et al., 2011; Simmons et al., 1992; Wiseman et al., 2014) have used $\text{NO}_3\text{-N}/\text{Cl}$ ratios to determine if $\text{NO}_3\text{-N}$ concentration decreases were due to dilution or apparent denitrification.

Oxidation–reduction (redox) potential in the soil was measured throughout much of the study period using platinum-tipped redox probes installed in buffers R1 and R2N in 2000 and in buffer R4W in 2002 (King, 2005; Knies, 2009; Kunickis,

2000; Ricks, 2002). The purpose of this effort was to determine if soil redox conditions were favorable for the process of denitrification. Three intermediate-depth (152 cm) and three deep-depth (300 cm) probes were located immediately adjacent to a salt bridge by each of the 20 monitoring well nests, for a total of 120 probes per buffer. Each researcher maintained the probes, took redox measurements, and found similar results, indicating that conditions were consistently favorable for denitrification in the soil around the deep-depth wells but inconsistently favorable for the intermediate-depth wells. Based in part on these data along with Cl data, each researcher concluded that denitrification was the most likely mechanism responsible for the observed $\text{NO}_3\text{-N}$ removal (Dukes et al., 2002; King, 2005; Knies, 2009; Kunickis, 2000; Ricks, 2002). This conclusion agrees with previous research that has consistently indicated that denitrification is the dominant $\text{NO}_3\text{-N}$ removal process in the subsoils of this region (Gilliam et al., 1978; Kliever and Gilliam 1995; Smith et al., 2006; Spruill 2000; Wafer 2004).

Statistical Analysis

For the $\text{NO}_3\text{-N}$ removal analysis, the relative change in $\text{NO}_3\text{-N}$ concentration was calculated per the generally accepted method based on the percentage difference entering and leaving the buffer (Mayer et al., 2007) (Eq. [1]). Equation [1] is the percentage change in groundwater $\text{NO}_3\text{-N}$ concentration as it passes through the buffer perpendicularly, which multiple researchers at this site demonstrated (Dukes et al., 2002; King 2005; Knies 2009).

$$[(\text{Initial } \text{NO}_3\text{-N} - \text{Final } \text{NO}_3\text{-N})/(\text{Initial } \text{NO}_3\text{-N})] \times 100\% \quad [1]$$

Percentage change in groundwater $\text{NO}_3\text{-N}$ concentration was constrained to 0% when the calculation became negative. This occurred when $\text{NO}_3\text{-N}$ groundwater concentrations entered the buffer (i.e., initial $\text{NO}_3\text{-N}$) near irreducible limits (around 1 mg L^{-1}). To ensure this constraint did not affect the overall quality of the analysis, data were processed through SAS both constrained and unconstrained, revealing that this constraint did not affect the overall results.

This study design was a split-split plot in four replications with 20 treatment combinations. Buffer width was the whole plot, vegetative type was factor 1 (split), and depth was factor 2 (split-split). No data were available for the switchgrass narrow buffer strip for either the deep or intermediate wells in Replication 1 because these wells were not installed at the beginning of the experiment. Thus, the total number of field plots was 79.

Data analyses were generated using SAS/STAT software, Version 9.3, of the SAS System for Windows (SAS Institute Inc.), and statistical significance was set as $\alpha = 0.05$. Because sampling position (field/buffer and buffer/stream) was not considered to be a variable, it was necessary to determine whether DOC concentrations represented the same population using MEANS ANOVA. Because 8 out of 20 plots represented different populations (data not shown), average DOC concentrations from both locations were used. The MIXED ANOVA procedure was used to test for treatment differences and change over the 12-yr monitoring period; residual plots were reviewed. Measurements were averaged within years, which eliminates within-year autocorrelation. Plots against year showed much variation and sometimes a

trend, so year was entered as a continuous variable in the model to investigate trend over time. Based on residual plots, there seemed to be no autocorrelation. Residual plots also appeared consistent with the assumption of normality.

Results and Discussion

Changes in NO₃-N Removal Effectiveness

Yearly percentage NO₃-N concentration reductions were plotted for each treatment and included a regression line (Fig. 2). Regression slopes in most treatments (11 of 20) suggested an increase in NO₃-N reduction effectiveness over time, although some trends decreased (3 of 20) or remained flat (6 of 20). When the ANOVA was performed, two main effects—depth ($P = 0.0166$) (Supplemental Table S1) and year ($P = 0.0197$) (Supplemental Table S1)—and a three-way interaction (year, vegetation, and width) were significant ($P = 0.0502$) (Supplemental Table S1).

