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Denitrification in soils of hydrologically restored wetlands relative to natural and converted wetlands in the Mid-Atlantic coastal plain of the USA

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ABSTRACT

In the last several decades, there has been considerable effort to protect and restore wetlands throughout the USA. These efforts have required significant investment of both private and public funds. Accordingly, it has become important to document the effectiveness of this protection and restoration. This study for the Mid-Atlantic Region (MIAR) Wetland Conservation Effects Assessment Project (CEAP) was part of the US Department of Agriculture CEAP. This study compared natural, converted, and hydrologically restored wetlands in the states of Delaware, Maryland, and Virginia. There were forty-eight total sites, and each site was sampled at 4 landscape elevations (wettest to driest) during a three year period. Here we report an assessment of soil denitrification conducted as one component of the MIAR Wetland-CEAP using denitrification enzyme activity (DEA). DEA values varied significantly with relative elevation and management DEA response to nitrate addition varied significantly with relative elevation and management. In stepwise regression, total C and moisture were the most influential physiochemical conditions for the converted and natural wetlands, respectively. Total C and Ca were the most important for the restored wetlands. Moreover, the percentage of denitrification as nitrous oxide and *nosZ* gene abundances, differed by relative elevation and management. In all aspects of DEA, the restored wetlands were more similar to the natural wetlands than to the converted wetland.

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1. Introduction

Throughout the USA, North America, and the world there is a general awareness of the extensive losses of wetlands (Coleman et al., 2008; Heal, 2000; Nicholls et al., 1999). There is also considerable documentation of the negative impact of these losses on wetlands services (Airoldi and Beck, 2007; Costanza et al., 1997; Hefting et al., 2013; Moreno-Mateos et al., 2012). Recognition of this fact has lead to various wetlands policies and legislation (Brinson and Eckles, 2011; Eckles, 2011; Farnese and Belcher, 2006). Accordingly, within the USA during recent decades, there have been substantive efforts and expenditures to restore wetlands. For instance, the Conservation Reserve Program and the Wetland Reserve Program (Eckles, 2011). With the expenditure of both public and private funds to restore wetlands, there was a need to assess the effectiveness of restored wetlands in reestablishing their original functions and services. Thus, to make this assessment as well as other related conservation assessments the U.S. Department of Agriculture Conservation Effects Assessment Project (CEAP) was initiated (Duriancik et al., 2008). It is a multi-agency effort to quantify the environmental benefits of conservation practices. The Mid-Atlantic Regional CEAP-Wetland Study is one of five regional studies undertaken as part of the national CEAP-Wetland effort. This project is assessing various wetland functions and services for natural, converted, and restored wetlands in the region.

In this region as well as many other regions of the world, one of the important biogeochemical functions of wetland ecosystems is their cycling of nitrogen. In particular, wetlands remove significant quantities of nitrogen by removing nitrogen via denitrification. This is especially true when the excess nitrogen enters the wetland in the form of nitrate. This nitrate is quite readily converted

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to dinitrogen gas via denitrification. The level of denitrification is frequently limited by available nitrogen rather than carbon (Hunt et al., 2004). Unfortunately, the denitrification does not always go to completion with the formation of dinitrogen gas. Under some conditions such as low C/N ratios, the denitrification will produce the end product gas nitrous oxide (Dodla et al., 2008; Hunt et al., 2007; Ullah and Moore, 2011). Not only is nitrous oxide a very potent greenhouse gas, its production is a significant air quality problem. While this is of concern, natural wetlands do not appear to be a uniformly overwhelming contributor of nitrous oxide (Audet et al., 2013; Jacinthe et al., 2012; Morse et al., 2012). Numerous methods have been employed to assess denitrification; two commonly used methods are denitrification enzyme activity (DEA) and quantification of denitrifying gene abundances. One denitrification gene commonly measured is *nosZ*, which encodes nitrous oxide reductase, the enzyme responsible for reducing N_2O to N_2 (Knowles, 1982). Abundances of *nosZ* have been demonstrated to correlate with $N_2O/(N_2+N_2O)$ levels (Ducey et al., 2011; Philippot et al., 2009).

To more fully understand denitrification in the restored wetland of the Mid-Atlantic's Delmarva region, the investigations of this paper was undertaken. The objectives were to assess the following: (1) physiochemical conditions; (2) denitrification enzyme activity; and (3) the *nosZ* gene abundances within soils of the natural, converted, and restored wetlands.

2. Materials and methods

2.1. Site description

The Mid-Atlantic Region CEAP-Wetland project included four states on the East Coast: Delaware, Maryland, Virginia, and North Carolina. This paper concerns the Delaware, Maryland, and Virginia sites. Three land use types were compared in this study, including restored, converted, and natural wetlands. For this study, natural wetlands were selected to serve as a measure of predisturbance conditions. The converted wetlands were derived from previously natural wetlands that had been drained to allow agriculture production. The restored wetlands were converted wetlands that had been hydraulically restored by the Natural Resources Conservation Service (NRCS). The wetlands were restored under Conservation Practice Standard 657 (Wetland Restoration) or Conservation Practice Standard 646 (Shallow Water Development and Management). Restored wetlands were selected from a series of candidate sites provided by the NRCS in each state. All of these wetland types had been restored between two to eight years prior to the beginning of this study. They were primarily selected to be depressional wetlands not affected by riverine or tidal waters. The converted wetlands were all sites which had been drained or filled for agricultural use prior to 1985. Natural wetlands were forested wetlands with no known history of drainage, although logging may have occurred at some point in their history. Following their initial selection from the NRCS candidates, restored sites were further evaluated through aerial photography and site visits before final selection. Both converted and natural wetlands were selected to be within 1 to 4 km from the restored sites. Many of the natural sites were managed by the nature conservancy or were state forests. There were a total of 39 sites (14 restored, 11 natural, 14 converted) split across the three states. Of these 39 total sites, 21 were in Maryland (8 restored, 7 natural, 6 converted), 10 in Virginia (3 restored, 3 natural, 4 converted), 8 in Delaware (3 restored, 1 natural, 4 converted). Access and permission made it difficult to have a totally balanced number of treatments.

2.2. Field sampling

For DEA, four points along a topographic gradient were sampled at each site. The four sampling points were selected prior to field sampling using ArcGIS (ESRI, Redlands, CA). A digital elevation model (DEM) was used to select the boundary of each study site. Within each wetland boundary, four evenly split topographic classes were defined using ArcGIS. Each topographic class represented one of the four sampling locations. These are referred to a relative elevation 1–4. Elevation class 1 was the lowest and 4 was the highest elevation class. Sampling points were randomly selected from each topographic class. For field sampling, geographic coordinates or these sampling sites uploaded into a global positioning unit (Trimble Navigation Limited, Sunnyvale, CA). The unit was then used for accurate placement of the sampling sites.

At each sampling location, three samples were collected within a 0.5 m radius of the sampling location from the upper 10 cm of the soil. These were combined into a composite sample. Each sample was placed in a cooler to be returned to the lab for DEA analysis. Soil temperature and electrical conductivity were measured in situ using an ECTestr11 + meter (Spectrum Technologies, East Plainfield, IL). Soil moisture was measured using a Delta-T HH2 Moisture Meter (Dynamax, Houston, TX).

Sampling of each site was performed over a three year period. The first sampling occurred in June 2009 and continued through November 2009. Sampling was continued from May 2010 through November 2010, and additional sampling was done in the May 2011. Over this period each site was sampled at least three times to obtain annual and seasonal variation in DEA.

2.3. Laboratory analysis

All samples collected from 2009 to 2011 were analyzed for DEA analysis using the acetylene inhibition method (Miller et al., 2012; Tiedje, 1994). All soils were kept under refrigeration at their initial field moisture. An additional set of samples were stored at $-80\,^{\circ}C$ for DNA extraction. From each sample, 10–15 g of soil was placed in 60 mL serum bottles (three replications). The treatments were as follow:

(A) Five mililiter of a solution containing chloramphenicol ($1\,g\,L^{-1}$) and 15×10^{-3} L of acetylene (produced from calcium carbide) to block denitrification at the nitrous oxide phase for measuring actual complete denitrification;
(B) Five mililiter of a solution containing chloramphenicol ($1\,g\,L^{-1}$) to inhibit protein synthesis and to measure actual incomplete denitrification;
(C) Five mililiter of a solution containing chloramphenicol ($1\,g\,L^{-1}$) and nitrates ($200\,mg\,L^{-1}$ NO_3–N), and 15×10^{-3} L of acetylene to measure potential complete denitrification;
(D) Five mililiter of a solution containing chloramphenicol ($1\,g\,L^{-1}$) and nitrates ($200\,mg\,L^{-1}$ NO_3–N) to measure potential incomplete denitrification.

In addition to direct comparison of treatments A–D, the ratios between actual and potential incomplete DEA rates were also compared. These ratios are an indication of the percentage of incomplete DEA occurring in a system at the time of measurement (ratioBA), or the potential maximum rate that can occur in a system if provided with an unlimited amount of nitrate (ratioDC).

Soil C and N were measured using a TruSpec CN analyzer (Leco Corp, St Joseph, MI). Soil pH was measured using a 1:1 (w/w) mixture of soil and water. Air dried soil samples were extracted using a Mehlich 1solution. The extracts were subsequently analyzed on an inductively coupled plasma (ICP)-atomic emission spectrometry

(ICP, Vista Pro, Varian Inc., Walnut Creek, CA) for Al, Ca, Cu, Fe, K, Mg, Na, P, and Zn. These soil samples were also extracted using water at a ratio of 5:1; extracts were filtered through a 0.2 μm filter. Anions were then measured by chemically suppressed ion chromatography (IC) using a Dionex 2000 Ion Chromatograph (ASTM Standard D4327-11). The following anions were measured: Cl, SO_4–S, and PO_4–P.

2.4. Soil DNA extraction

Soil DNA extraction was performed using a PowerSoil DNA Extraction Kit (MO BIO Laboratories Inc., Carlsbad, CA) according to manufacturer specifications. DNA was extracted from a total of 472 samples (107 in 2009, 299 in 2010, 66 in 2011). DNA quality and quantity were determined via Biophotometer (Eppendorf, Hamburg, Germany), and electrophoresis on a 1% agarose gel stained with SYBR Safe.

2.5. nosZ quantitative real-time PCR (qPCR) assays

All qPCR assays were performed using the LightCycler 480 Real-Time PCR Detection System (Roche Diagnostics, Indianapolis, IN). Two primers nosZF (CGYTGTTCMTCGACAGCCAG) and nosZ-1622R (CGSACCTTSTTGCCSTYGCG) capable of producing a ~450 bp amplification product were used in the study; these were obtained from Integrated DNA Technologies (Coralville, IA). Assays were carried out using SYBR GreenER qPCR SuperMix (Invitrogen, Carlsbad, CA) in a total volume of 25 μL. The final reaction concentration of reagents was as follows: 1× SYBR GreenER qPCR SuperMix; 200 nM each of forward and reverse primers; and 10 ng of DNA template. The qPCR reaction conditions were as follows: (1) an initial denaturation at 95 °C for 5 min; (2) 50 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s; and (3) a final melting curve analysis to confirm amplification product specificity. Fluorescent measurements were taken during the annealing phase of each cycle. Data was collected and processed using the LightCycler 480 software package. All qPCR assays included control reactions without template, and were performed in triplicate. A *nosZ* DNA standard, derived from the linearized plasmid pCPDnosZ1 (Ducey et al., 2011), was utilized to develop a standard curve from between 10^1 and 10^9 copies per reaction; this standard was also used to calculate an amplification efficiency of 1.90 according to the equation: $E = 1 + 10^{[-1/\mathrm{slope}]}$ (Pfaffl, 2001).

2.6. Statistics

The data were statistically analyzed using SAS v 9.3 (SAS Institute and Inc, 2002). Analysis of variance for DEA rates of NosZ gene copies was done using GLIM-MIX procedure. The replications were considered random; sites, sampling dates, and laboratory replication were pooled for replication. The treatments were considered fixed; these were wetland management type and relative elevation class. Treatments were analyzed using the least squares mean (LSM) method. The treatment differences of analyzed variables were compared using the pdiff option. The T value grouping for treatment LSM was at $P \leq 0.05$. The DEA and *nosZ* gene copies were also analyzed using stepwise regression. The regression was done within each wetland management. In the case of DEA, the stepwise regression was more specific being done for each DEA treatment (A–D) within each wetland management type. Twenty one parameters used in the stepwise regression were moisture, pH, EC, TC, TN, CN ratio, Al, Ca, Cu, Fe, K, Mg, Na, P, Zn, Cl, PO_4–P, SO_4–S, Al/(Ca + Mg), K/(Ca + Mg), and P/(Al + Fe). With the stepwise analyses, there was a first step linear regression of the best linear fitted variable. Subsequently, the regression was expanded by stepwise regressions to determine if additional parameters provided significant improvement to the regression. Generally, three or more of the variables were significant for the linear regression with a P value of ≤0.001. Additionally, the Mallow's Cp values for the final step of the stepwise regressions were typically near the desired value that corresponded to the number of variables used in the final regression step. Thus, these Cp values were consistent with an acceptably low collinearity in the stepwise regression model.

2.7. Soil physiochemical characteristics

The natural wetlands were statistically different from the converted wetlands in all soil physiochemical characteristics (Tables 1–3). For most, but not all, of the measured parameters, the restored wetlands showed greater similarity to the converted wetlands than the natural wetlands. While this is not the most desirous effect of wetland restoration, it is not an unsuspected result (Brinson and Eckles, 2011). It is reasonable to hypothesize however, that time will lessen the agricultural legacy of the restored wetlands, and they will more closely approximate the physicochemical makeup of the natural wetlands analyzed in this study.

In regards to soil C, natural wetland soils had contents three times higher than either the restored or converted wetlands. In the natural wetlands, the soil carbon was greatest (7.5%) in the lowest landscape position, relative depth 1. This result is consistent with the wetter soil conditions of the lower elevation. It decreased with increasing relative landscape position to the highest position, relative depth 4, with a soil C content of 5.6%. It should be noted that this content was still nearly threefold higher than the highest soil C content of either the converted or restored wetlands. In contrast to the considerable effect of elevation on C content in the natural wetlands, the restored wetlands varied very little with relative depth, with a range from 1.8% to 2.0%. In the converted wetlands, the soil C contents were lower, ranging from 1.5% to 2.0%, and was effected by elevation. Thus, the highest value was at the lowest relative depth in the natural wetlands, and the lowest soil C content was at the highest elevation of the converted wetlands.

Similar to soil C, the soil N contents of the natural wetlands were highest, with a significant increase over both the restored and converted sites at all elevations. The soil N content of the natural wetlands ranged from 0.28% to 0.48%. The lowest relative depth had the greatest N content; the highest relative elevation had the least soil N content. In the restored and converted wetlands, the soil N contents were all ≤0.2%, and they varied very little with relative depth. These values for C and N are similar to those of forested and non-forested riparian wetlands for the Delaware River basin reported by Bedison et al. (2013). Their forested soil had C and N contents of 4.8 and 0.3%, respectively. The non-forested soil had C and N contents of 2.3 and 0.1%, respectively.

As a result of the soil C and N concentrations, the C/N ratios were highest in the natural wetlands. In the natural wetlands the C/N ratio was greatest for the highest elevation (19.7) and smallest for the lowest elevation (13.8). Elevation had no significant impact on the soil C/N ratio in the restored wetlands; they were all above 9.0. The lowest C/N ratio (7.5) was found in the converted wetlands at the highest elevation. These low carbon and nitrogen ratios could certainly be expected to enhance incomplete denitrification (Hwang et al., 2006; Zhang and Wang, 2009). However, even the natural wetland C/N ratios could be suspect for incomplete denitrification, and the restored and converted could be highly suspect for this incomplete denitrification (Hunt et al., 2007; Klemedtsson et al., 2005).