The statistical significance of the groundwater sampling depth main effect was unsurprising because it had repeatedly and consistently demonstrated significance for each of the experimental phases (Dukes et al., 2002; King, 2005; Knies, 2009; Kunickis, 2000; Ricks, 2002). From a physical and biological perspective, the intermediate (~152 cm) and deep (~300 cm) depths exhibited very different soil and hydrologic conditions. The deep groundwater sampling depth was always reduced, whereas vacillating reducing/oxidation conditions were observed at the

intermediate depth (Dukes et al., 2002; King, 2005; Knies, 2009). As a consequence of the different physicality of sampling depths, depth was removed as a variable, and the data set was reanalyzed at each depth; thus, the experimental design became a split-plot.

At the intermediate groundwater sampling depth, NO₃-N concentration reduction was significant only for two main effects (width, $P = 0.0098$; year, $P = 0.0131$) (Supplemental Table S2). Wider buffers (15 m) were almost 2.5 times more effective for NO₃-N concentration reduction (49.8%) than narrow (8 m) buffers (19.1%) in Years 0, 6, and 12 (Table 2). Because both year and width main effects were significant, the changes in NO₃-N reduction effectiveness over time required separate equations for the narrow (8 m; Eq. [2]) and wide buffers (15 m; Eq. [3]).

$$\text{NO}_3\text{-N Reduction \%} = 15.9\% + 0.62\% \times \text{yr} \quad [2]$$

$$\text{NO}_3\text{-N Reduction \%} = 46.1\% + 0.62\% \times \text{yr} \quad [3]$$

These equations have a common slope, indicating that yearly increases in NO₃-N reduction effectiveness were the same (0.62% yr⁻¹) regardless of buffer width. This increase corresponds to an equivalent 7.2% reduction during the 12-yr period. Thus, although NO₃-N reductions significantly improved over time for both the 8-m and the 15-m buffers, the difference in observed effectiveness between the two widths was present at the onset of buffer installation (Dukes, 2000) and continued during the 12-yr monitoring period; wide buffers were consistently better at decreasing NO₃-N concentrations relative to the narrow buffers at the intermediate groundwater depth, most probably as a result of increased resident time.

Vegetation, however, did not affect the NO₃-N reduction capacity over the 12-yr duration. Nitrate-N reduction effectiveness ranges at Year 12 were tree (44%), native (41%), fescue (37%), switchgrass (35%), and control (32%). Although numerically trees were superior, the variability in the data obscured any significance despite the trends observed (Fig. 3). The control treatment had the lowest NO₃-N reduction, but rates were nevertheless statistically indistinguishable between buffer vegetation treatments. The same trend held when replications were analyzed separately (Knies, 2009). The lack of differences in NO₃-N reduction as a function of vegetation is similar to what many other researchers have shown (Addy et al., 1999; Haycock and Pinay, 1993; Hubbard et al., 1998; Lowrance et al., 2000; Mayer et al., 2007; Osborne and Kovacic, 1993), except that none of those experiments used a control treatment or began their monitoring at buffer inception. The NO₃-N