Table 1
Wetland soil physicochemical characteristics for different managements and relative elevations.

Management	Relative Elevation	TC	TN	CN ratio	pH	EC (μS/cm)	ORP (mV)	Moisture (%)	Soil temp (°C)
		%							
Natural	1	7.5a†	0.48a	13.8d	4.43f	41.1e	621b	34.4a	21.8d
	2	7.1b	0.42b	15.2c	4.23g	37.7e	606b	32.5b	22.0d
	3	6.1c	0.35c	16.7b	4.17g	46.6e	624b	28.6c	21.4d
	4	5.6d	0.28d	19.7a	4.25g	44.0e	654a	22.1e	22.1d
Restored	1	2.0e	0.18ef	9.3e	5.61e	69.2cd	500f	24.6d	25.0abc
	2	2.1e	0.19ef	9.1e	5.78d	53.0de	468g	19.5f	24.6bc
	3	2.1e	0.19ef	9.3e	5.72d	50.5e	538e	17.2gh	24.3c
	4	1.8ef	0.18ef	9.3e	6.12a	54.3de	549de	13.5i	24.7bc
Converted	1	2.0e	0.20e	9.3e	6.01b	141.9a	540de	18.6fg	25.3ab
	2	1.9ef	0.19ef	9.0e	5.92c	144.1a	543de	16.2h	25.0abc
	3	1.6fg	0.18ef	8.0f	6.02b	108.9b	574c	14.0i	24.9abc
	4	1.5g	0.17f	7.5g	5.90c	83.6c	558cd	11.2j	25.5a

† Based on least significant means values ($P < 0.05$).

In the case of soil pH the natural wetlands were quite acidic ranging from 4.17 to 4.43. The restored and converted wetlands were more neutral in pH, with ranges of 5.72 to 6.12 for restored sites, and 5.9 to 6.02 for converted sites. With the exception of the highest elevation in the restored sites which was statistically the soil with the highest pH in the study, the converted sites were more neutral than the restored. This is to be generally expected and indicative of liming to achieve soil pH's conducive for micronutrient solubility (Hue and Licudine, 1999).

Somewhat similar to pH, the EC values were lower for either the natural or restored wetlands relative to the converted wetlands. Converted wetlands had EC values > 83 $\mu S\,cm^{-1}$. The restored had had EC values were <70 $\mu S\,cm^{-1}$, and the natural wetlands had EC values were <47 $\mu S\,cm^{-1}$. Moreover, the EC decrease with depth was most pronounced for the converted wetlands. This is indicative of an agricultural legacy of the converted and restored sites, as fertilizer use has been demonstrated to increase soil EC (Agassi et al., 1981). In the case of soil moisture, the natural wetlands were significantly wetter than the converted wetlands; the moisture contents ranged from 22.1% to 34.4% and 11.2% to 18.6%, respectively, for the natural and converted wetlands. The restored wetlands showed an intermediate pattern. At each relative elevation they were wetter than the converted wetlands, but dryer than the natural wetlands, with a range of moisture contents from 13.5% to 24.6%. The higher moisture content of the natural wetlands would be expected to influence a lower soil temperature and that was the case. While their soil temperatures were <23 °C, the soil temperatures of both the restored and converted wetlands were >24 °C.

Significant differences occurred in the plant available nutrient content among soils of natural, restored, and converted wetlands. For plant available Al, Ca, Cu, K, and Mg, the natural wetlands were statistically different from both restored and converted wetlands (Table 2). For Fe, P and Zn, the restored wetlands were more similar to the natural wetlands than to the converted wetlands. As for Na and Mg, the restored wetlands were more similar to converted wetlands. At the individual landscape level, the highest nutrient contents were mostly in the lowest elevation for the natural and converted wetlands. However, only Al, Fe, K, Na, P, and Zn were highest in the lowest elevation of the restored wetland.

The Al/(Ca + Mg) and P/(Al + Fe) molar ratios was used to assess if restored wetlands were recovering the plant available nutrient composition of natural wetlands. Compared to the natural wetlands, significant lower Al/(Ca + Mg) ratios (0.21–1.12) in both the restored and converted wetlands were indicative of Ca and Mg addition to converted wetlands as lime amendment. Lime amendment maintained the soil pH of converted wetlands in the range of 5.90 to 6.02 (Table 1). In restored wetlands, the agricultural legacy of these lands – Ca and Mg inherited from their previous converted wetlands status – contributed to maintaining the soil pH in the range of 5.61 to 6.12. On the other hand the significantly higher Al/(Ca + Mg) ratios for the acidic natural wetland soils were the result of Al predominance in the more acidic soil environments that existed between pH 4.17 and 4.43 (Table 1). These significant differences in pH among the three wetland management types also affected P solubility.

In restored wetlands, solubilization of residual P fertilizer is possible because the redox potential can vary with fluctuating water table (Scholz, 2011). Inorganic P is retained in phosphate form by ferric-iron oxides but decreasing redox potential can induce iron reduction into soluble ferrous form with simultaneous release of P (Reddy et al., 2000). However, P can be strongly bound to Al compounds in restored wetland soils even during fluctuating redox potentials (Scholz, 2011). Compared to the natural and restored wetlands, the plant available P levels were significantly higher for converted wetland (Table 2). Since the highest plant available P is at a maximum near neutral to slightly acid pH of 6.5 (Havlin et al., 1999), the highest available P concentrations were found in the slightly acidic converted wetland soils. The P/(Al + Fe) molar ratio has been used as an index to infer P availability across soils of the Mid-Atlantic area including restored wetland soils (Moser et al., 2009; Sims et al., 2002). Compared to natural wetlands, high significant P/(Al + Fe) indices (0.215–0.233) indicated higher P availability in converted wetlands across the four elevations. In turn, low P/(Al + Fe) indices (0.028–0.035) in natural wetland soils indicated low P availability in all four landscape positions. With respect to P availability in restored wetlands, they had significantly higher P/(Al + Fe) values than the natural wetlands. Conversely, they had much lower P/(Al + Fe) values than the converted wetlands. This indicated transition of the restored wetlands toward natural wetland conditions—a much desired condition for water quality.

2.8. Soil denitrification enzyme activity

The DEA values were converted to the natural log to obtain better analyses of the substantially variable rates. The natural log of DEA will be hence represented by LDEA. The DEA treatments produced results with the expected relationships (Miller et al., 2012). The greatest rates were found where non-nitrate limiting N was added. For these treatments when acetylene was added, the mean LDEA rate was 2.82 $\mu g\,N_2O–N\,kg^{-1}\,soil\,h^{-1}$; this rate was significantly higher than the other three treatments. When acetylene was not added, the natural log of nitrous oxide (LN_2O) accumulation rate was 2.00 $\mu g\,N_2O–N\,kg^{-1}\,soil\,h^{-1}$. When no additional nitrate

Table 2
Wetland soil physicochemical characteristics for different managements and relative elevations.

Management	Relative Elevation	Al	Ca	Cu	Fe	K	Na	Mg	P	Zn	Ratio K K/(Ca + Mg)	Ratio Al Al/(Ca + Mg)	Ratio P P/(Al + Fe)
		mg/kg											
Natural	1	672a†	111f	0.31e	79ef	75f	24.7a	58f	25.1f	2.22bc	0.538c	7.26c	0.04e
	2	692a	55g	0.29ef	75f	62g	20.0b	41g	21.6gh	1.74d	0.560b	8.09b	0.04e
	3	609b	47g	0.20g	85e	54h	18.3c	35gh	19.0h	1.35fg	0.651a	10.07a	0.03e
	4	443c	53g	0.24fg	137a	51h	14.0g	31h	13.0i	1.70d	0.559bc	6.83d	0.03e
Restored	1	277e	500e	0.45d	113b	83e	16.9de	111d	25.3f	1.53e	0.145ef	0.48f	0.07d
	2	232fg	522e	0.44d	101c	72f	15.5f	110d	23.4fg	1.31g	0.126fg	0.42f	0.08d
	3	341d	507e	0.44d	83ef	70f	16.2ef	102e	25.7f	1.10h	0.140efg	1.12e	0.08d
	4	247f	613d	0.43d	51g	82e	12.4h	117c	31.7e	1.49ef	0.123g	0.43f	0.14c
Converted	1	300e	1086a	1.05a	93d	149a	20.2b	178a	81.7a	2.56a	0.149e	0.21f	0.23b
	2	282e	890b	0.98b	77f	132b	17.4cd	143b	69.9b	2.31b	0.157de	0.26f	0.22b
	3	246f	778c	0.93b	49g	119c	15.3f	123c	66.4c	2.12c	0.154e	0.26f	0.25a
	4	207g	624d	0.86c	29h	108d	12.4h	106de	51.9d	2.08c	0.177d	0.32f	0.23b

† Based on least significant means values ($P < 0.05$).

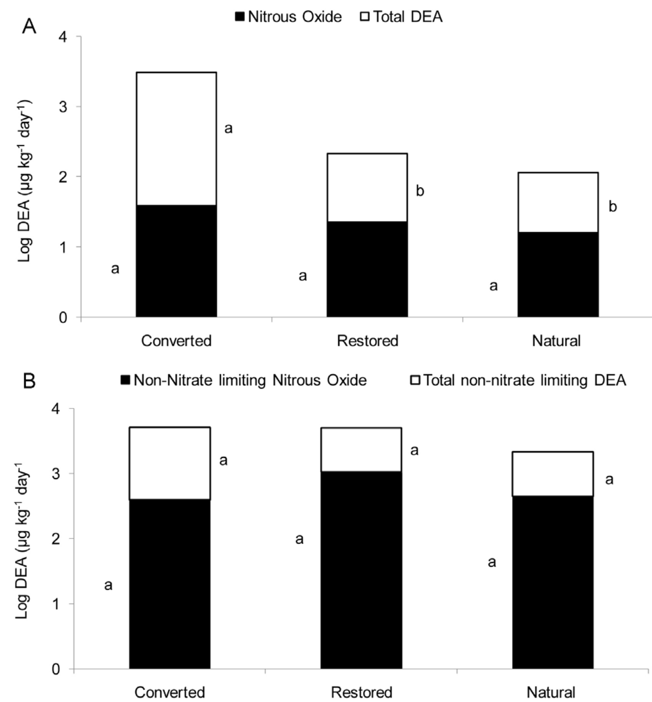

Fig. 1. DEA (a) and non-nitrate limited DEA (b) in CEAP management types at landscape position 1 (lowest elevation).

was added, the LDEA rate was 2.08 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1}. In the control where neither acetylene nor nitrate additives were present, the LN_2O accumulation rate of 0.95 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1} was significantly lower than any of the other treatments. The wetland management type and relative elevation were significant in the ANOVA at the 0.01 level. Additionally, the interaction of type and elevation was significant in the ANOVA at the 0.01.

2.9. Relative elevation 1 (lowest elevation)

The converted wetlands had an LDEA rate of 3.49 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1}(Fig. 1a). This rate was significantly higher than the LDEA rates of either natural or restored wetlands, 2.06 or 2.33 $\mu g\ N\ kg^{-1}$ soil h^{-1}, respectively. These DEA rates were within the reported range for riparian buffers in a Coastal Plain of North Carolina (Hunt et al., 2007). As would be expected, they were lower than the mean LDEA of 4.7 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1} in a riparian buffer that was significantly impacted with swine wastewater application (Hunt et al., 2004). When acetylene was not added to block nitrous oxide conversion to dinitrogen gas, the nitrous oxide accumulation was substantive for all the management types. This indicated a considerable potential for incomplete denitrification. The LN_2O accumulation rate for the converted wetlands, 1.59 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1}, was not significantly higher than the other two management types. For the natural and restored wetlands, the LN_2O accumulation rates were 1.20 and 1.36 $\mu g\ N_2O{-}N\ kg^{-1}$ soil h^{-1}, respectively. The percentages of nitrous oxide accumulation in the non-acetylene control compared to the DEA rates were 25% for the converted wetlands and <44% for either of the other management types (ratioBA, Table 4). This percentage is higher than the 25% found in the Coastal Plain riparian buffers by Hunt et al. (2007). However, all of the wetland sites of this CEAP evaluation had C/N ratios <20, and many of the riparian buffer sites had C/N ratios >25. These higher ratios are generally associated with less base level accumulation of nitrous oxide (Klemedtsson et al., 2005). When

Table 3
Wetland soil physicochemical characteristics for different managements and relative elevations.

Management	Relative Elevation	Cl	NO_3–N	SO_4–S	PO_4–P
		mg/kg			
Natural	1	11.55cd[†]	4.91ef	49.93a	2.37d
	2	10.47de	5.32e	40.62b	2.65d
	3	11.05cd	5.29e	30.02c	2.41d
	4	11.73cd	4.51ef	17.21de	2.59d
Restored	1	8.00e	6.13e	14.68ef	0.89f
	2	9.46de	2.81f	13.19fg	1.25e
	3	9.89de	6.92e	12.84fg	1.32e
	4	10.59d	11.13d	11.07g	2.35d
Converted	1	33.06a	21.36b	19.17d	4.73a
	2	21.97b	25.10a	14.76ef	4.12b
	3	21.76b	22.15b	11.11g	4.64a
	4	13.62c	17.81c	7.51h	3.74c

† Based on least significant means values ($P < 0.05$).

nitrate was added, the LDEA increased in both the converted and restored wetlands to 3.71 and 3.70 μg N_2O–N kg^{-1} soil h^{-1}, respectively (Fig. 1b). Moreover, the natural wetlands increase to a 3.34 μg N_2O–N kg^{-1} soil h^{-1} which was not significantly different than the other two management practices. Thus, denitrification rates in soils of all of the management types were limited by nitrate, but the converted wetlands were less limited. This would be consistent with a reasonable assumption that the converted wetlands received direct or indirect N fertilization associated with production of agriculture crops. In all of the management conditions, the addition of nitrate increased the amount of nitrous oxide that accumulated even when acetylene blockage was absent. This is similar to the lack of significant difference in DEA between natural and created wetlands reported by Ahn and Peralta (2012) when the DEA samples were amended with nitrate. The DEA of their sites ranged from 41 to 228 μg N_2O–N kg^{-1} soil h^{-1}. While the N_2O accumulations were not significant different for management types, they were a significant percentage of the total DEA. They ranged from 2.61 μg N_2O–N kg^{-1} soil h^{-1} in the restored wetlands to 3.03 μg N_2O–N kg^{-1} soil h^{-1} in the converted wetlands (Fig. 1b). This was 49 to 66% of the non-nitrate limited DEA; the natural wetlands were the highest (ratioDC, Table 4).

2.10. Relative elevation 2

The LDEA rate was again the significantly highest for the converted wetlands, 2.91 μg N_2O–N kg^{-1} soil h^{-1} (Fig. 2a). While lower, the restored and natural wetlands were very similar for LDEA rates, 2.10 and 2.18 μg N_2O–N kg^{-1} soil h^{-1}, respectively. When no acetylene was added, the level of N_2O accumulated was not significantly different for the management conditions (Fig. 2a). The LN_2O ranged from 1.13 to 1.32 μg N_2O–N kg^{-1} soil h^{-1}. The percentages of non-acetylene block N_2O–N (ratioBA) was lowest in the converted wetlands and highest in the natural wetlands, 24 and 52%, respectively (ratioBA, Table 4). When nitrate was added, the LDEA were