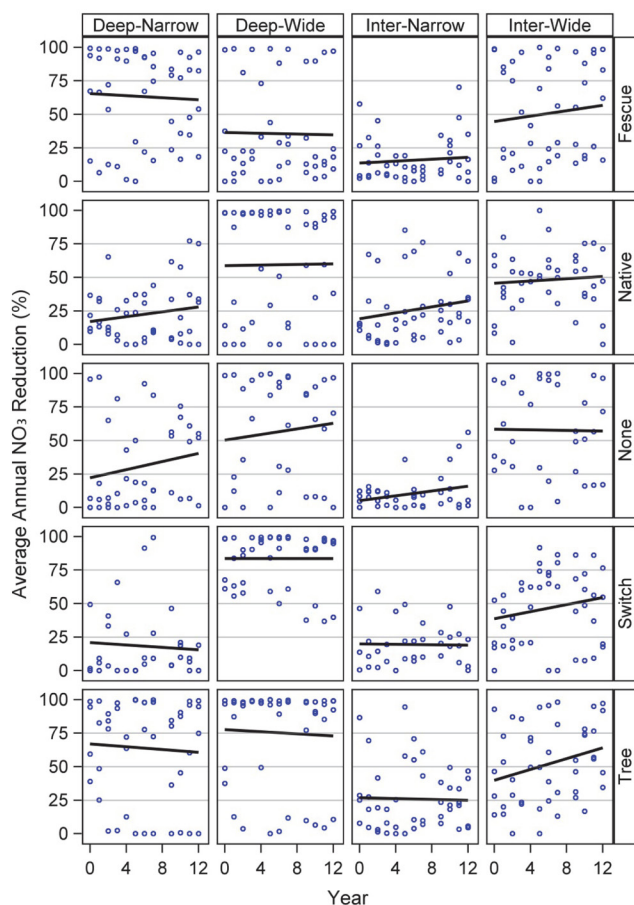


Fig. 2. Nitrate-nitrogen reduction (%) and regression line for each vegetation type (tree, switch [switchgrass], native, fescue, and none [control]), width, and groundwater sampling depth over time.

Table 2. Nitrate-N removal at the intermediate groundwater sampling depth for the narrow and wide buffers at the beginning (Year 0), mid-point (Year 6 yr), and end (Year 12) of the sampling period.

Year of buffer establishment	NO ₃ -N removal by buffer width	
	Narrow (8 m)	Wide (15 m)
Year 0	17.7 (7.6)b†	44.8 (7.5)a
Year 6	19.4 (7.4)b	49.8 (7.2)a
Year 12	21.1 (7.6)b	54.8 (7.5)a

† Values are means (%) with SE in parentheses. Means across rows/years for each year followed by different lowercase letters are significantly different ($P < 0.05$).

reduction rates, on the whole, were generally lower in this experiment than in the aforementioned studies, likely due to complex and inter-related processes at this location, low incoming $\text{NO}_3\text{-N}$ concentration ($\leq 8 \text{ mg L}^{-1}$) (Fig. 3), variable redoximorphic (range, 350–450 mV) conditions in the intermediate groundwater depths (Dukes et al., 2002; King, 2005; Knies, 2009; Kunickis, 2000; Ricks, 2002), and the heterogeneity of the soil system.

At the deep groundwater depths, only the interaction of vegetation type-by-width was significant ($P = 0.0161$) (Supplemental Table S2). All other main effects and interactions were insignificant, including year, meaning that the $\text{NO}_3\text{-N}$ concentration reductions at the deep depth did not improve over the 12-yr study period. In the narrow buffers (8 m), buffers with tree and fescue vegetation exhibited significantly higher $\text{NO}_3\text{-N}$ reduction effectiveness than those with native and switchgrass vegetation, whereas trees alone demonstrated significantly greater reduction effectiveness than the control (Table 3). In the wide buffer (15 m), the fescue treatment had significantly lower $\text{NO}_3\text{-N}$ reduction effectiveness when compared with switchgrass and tree vegetation but was no different from the control and native vegetation (Table 3). The same vegetation type often varied significantly in effectiveness across replications and widths (Knies, 2009). For example, switchgrass replicates were the most effective for removing $\text{NO}_3\text{-N}$ in the wide buffers and the least effective in the narrow buffers (Table 3). Incoming median $\text{NO}_3\text{-N}$ concentrations at the deep groundwater depth were lower than in the intermediate groundwater depth and ranged from approximately 0 to 5 mg L^{-1} (Fig. 3). Early in this study, detailed characterization of the floodplain soils on which the buffers were established indicated significant soil texture and layer variability between plots and within plots. This soil heterogeneity no doubt affected groundwater flow paths and resident times of $\text{NO}_3\text{-N}$ more than vegetative type (including the control), thereby affecting denitrification potential (Kunickis, 2000). These variations in soil and hydrologic properties and variations in low incoming $\text{NO}_3\text{-N}$ concentrations appeared to exert more control over $\text{NO}_3\text{-N}$ removal than vegetative type; vegetation had little effect on the soil and hydrologic processes responsible for the removal of NO_3 .