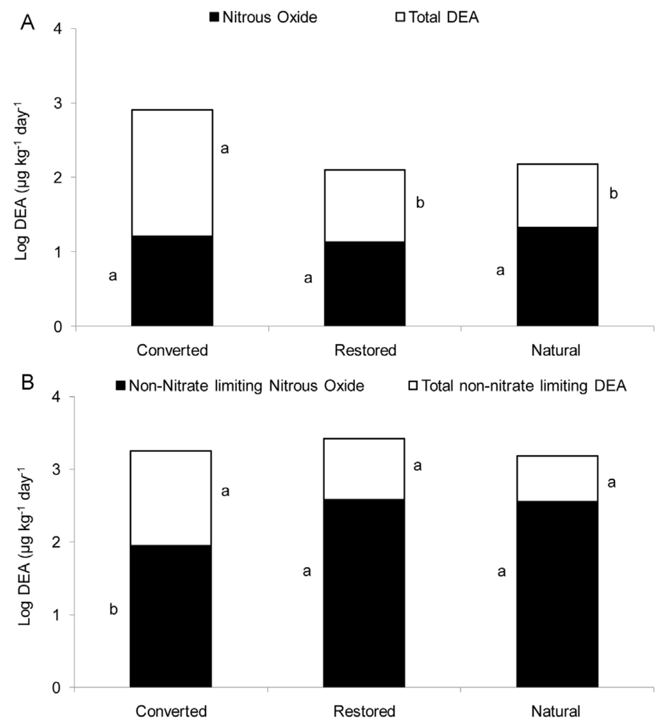

Fig. 2. DEA (a) and non-nitrate limited DEA (b) in CEAP management types at landscape position 2.

higher, but they were not significantly different for management type. The LDEA ranged from 3.43 to 3.19 μg N_2O–N kg^{-1} soil h^{-1}. When nitrate was added in the absence of acetylene, the natural and restored wetlands were not significantly different from each other and >2.58 μg N_2O–N kg^{-1} soil h^{-1}. Yet, both were higher than the converted management 1.95 μg N_2O–N kg^{-1} soil h^{-1}.

Table 4
DEA ratio's.

CID	Natural		Restored		Converted	
	RatioBA	RatioDC	RatioBA	RatioDC	RatioBA	RatioDC
	%					
1	44.0abc[†]	65.9a	37.1cd	56.5abc	25.4ef	48.7cd
2	51.6a	63.3ab	38.6bcd	51.6bcd	24.2f	40.9d
3	50.3ab	56.0abc	46.1abc	55.2abc	23.4f	39.2d
4	51.7a	64.1ab	36.5cde	45.9cd	31.1def	44.2cd

† Based on least significant means values ($P < 0.05$).

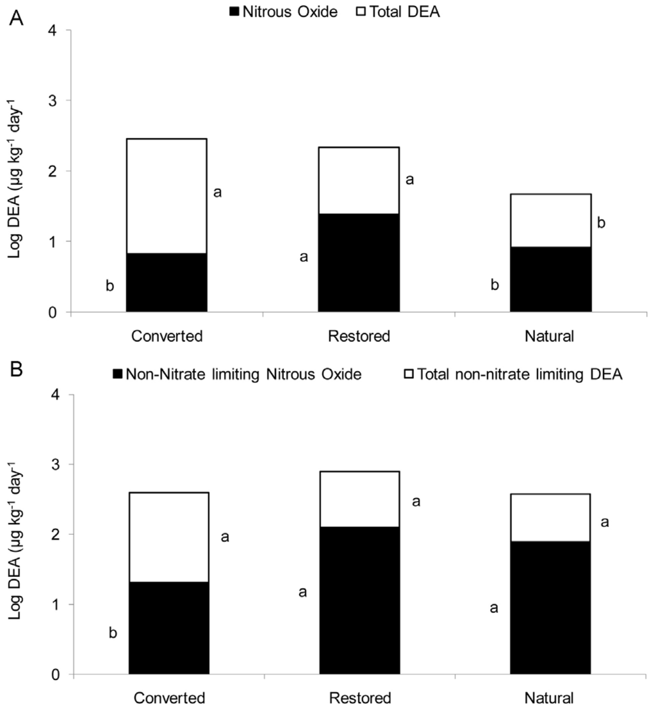

Fig. 3. DEA (a) and non-nitrate limited DEA (b) in CEAP management types at landscape position 3.

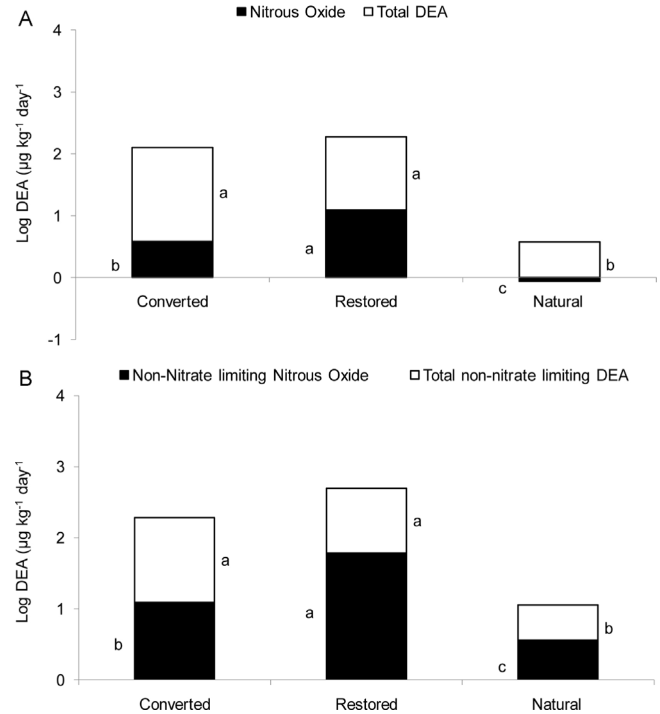

Fig. 4. DEA (a) and non-nitrate limited DEA (b) in CEAP management types at landscape position 4 (highest elevation).

Accordingly, the converted wetlands had a considerably lower percentage of incomplete denitrification, with 41% of the non-nitrate limited DEA; the other wetland types had percentages >63% (ratioDC, Table 4).

2.11. Relative elevation 3

As anticipated, the soils of this higher relative elevation had lower LDEA rates (Fig. 3). Furthermore, the natural wetlands had significantly lower LDEA, 1.67 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$ than either of the other wetland management types (Fig. 3a). The restored and converted wetlands were both >2.33 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$ and non-significantly different from each other. When acetylene was withheld, the LN_2O of 1.39 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$ in the restored wetlands was significantly higher than the other management types; they were <0.92 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$. The reason for the restored wetlands being so high is not clear. When nitrate was added, the LDEA was not significantly different for the treatments; they ranged from 2.57 to 2.90 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$ (Fig. 3b). The nitrate had again eliminated the management differences. However, when acetylene was withheld, the LDEA derived from incomplete denitrification was least in the converted wetlands, 1.31 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$. This was 39% incomplete denitrification; the other management types were >55% (ratioDC, Table 4).

2.12. Relative elevation 4

At the highest relative elevation, the highest LDEA of 2.27 $\mu g\, N_2O-N\, kg^{-1}\, soil\, h^{-1}$ occurred in the restored wetlands (Fig. 4a). This rate was significantly lower than the > 2.1 $\mu g\, N_2O–N\, kg^{-1}\, soil\, h^{-1}$ of other two management types. When acetylene was withheld all of the management types were statistically different from each

Table 5
Stepwise regression of DEA (control) by landscape management.

Type	Treatment	step	Variable	Partial R^2	Model R^2	C	Prob. F
Converted	A	1	CN ratio	0.24	0.24	102.61	<0.0001
		2	Zn	0.07	0.31	52.91	<0.0001
		3	TN	0.03	0.34	30.62	<0.0001
	B	1	CN ratio	0.30	0.30	87.02	<0.0001
		2	Fe	0.06	0.36	43.06	<0.0001
		3	Zn	0.03	0.38	24.94	<0.0001
Natural	A	1	P ratio	0.14	0.14	99.86	<0.0001
		2	Moisture	0.05	0.19	73.88	<0.0001
		3	CN ratio	0.03	0.21	61.29	0.0004
	B	1	P ratio	0.21	0.21	114.75	<0.0001
		2	Moisture	0.06	0.28	79.54	<0.0001
		3	K ratio	0.04	0.31	58.49	<0.0001
Restored	A	1	Ca	0.15	0.15	140.14	<0.0001
		2	K	0.05	0.20	100.34	<0.0001
		3	K ratio	0.04	0.25	68.05	<0.0001
	B	1	TC	0.16	0.16	160.78	<0.0001
		2	P	0.06	0.22	117.23	<0.0001
		3	Al	0.03	0.25	97.89	<0.0001

Table 6
Stepwise regression of DEA (non-nitrate limiting) by landscape management.

Type	Treatment	step	Variable	Partial R^2	Model R^2	Cp	Prob. F
Converted	C	1	TC	0.30	0.30	159.39	<0.0001
		2	CN ratio	0.06	0.36	101.88	<0.0001
		3	Zn	0.06	0.42	51.53	<0.0001
	D	1	CN ratio	0.34	0.34	153.51	<0.0001
		2	Mg	0.08	0.42	76.46	<0.0001
		3	Fe	0.04	0.46	44.34	<0.0001
Natural	C	1	Moisture	0.34	0.34	152.25	<0.0001
		2	Mg	0.04	0.39	119.33	<0.0001
		3	P ratio	0.03	0.41	98.99	<0.0001
	D	1	Moisture	0.31	0.31	145.72	<0.0001
		2	Mg	0.05	0.36	109.30	<0.0001
		3	P ratio	0.02	0.38	93.52	0.0002
Restored	C	1	Ca	0.18	0.18	207.69	<0.0001
		2	Fe	0.06	0.24	152.66	<0.0001
		3	K	0.05	0.29	107.96	<0.0001
	D	1	TC	0.15	0.15	230.62	<0.0001
		2	Cu	0.07	0.22	171.83	<0.0001
		3	K	0.03	0.25	149.05	<0.0001

other, and the natural wetland, were again the least with a LDEA of 0.01 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$. The restored wetlands were highest with a rate of 1.09 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$. When nitrate was added, the restored and converted wetlands were not significantly different, and they were >2.28 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$ (Fig. 4b). The natural wetlands were lowest with 1.06 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$. When nitrate was added without acetylene, the wetland management types were all significantly different from each other. The restored wetlands were the highest with a LN_2O rate of 1.79 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$. The natural wetlands were lowest with 0.56 $\mu g\ N_2O–N\ kg^{-1}\ soil\ h^{-1}$; this was 52% incomplete denitrification. The other management types had <37% incomplete denitrification (ratioDC, Table 4).

2.13. Stepwise regression

Stepwise analysis allow for insight into the relationship of soil physiochemical condition impacting DEA. In some past investigation of wetland riparian buffers Hunt et al. (Hunt et al., 2004) have found soil C and N to have R^2 correlations of >0.75. Such was not the case in this investigation different factors appeared to be impacting DEA in the natural, converted, and restored wetlands.

Treatment A is an acetylene blocked DEA measurement which results in the block of denitrification at the nitrous oxide phase, and is therefore used to measure actual complete denitrification. For treatment A of the converted wetlands, the DEA was best correlated to CN ratio with an R^2 of 0.24 (Table 5). The CN ratio of the converted wetlands was the lowest. Yet, the likelihood for fertilizer nitrate inducing enhanced denitrification was considerable, and the denitrification of this nitrogen would have been impeded by low soil carbon. This difference in denitrification is consistent with that of Ding et al. (2012) where varying levels for CN ratio were added to treatment wetlands. The addition of Zn and TN in the stepwise regression increased the R^2 to 0.34. The final regression equation was DEA = 0.40 CN ratio + 0.19 Zn + 10.19 TN - 1.91. The impact of TN on denitrification in wetland is quite expected (Hunt et al., 2004), and Zn and Al have also been reported to impact denitrification in wetland systems (Sakadevan et al., 1999; Zhang and Wang, 2009). In the case of the restored wetlands and treatment A, the best correlation was with Ca, R^2 of 0.15. Adding the K and K ratio to the stepwise regression increased the R^2 to 0.25. The final regression equation was DEA = −0.00003 Ca + 0.03 K–9.08 K/(Ca + Mg) + 1.88. The natural wetlands were best correlated with P ratio with an R^2 of 0.14. The addition of moisture and CN ratio increased the correlation to an R^2 of 0.21. The final regression equation was DEA = 23.7 P/(Ca − Mg) + 0.02 moisture −0.07 CN ratio + 1.63.

Treatment B is the measure of $N_2O–N$ accumulation absent the acetylene to block of the final conversion of denitrification to di-nitrogen. In the case of treatment B for the converted wetlands, the best correlation was the CN ratio; it had an R^2 of 0.30 (Table 5). The addition of Fe, Zn, and moisture to the stepwise regression increased the R^2 to 0.38. The final regression equation was $N_2O–N$ = 0.35 CN ratio + 0.005 Fe + 0.18 Zn − 2.49. The best correlation for the restored wetlands was the TC with an R^2 of 0.16. When P and Al were added, the R^2 improved to 0.25 (Table 5). The final regression equation was $N_2O–N$ = 0.89 TC + 0.03 P − 0.002 Al − 0.09. As with treatment A in the case of the natural wetlands, the best fit was P ratio with an R^2 of 0.21. The addition of moisture and K ratio increased the R^2 to 0.31. The final regression was $N_2O–N$ = 25.6 P/(Al + Fe) + 0.03 moisture −0.97 K/(Ca + Mg) − 0.09.

Treatment C was the measure of DEA with non-limiting $NO_3–N$ added. For the converted wetlands, the best stepwise regression

Table 7
nosZ gene abundances by management and elevation.

Management	Samples (n)	Mean[†] ± S.E.	Grouping[‡]
Converted	129	6.783 ± 0.045	A
Restored	160	6.686 ± 0.046	B
Natural	97	6.439 ± 0.045	C
Elevation	**Samples (n)**	**Mean ± S.E.**	**Grouping**
1 = Lowest	96	6.705 ± 0.046	A
2	96	6.662 ± 0.045	B
3	96	6.674 ± 0.046	A,B
4 = Highest	98	6.501 ± 0.045	C
Management	**Elevation**	**Mean ± S.E.**	**Grouping**
Converted	1	6.897 ± 0.049	A
Converted	2	6.799 ± 0.049	B
Converted	3	6.782 ± 0.049	B
Restored	2	6.716 ± 0.048	C
Restored	4	6.703 ± 0.048	C,D
Restored	3	6.678 ± 0.048	C,D
Converted	4	6.653 ± 0.049	D
Restored	1	6.646 ± 0.048	E
Natural	1	6.573 ± 0.049	F
Natural	3	6.563 ± 0.051	F
Natural	2	6.472 ± 0.050	G
Natural	4	6.146 ± 0.051	H

† Copy numbers per gram of soil are $\log_{10}$ transformed.
‡ Based on Duncan's multiple range values ($P < 0.05$).

Table 8
Stepwise regressions between *nosZ* gene abundance and environmental variables.

Management	Variable (R^2)†				
All	Ca (0.21)	Moisture (0.03)	RK (0.03)	SO4 (0.01)	Na (0.01)
Converted	Ca (0.20)	C:N (0.04)	Cu (0.04)	SO4 (0.04)	Na (0.02)
Restored	Ca (0.28)	Moisture (0.04)	Cu (0.03)	RK (0.02)	Na (0.02)
Natural	C:N (0.11)	RK (0.05)	Moisture (0.03)	pH (0.02)	EC (0.01)

† Variables placed in order of ranking in stepwise regression. Top five for each management are listed. All variables are significant ($P < 0.0001$).

correlation, R^2 of 0.30 was with TC (Table 6). The R^2 was increased to 0.42 with the addition of the CN ratio and Zn. The final regression equation was DEA with non-limiting NO_3–N = 0.86 TC + 0.27 CN ratio + 0.27 Zn − 0.95. In contrast, none of these wetland parameters were greatly correlated to treatment C in the restored wetlands. Their best correlation was an R^2 of 0.18 with Ca. The correlation was increased to an R^2 of 0.29 by the addition of Fe and K (Table 6). The final regression equation was DEA with non-limiting NO_3–N = 0.002 Ca + 0.006 Fe + 0.01 K + 1.12. The natural wetlands the best correlation, R^2 of 0.34 was with soil moisture. The correlation was increased to an R^2 of 0.41 by the addition of Mg and P ratio (Table 6). The final regression equation was DEA with non-limiting NO_3–N = 0.05 moisture + 0.010 Mg + 14.6 P/(Al + Fe) + 0.27.