Both buffer widths used in this experiment are considered comparatively narrow relative to the literature; narrow buffers are generally considered to be $< 31 \text{ m}$. Some research, however, has suggested that narrow buffers can be very effective in processing NO_3 (Jacobs and Gilliam 1985; Johnson et al., 2012; Jordan et al., 1993; Osborne and Kovacic, 1993; Wafer, 2004). Meta-data analysis of riparian buffer width by Mayer et al. (2007) also suggests that narrow widths can be highly effective in processing of groundwater $\text{NO}_3\text{-N}$. Our data suggest that slightly wider (15 m) “narrow” buffers were more effective and that, depending on the groundwater depth, their effectiveness increased with time.

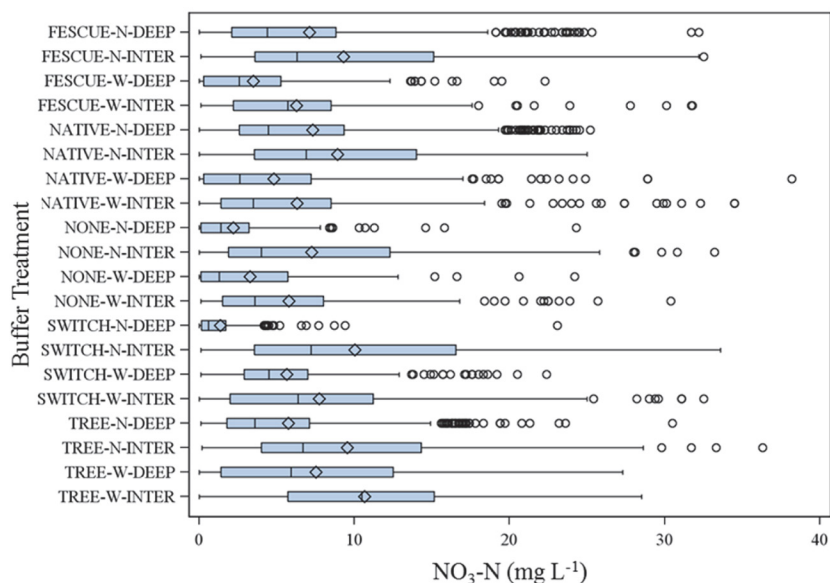


Fig. 3. Incoming nitrate-N concentrations for each vegetation type (tree, switch [switchgrass], native, fescue, and none [control]), width, and groundwater sampling depth and each sampling time plotted as a box and whisker plot that includes the median (straight line) and the mean (diamond).

There was a slow but clear increase in $\text{NO}_3\text{-N}$ removal over the 12-yr study at the intermediate groundwater sampling depth but not at the deep depth. Lowrance (1992) implied that it may take up to 20 yr for a riparian forest to become an effective N sink and that the $\text{NO}_3\text{-N}$ removal ability of newly restored forested buffers would not be realized in the near term (defined in that study as 12 yr after restoration). Inherent site characteristics at our buffer experiment (e.g., hydrology, water table depth, and soils) supported Lowrance’s proposition at this location. Newbold et al. (2010) demonstrated a 26% reduction in stream $\text{NO}_3\text{-N}$ concentrations 15 yr after buffer establishment. Based on our results, we would not expect near-term changes in NO_3 delivery and losses to the stream from newly established buffers because conservation practices often take time to become effective.

Changes in Dissolved Organic Carbon Concentration

Dissolved organic carbon concentrations were plotted separately over time for field/buffer (Fig. 4) and buffer/stream (Fig. 5) sampling locations. Temporal patterns emerged and were reconfirmed through ANOVA (Supplemental Table S1) as year was significant; DOC increased significantly at both sampling locations over the 12-yr study period. Groundwater depth was also significant (field/buffer, $P = 0.0006$; buffer/stream, $P =$

Table 3. Nitrate-N removal at the deep groundwater sampling depth related to vegetation type and buffer width

Buffer vegetation type	Buffer width	
	8 m	15 m
Trees	63.8 (17.2)ad†	72.6 (17.2)abc
Fescue	62.7 (17.2)abd	34.6 (17.2)de
Control	28.6 (17.2)bce	53.8 (17.3)abcd
Native	23.3 (17.2)ce	57.0 (17.2)abcd
Switchgrass	18.7 (18.8)ce	82.2 (17.2)abc

† Values are means (%) with SE in parentheses. Means followed by different lowercase letters are significantly different ($P < 0.05$).

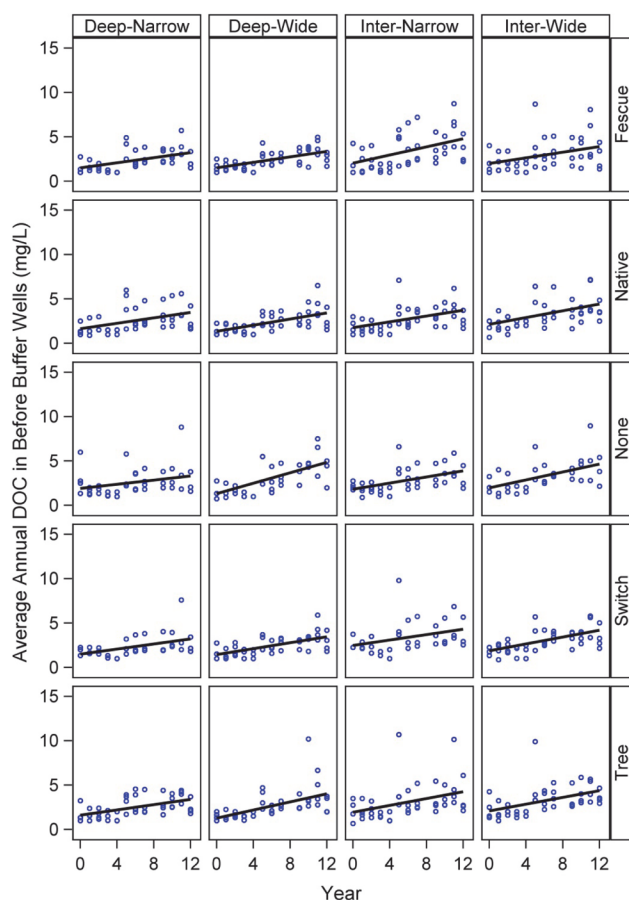


Fig. 4. Carbon concentrations (mg L^{-1}) at the field/buffer sampling location and regression line for each vegetation type (tree, switch [switchgrass], native, fescue, and none [control]), width, and groundwater sampling depth over time. DOC, dissolved organic carbon.

0.0024) (Supplemental Table S1), so we split our analysis by groundwater depth.

Field/Buffer Sampling Location

At the field/buffer intermediate sampling depth, only the year was significant ($P < 0.0001$) (Supplemental Table S3), with DOC increasing over time (Eq. [4]).

$$\text{DOC (mg L}^{-1}\text{)} = 2.1 + 0.17 \times \text{yr} \quad [4]$$

Dissolved organic carbon increased just $0.17 \text{ mg L}^{-1} \text{ yr}^{-1}$ or from 2.11 to 4.11 mg L^{-1} over the 12-yr study. Vegetation type was not significant for DOC concentrations (Table 4).