Treatment D was the measure of N_2O–N accumulation when non-limiting NO_3–N added in the absence of acetylene to block of the final conversion of denitrification to di-nitrogen. In the converted wetlands the best correlation, R^2 of 0.34 was with the CN ratio (Table 6). The R^2 was increase to 0.46 by addition to the stepwise of Mg and Fe (Table 6). The final regression equation was N_2O-N with non-limiting NO_3–N = 0.39 CN ratio + 0.005 Mg + 0.007 Fe − 2.34. In the case of the restored wetlands, the best R^2 was only 0.15 with TC. The R^2 was increased to 0.25 by the addition of Cu and K (Table 6). The final regression equation was N_2O-N with non-limiting NO_3–N = 0.60 TC + 1.57 Cu + 0.009 K + 0.20. As with treatment C, the natural wetlands were best correlated for treatment D by soil moisture. The R^2 was 0.31. The R^2 was increased to 0.38 by the addition of Mg and P ratio (Table 6). The final regression equation was N_2O–N with non-limiting NO_3–N = 0.05 moisture + 0.01 Mg + 13.5 P/(Al + Fe) − 0.13.

2.14. Nitrous oxide reductase (nosZ) gene abundances

Quantitative Real-Time PCR (qPCR) results for soil *nosZ* gene abundances can be found in Table 7. Comparison of land management types reveals that converted and restored wetlands had significantly ($P < 0.05$) greater abundances of *nosZ* than natural wetlands; natural wetlands contained roughly half the number of *nosZ* gene copies per gram of soil (∼2750,000), as converted (∼6000,000) and restored (∼5000,000) wetlands. Additionally, converted and restored wetlands were also statistically different from each other ($P < 0.05$). Levels of *nosZ* in these soils reflected levels previously reported in wetland and cropland soils (Ji et al., 2012; Miller et al., 2009). The difference of *nosZ* abundances as a function of land management is reflected in a majority of the chemical characteristics as well. The direct impact of land management on both soil pH and soil nutrients has been previously demonstrated as having a significant effect on other genes within the denitrification pathway (Enwall et al., 2010). In a study by Miller et al. (2009), they hypothesized that high N fertilization rates in cropping system soils may have resulted in increased denitrifier populations.

Examination of *nosZ* gene abundances along the elevation gradient demonstrated increased *nosZ* gene abundances as elevation decreased (Table 7). There were significantly ($P < 0.05$) greater abundances at the lower elevations, with ∼5000,000 copies of *nosZ* per gram of soil at elevation 1, and ∼3250,000 copies of *nosZ* per gram of soil at the highest elevation (elevation 4). The middle elevations (elevations 2 and 3) had ∼4250,000 copies of *nosZ* per gram of soil. Higher abundances of the *nosZ* gene at the lowest elevation coincide with the average soil moisture, which also peaked at lower elevations (Table 7). These depression-situated soils are more likely to become saturated, resulting in a reduced environment favorable to denitrification (Fellows et al., 2011; Hunter and Faulkner, 2001). Closer inspection however revealed that while this trend held true for both converted and natural management soils, the inverse was true for the restored management soils. In the restored management, soils at the highest elevation had ∼5000,000 copies of *nosZ* whereas soils at the lowest elevation had only 87.7% that abundance (∼4400,000 copies; $P < 0.05$). Given the recent activity, within the past two to eight years, in restoring these soils to wetland status however, the switch in abundance patterns could be indicative of a microbial community currently in flux. When looking at denitrification gene abundances in a series of agricultural and successional sites, Morales et al. (2010) noted that the impacts of agricultural management on soil microbial populations could last for decades after the practices have ceased.

When looking at the relationship between *nosZ* gene abundances and environmental variables across all treatments, the strongest link identified was to Ca ($R^2 = 0.21$, $P < 0.0001$; Table 8). When looking at each management separately, Ca was also the strongest related variable for both converted ($R^2 = 0.20$, $P < 0.0001$) and restored ($R^2 = 0.28$, $P < 0.0001$). As mentioned previously, this is most likely indicative of the agricultural legacy of these soils. For natural wetlands, the strongest relationship was between *nosZ* and CN ratio ($R^2 = 0.11$, $P < 0.0001$).

It has been previously demonstrated that while *nosZ* is responsible for reducing nitrous oxide to dinitrogen gas, gene abundances of this gene are not directly correlated to DEA. However, a relationship between *nosZ* gene abundance and $N_2O/(N_2 + N_2O)$ has been previously reported, whereby higher abundances of the *nosZ* gene correlate with lower N_2O production (Ducey et al., 2011; Philippot et al., 2009). Examining the relationship between mean *nosZ* gene abundances and mean ratioBA percentages (Table 4) revealed a similar relationship. A strong negative relationship ($y = -30.504x + 239.37$, $P = 0.01$, $R^2 = 0.44$) was demonstrated, indicating that as *nosZ* gene abundances increased, the amount of incomplete denitrification decreased. Of note is that these results follow along management type, with converted wetlands having lower percentages of incomplete denitrification and natural wetlands having the highest percentages of incomplete denitrification.

3. Conclusion

Two major goals of the Conservation Effects Assessment Project (CEAP) are to examine the effectiveness of conservation practices, and develop management practices to ensure continued environmental quality standards for agriculture. Given those goals, this research focused on wetlands regions of the Mid-Atlantic US to study the effects of wetland restoration on nitrogen cycling processes. Nitrous oxide, a product of incomplete denitrification, is a greenhouse gas with potentially major environmental implications. A series of hydrologically-restored wetlands were compared to currently existing wetlands (natural wetlands) and wetlands that were drained to use for agricultural purposes (converted wetlands). Physicochemically, restored wetlands were statistically different from both converted and natural wetlands. Denitrification enzyme activity (DEA) and abundances of the gene responsible for the reduction of nitrous oxide (*nosZ*), also demonstrated that restored wetlands differed from both converted and natural wetlands. Comparisons of all three management types reveal that while restored wetlands can be demonstrated to have an agricultural legacy, it appears that efforts to restore their hydrological features is having an environmentally beneficial impact on changing denitrification towards a more natural wetland condition.