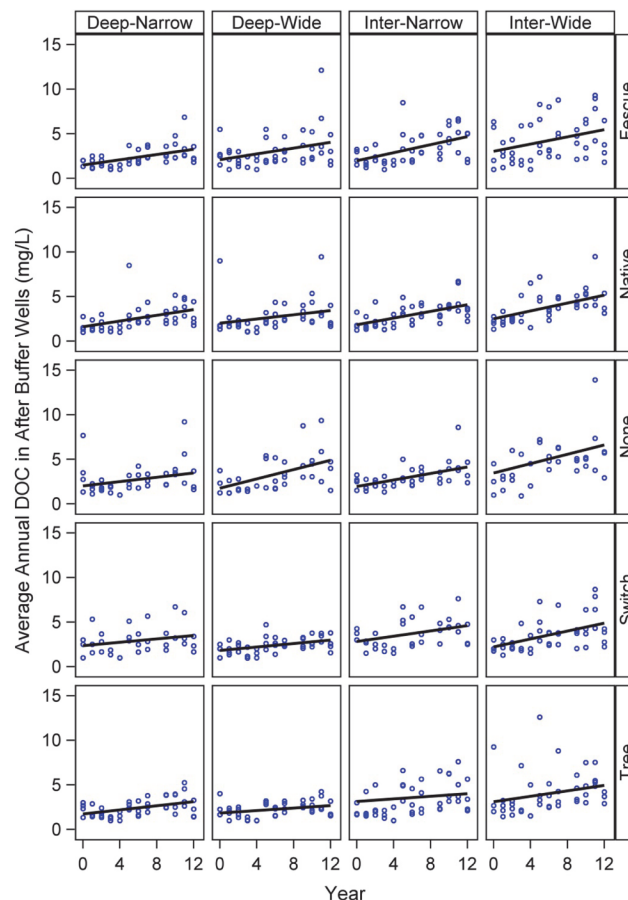


Fig. 5. Carbon concentrations (mg L^{-1}) at the buffer/stream sampling location and regression line for vegetation type (tree, switch [switchgrass], native, fescue, and none [control]), width, and groundwater sampling depth over time. DOC, dissolved organic carbon.

At the field/buffer deep groundwater sampling depth, both the year main effect ($P < 0.0001$) and the interaction of year with width ($P = 0.0069$) (Supplemental Table S3) were significant. Due to this interaction, DOC change in concentrations was represented in two separate equations: (i) narrow buffers (8 m), deep wells (Eq. [5]) and (ii) wide buffers (15 m), deep wells (Eq. [6]).

$$\text{DOC (mg L}^{-1}\text{)} = 1.6 + 0.13 \times \text{yr} \quad [5]$$

$$\text{DOC (mg L}^{-1}\text{)} = 1.6 + 0.18 \times \text{yr} \quad [6]$$

Table 4. Dissolved organic carbon concentration in field/buffer and buffer/stream locations compared with NO_3 reduction in Year 12 of the study.

Vegetation type†	Field/buffer DOC‡		Buffer/stream DOC		NO ₃ reduction	
	Intermediate depth	Deep depth	Intermediate depth	Deep depth	Intermediate depth	Deep depth
	mg L^{-1}				%	
Trees	4.3	3.7	4.5	2.9	44.4	66.7
Native	4.0	3.4	4.6	3.5	41.4	44.0
Fescue	4.3	3.3	5.0	3.6	37.3	47.7
Switchgrass	4.1	3.2	4.6	3.2	35.0	50.0
Control	3.9	3.9	5.0	4.0	31.7	43.6

† The vegetative effect was not significant for any of these constituents.

‡ Dissolved organic carbon.

Dissolved organic carbon levels began at the same low concentration but increased slightly more rapidly in wide buffers (2.2 mg L⁻¹) than in narrow buffers (1.6 mg L⁻¹) over the 12-yr study.

Buffer/Stream Sampling Location

The only significant effect in the analysis of the DOC concentration for the buffer/stream intermediate- and deep-depth groundwater wells was year ($P < 0.0001$) (Supplemental Table S4). Vegetation type was not significant, and DOC concentrations in the buffer/stream intermediate-depth wells in the final sampling year had a narrow range (4.5–5.0 mg L⁻¹) (Table 4). Concentrations of DOC increased by 0.15 mg L⁻¹ yr⁻¹ at both the intermediate depth (Eq. [7]) and the deep depth (Eq. [8]) over the 12-yr study

$$\text{DOC (mg L}^{-1}\text{)} = 2.8 + 0.15 \times \text{yr} \quad [7]$$

$$\text{DOC (mg L}^{-1}\text{)} = 1.8 + 0.15 \times \text{yr} \quad [8]$$

Thus, over the 12-yr study period, the intermediate- and deep-depth groundwater at the buffer/stream location had a total DOC increase of 1.8 mg L⁻¹. Increases in DOC within the buffer over time are probably due to increased organic contributions from the riparian vegetation because tillage ceased or was significantly reduced on all treatments, including the control.

Regardless of location, although DOC concentrations were greater in the intermediate-depth groundwater wells (2.8 mg L⁻¹) than in the deep-depth wells (1.8 mg L⁻¹) at the start of the experiment, these levels may have limited biological removal because DOC concentrations were all relatively low. Because additional increases of DOC over time were also relatively slow (~0.15 mg L⁻¹ yr⁻¹), biological removal was probably limited over the experimental time period. In a soil column study, Wu et al. (2012) found that citric acid, with its comparatively low molecular mass, was shown to increase NO₃-N removal 4-fold as the DOC concentration doubled from 4 to 8 mg L⁻¹ because additional DOC increased denitrification. Other researchers have found similar results (Obenhuber and Lowrance, 1991; Starr and Gillham, 1993; Wu et al., 2012). In this study, DOC concentrations in Year 12 were 5.0 mg L⁻¹ or less for all vegetative types in the intermediate and deep groundwater at both the field/buffer and buffer/stream locations.

The low DOC concentration differences in this study do not immediately appear to have affected NO₃-N reduction because the range of NO₃-N reductions was less in the intermediate-depth wells where DOC concentrations were greater. As stated earlier, redox data from the intermediate-depth wells vacillated between oxidizing and reducing conditions as compared with the relatively constant reduced conditions in the deep wells (Dukes et al., 2002; King, 2005; Knies, 2009; Kunickis, 2000). Any increase in NO₃-N removal ability that may potentially have been provided by the greater DOC concentrations in the intermediate-depth wells may have been inhibited by the relative lack of reduced conditions. Likewise, any increased NO₃-N removal rates in the deep-depth wells provided by their more constant reduced conditions might have been inhibited by their comparatively low DOC concentrations. Smith et al. (2006) found NO₃-N reductions of 53 and 40% for two 9-m-wide buffers in shallow-depth wells with DOC values of 11 and 5.4 mg

L⁻¹ but found reductions of only 17 and 18% for those same buffers in the deeper depth wells, with DOC values of just 3 mg L⁻¹ each.

Some researchers have suggested that it is the relationship between soil organic matter (e.g., DOC), incoming NO₃-N concentrations, hydrology, and landscape position that determines subsurface effectiveness of buffer function (Mayer et al., 2007; Wafer, 2004). In evaluating those conditions for the buffers at this project site, we observed several factors potentially inhibiting stronger NO₃-N removal rates: (i) relatively low incoming nitrate concentrations (generally <6 mg L⁻¹) due to limited N application to adjacent cropping and pasture systems, (ii) DOC concentrations almost always <5.0 mg L⁻¹ and even lower in the first years after buffer installation, (iii) a fluctuating hydrology such that the intermediate wells experienced significant periods of oxidizing conditions (Kunickis, 2000), and (iv) a landscape position adjacent to a channelized stream network (Dukes et al., 2002). At current rates of increase, DOC concentrations would increase to 8 mg L⁻¹ within approximately 32 yr after establishment in the intermediate wells and within 56 yr in the deep wells.

Conclusions

A unique replicated experiment explored the effectiveness of riparian buffer widths (8 and 15 m) and five vegetation types (trees, switchgrass, fescue, native, and control) from establishment to Year 12 on incised channelized streams in the middle coastal plain of North Carolina. Nitrate removal and DOC concentrations were determined at two groundwater sampling depths. At the intermediate groundwater depths, NO₃-N removal was 2.5 times greater in the 15-m than in the 8-m buffer, but yearly increases in effectiveness (0.62%) were the same. At the deep groundwater depth, a width-by-vegetation interaction was significant, and NO₃-N removal ranged from 19 to 82% depending on width and vegetative type; there was no increase in removal over time. Concentrations of DOC ranged from a starting concentration of 1.6 to 2.8 mg L⁻¹ and increased between 0.13 and 0.17 mg L⁻¹ yr⁻¹. The DOC concentrations were consistently lower than levels shown to enhance NO₃-N removal in other studies (~8 mg L⁻¹), yet there appeared to be some denitrification occurring based on redox and NO₃-N/Cl ratios measured throughout the study, although each groundwater depth appeared to be denitrification limited by low DOC concentrations (<5 mg L⁻¹) and a lack of consistent anaerobic/reducing conditions in the intermediate groundwater and by low DOC concentrations (<4 mg L⁻¹) and low incoming NO₃-N (<5 mg L⁻¹) in the deep groundwater. Vegetation alone, including the control, had no effect on NO₃-N removal or DOC concentrations, although trends suggested that trees provided the greatest NO₃-N removal and that the control provided the least. Due to the complex relationships and interactions between site characteristics (water table depth, soil type, low incoming N, and low DOC), it is not surprising that only width and time have thus far emerged as important factors affecting NO₃ removal at this site.

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