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References

Agassi, M., Shainberg, I., Morin, J., 1981. Effect of electrolyte concentration and soil sodicity on infiltration-rate and crust formation. Soil Sci. Soc. Am. J. 45, 848–851.
Ahn, C., Peralta, R.M., 2012. Soil properties are useful to examine denitrification function development in created mitigation wetlands. Ecol. Eng. 49, 130–136.
Airoldi, L., Beck, M.W., 2007. Loss, status and trends for coastal marine habitats of Europe. In: Gibson, R.N., Atkinson, R.J.A., Gordon, J.D.M. (Eds.), Oceanography and Marine Biology: An Annual Review. CRC Press, London.
Audet, J., Elsgaard, L., Kjaergaard, C., Larsen, S.E., Hoffmann, C.C., 2013. Greenhouse gas emissions from a Danish riparian wetland before and after restoration. Ecol. Eng. 57, 170–182.
Bedison, J.E., Scatena, F.N., Mead, J.V., 2013. Influences on the spatial pattern of soil carbon and nitrogen in forested and non-forested riparian zones in the Atlantic coastal plain of the Delaware River basin. For. Ecol. Manage. 302, 200–209.
Brinson, M.M., Eckles, S.D., 2011. U.S. Department of Agriculture conservation program and practice effects on wetland ecosystem services: a synthesis. Ecol. Appl. 21, S116–S127.
Coleman, J.M., Huh, O.K., Braud Jr., D., 2008. Wetland loss in world deltas. J. Coastal Res. 24, 1–14.
Costanza, R., D'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., Limburg, K., Naeem, S., O'Neill, R.V., Paruelo, J., Raskin, R.G., Sutton, P., Van Den Belt, M., 1997. The value of the world's ecosystem services and natural capital. Nature 387, 253–260.
Ding, Y., Song, X., Wang, Y., Yan, D., 2012. Effects of dissolved oxygen and influent COD/N ratios on nitrogen removal in horizontal subsurface flow constructed wetland. Ecol. Eng. 46, 107–111.
Dodla, S.K., Wang, J.J., DeLaune, R.D., Cook, R.L., 2008. Denitrification potential and its relation to organic carbon quality in three coastal wetland soils. Sci. Total Environ. 407, 471–480.
Ducey, T.F., Shriner, A.D., Hunt, P.G., 2011. Nitrification and denitrification gene abundances in swine wastewater anaerobic lagoons. J. Environ. Qual. 40, 610–619.
Duriancik, L.F., Bucks, D., Dobrowolski, J.P., Drewes, T., Eckles, S.D., Jolley, L., Kellogg, R.L., Lund, D., Makuch, J.R., O'Neill, M.P., Rewa, C.A., Walbridge, M.R., Parry, R., Weltz, M.A., 2008. The first five years of the: conservation effects assessment project. J. Soil Water Conserv. 63, 185A–197A.
Eckles, S.D., 2011. Linking science, policy, and management to conserve wetlands in agricultural landscapes. Ecol. Appl. 21, S1–S2.
Enwall, K., Throback, I.N., Stenberg, M., Soderstrom, M., Hallin, S., 2010. Soil resources influence spatial patterns of denitrifying communities at scales compatible with land management. Appl. Environ. Microbiol. 76, 2243–2250.
Farnese, P.L., Belcher, K.W., 2006. Wetlands in agricultural landscapes: a legal and policy context. Law Sci. Policy 3, 33–58.
Fellows, C.S., Hunter, H.M., Eccleston, C.E.A., De Hayr, R.W., Rassam, D.W., Beard, N.J., Bloesch, P.M., 2011. Denitrification potential of intermittently saturated floodplain soils from a subtropical perennial stream and an ephemeral tributary. Soil Biol. Biochem. 43, 324–332.
Havlin, J.L., Beaton, J.D., Tisdale, S.L., Nelson, W.L., 1999. Soil Fertility and Fertilizers. An Introduction to Nutrient Management. Prentice Hall, Upper Saddle River, NJ.
Heal, G., 2000. Valuing ecosystem services. Ecosystems 3, 24–30.
Hefting, M.M., van den Heuvel, R.N., Verhoeven, J.T.A., 2013. Wetlands in agricultural landscapes for nitrogen attenuation and biodiversity enhancement: opportunities and limitations. Ecol. Eng. 56, 5–13.
Hue, N.V., Licudine, D.L., 1999. Amelioration of subsoil acidity through surface application of organic manures. J. Environ. Qual. 28, 623–632.
Hunt, P.G., Matheny, T.A., Ro, K.S., 2007. Nitrous oxide accumulation in soils from riparian buffers of a coastal plain watershed-carbon/nitrogen ratio control. J. Environ. Qual. 36, 1368–1376.
Hunt, P.G., Matheny, T.A., Stone, K.C., 2004. Denitrification in a coastal plain riparian zone contiguous to a heavily loaded swine wastewater spray field. J. Environ. Qual. 33, 2367–2374.
Hunter, R.G., Faulkner, S.P., 2001. Denitrification potentials in restored and natural bottomland hardwood wetlands. Soil Sci. Soc. Am. J. 65, 1865–1872.
Hwang, S., Jang, K., Jang, H., Song, J.Y., Bae, W., 2006. Factors affecting nitrous oxide production: a comparison of biological nitrogen removal processes with partial and complete nitrification. Biodegradation 17, 19–29.
Jacinthe, P.A., Bills, J.S., Tedesco, L.P., Barr, R.C., 2012. Nitrous oxide emission from riparian buffers in relation to vegetation and flood frequency. J. Environ. Qual. 41, 95–105.
Ji, G.D., Wang, R.J., Zhi, W., Liu, X.X., Kong, Y.P., Tan, Y.F., 2012. Distribution patterns of denitrification functional genes and microbial floras in multimedia constructed wetlands. Ecol. Eng. 44, 179–188.
Klemedtsson, L., Von Arnold, K., Weslien, P., Gundersen, P., 2005. Soil CN ratio as a scalar parameter to predict nitrous oxide emissions. Global Change Biol. 11, 1142–1147.
Knowles, R., 1982. Denitrification. Microbiol. Rev. 46, 43–70.
Miller, J.O., Hunt, P.G., Ducey, T.F., Glaz, B.S., 2012. Denitrification and gas emissions from organic soils under different water table and flooding management. Trans. ASABE 55, 1793–1800.
Miller, M.N., Zebarth, B.J., Dandie, C.E., Burton, D.L., Goyer, C., Trevors, J.T., 2009. Denitrifier community dynamics in soil aggregates under permanent grassland and arable cropping systems. Soil Sci. Soc. Am. J. 73, 1843–1851.
Morales, S.E., Cosart, T., Holben, W.E., 2010. Bacterial gene abundances as indicators of greenhouse gas emission in soils. ISME J. 4, 799–808.
Moreno-Mateos, D., Power, M.E., Comín, F.A., Yockteng, R., 2012. Structural and functional loss in restored wetland ecosystems. PLoS Biol. 10 (1), 1–8, e1001247.
Morse, J.L., Ardón, M., Bernhardt, E.S., 2012. Greenhouse gas fluxes in southeastern U.S. coastal plain wetlands under contrasting land uses. Ecol. Appl. 22, 264–280.
Moser, K.F., Ahn, C., Noe, G.B., 2009. The influence of microtopography on soil nutrients in created mitigation wetlands. Restor. Ecol. 17, 641–651.
Nicholls, R.J., Hoozemans, F.M.J., Marchand, M., 1999. Increasing flood risk and wetland losses due to global sea-level rise: regional and global analyses. Global Environ. Change 9, S69–S87.
Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res. 29, e45.
Philippot, L., Cuhel, J., Saby, N.P., Cheneby, D., Chronakova, A., Bru, D., Arrouays, D., Martin-Laurent, F., Simek, M., 2009. Mapping field-scale spatial patterns of size and activity of the denitrifier community. Environ. Microbiol. 11, 1518–1526.
Reddy, K.R., D'Angelo, E.M., Harris, W.G., 2000. Biogeochemistry of wetlands. In: Sumner, M.E. (Ed.), Handbook of Soil Science. CRC Press, Boca Raton, FL.
Sakadevan, K., Zheng, H., Bavor, H.J., 1999. Impact of heavy metals on denitrification in surface wetland sediments receiving wastewater. Water Sci. Technol.
SAS Institute Inc., 2002. Statistical Analysis System. SAS Institute, Inc., Cary, NC.
Scholz, M., 2011. Wetland Systems. Springer-Verlag, London.
Sims, J.T., Maguire, R.O., Leytem, A.B., Gartley, K.L., Pautler, M.C., 2002. Evaluation of Mehlich 3 as an agri-environmental soil phosphorus test for the Mid-Atlantic United States of America. Soil Sci. Soc. Am. J. 66, 2016–2032.
Tiedje, J.M., 1994. Denitrifier enzyme activity (DEA). In: Mickelson, S.H., Bigham, J.M. (Eds.), Methods of Soil Analysis Part 2, second ed. SSSA, Madison, WI, SSSA Book Series 5.
Ullah, S., Moore, T.R., 2011. Biogeochemical controls on methane, nitrous oxide, and carbon dioxide fluxes from deciduous forest soils in eastern Canada. J. Geophys. Res. G: Biogeosci. 116, 2156–2202.
Zhang, J., Wang, S., 2009. Effect of influent COD/N ratio on nitrous oxide production during denitrification using different electron acceptors. In: International Conference on Energy and Environment Technology, Guilin, China.