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SPATIAL VARIABILITY OF DENITRIFICATION POTENTIAL AND RELATED SOIL PROPERTIES IN CREATED, RESTORED, AND PAIRED NATURAL WETLANDS

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Abstract: To gain a better understanding of the spatial patterns of denitrification potential and related soil properties in created (CW), restored (RW), and natural wetlands (NWs), four CW/RW-NW pairs in North Carolina, USA were sampled. These site pairs spanned a range of hydrogeomorphic (HGM) settings common in the Coastal Plain. It was hypothesized that denitrification enzyme activities (DEAs) and related soil properties of CW/RWs would have less spatial variability than DEAs and soil properties of NWs, as prior land-use and mitigation activities tend to homogenize soil properties. Cochran's C tests indicated that variances were significantly lower in CW/RWs than in NWs for most soil properties, and that for nitrate (NO_3-N), variances were significantly lower in CW/RWs across all HGM settings. Interpolated maps of the soil properties revealed homogeneous distributions of NO_3-N across the CW/RW plots compared to much more heterogeneous distributions of NO_3-N across the NW plots. Multiple stepwise regressions confirmed that either NO_3-N or soluble organic carbon were significant predictors of the DEA at each plot. Interpolated maps of predicted DEA generally showed similar patterns to those of NO_3-N. While some nitrate and DEA hotspots were observed in the CW/RWs, more were present in the NWs. These results indicated that spatial distributions of soil chemical properties and DEAs were considerably different in CW/RWs than in paired NWs. This is the first study to document such differences, suggesting that CWs and RWs with homogeneous soil chemical distributions may not develop the full range of soil biogeochemical processes that occur in NWs.

Key Words: denitrification enzyme activity, geostatistics, hydrogeomorphic subclasses, nitrate, North Carolina, restoration, soil, spatial variability, wetland

INTRODUCTION

This study investigated the spatial variability of denitrification and related soil properties in created (CW), restored (RW), and paired natural wetlands (NWs). Comparing the spatial distribution of denitrification in soils of CW/RWs to those of paired NWs is important because forested wetlands have been shown to support high denitrification rates and, in certain cases, to transform the majority of nitrate inputs to nitrogen gases (Jacobs and Gilliam 1985, Lowrance 1992). Denitrification in these forested wetlands has also shown high spatial variability (Pinay et al. 1993, Schipper et al. 1993, Casey et al. 2001, Dhondt et al. 2004) due to the presence of patches of organic matter and anaerobic microsites in the soil profile (Parkin 1987, Gold et al. 1998, Jacinthe et al. 1998, Casey et al. 2004). The term "hot spots" was coined to describe these areas of high denitrification (Parkin 1987, Christensen et al. 1990). It is unclear whether such "hot spots" occur in the soils of CW/RWs or if they occur in similar frequencies or at similar spatial scales. A better understanding of the factors that produce denitrification hotspots may facilitate the development of these processes in created and restored wetlands (McClain et al. 2003).

As the spatial distribution of soil properties such as soil moisture, nitrate, and soluble organic carbon content directly influences denitrification (Grundmann et al. 1988, Robertson et al. 1988, Ball et al. 1997), a description of the spatial variability of these soil properties should improve our understanding of the spatial distribution of denitrification (van den Pol-van Dasselaar et al. 1998). However, only a few studies have combined the analysis of the spatial variability of denitrification with an analysis of soil properties, and it is still unknown whether soil properties display variability at scales similar to denitrification (van den Pol-van Dasselaar et al. 1998). Interestingly, recent studies have shown significantly lower denitrification potential in wetlands with nutrient-poor substrates (sand, light till) than in wetlands with nutrient-rich substrates (alluvium, dark till) (Groffman and Hanson 1997), significant differences in denitrifier communities in a successional field compared to a nearby conventionally-tilled agricultural field (Cavigelli and Robertson 2000), significantly lower denitrification enzyme activity (DEA) levels in RWs compared to NWs (Hunter and Faulkner 2001), and a positive relationship between plant species richness and denitrification potential (Chabrerie et al. 2001). Studies that have compared microtopography (Stolt et al. 2000) and soil properties of CW/RWs and NWs (Stolt et al. 2000, Bruland and Richardson 2005) have also shown that there is often much less variability in the CW/RWs than in adjacent or nearby NWs. Thus, we might also expect to observe considerable differences in the spatial patterns of denitrification in CW/RWs versus NWs.

When comparing a process such as denitrification across different wetland types, it is also important to consider the hydrogeomorphic (HGM) setting of the wetlands in question (Brinson 1993a, Brinson 1993b, Cole et al. 1997). For example, wetlands in riverine HGM settings will have a different structure and perform different functions than wetlands in non-riverine HGM settings. It has been speculated that due to differences in overbank transport, water flow paths, and flood duration, variability of soil properties of wetlands in headwater subclasses (stream order ≤ 2) would differ considerably from variability of wetlands in mainstream subclasses (stream order > 2) (Brinson 1993a). This idea has been confirmed in a recent study where soil properties of a headwater riverine wetland were much more heterogeneous than those of a mainstream riverine wetlands, possibly due to differential frequency, duration, and intensities of flooding (Bruland and Richardson 2004). Headwater riverine wetlands have also been shown to possess significantly different vegetative communities than wetlands in mainstream riverine wetlands (Rheinhardt et al. 1998), which has implications for litter quality and quantity, as well as nutrient dynamics. In contrast to riverine wetlands, spatial variability of soil properties and processes in non-riverine wetlands have shown patchy distributions that correspond to microtopography and local vegetation (Hanchey 2001, Bruland and Richardson 2005).

The primary objective of this study was to compare the spatial variability of denitrification potential and related soil properties of CW/RWs to those of paired NWs across a range of HGM settings (Brinson 1993b). It was hypothesized that denitrification and related soil properties of CW/RWs would show less variability than soil properties of NWs, as prior land-use and creation/restoration activities tend to homogenize wetland soil properties.

METHODS

Study Sites

The sites selected for this study were located in the Coastal Plain physiographic region of North Carolina and were part of the North Carolina Department of Transportation compensatory mitigation program (Figure 1). Representative sites were selected from four different HGM subclasses including (1) headwater riverine, (2) mainstream riverine, (3) non-riverine mineral soil flat, and (4) non-riverine organic soil flat (Brinson 1993b, Cole et al. 1997). Paired plots were used to compare differences in the spatial variability of soil properties of CW/RWs and NWs. Both CW/RW and NW pairs were located in similar hydrogeomorphic settings and in areas of a related or identical soil series based on county soil survey maps. All four of the CW/RWs were between three and six years since construction. Below a brief description is given for each site. More detailed site information can be found in Bruland and Richardson (2004 and 2005).

Both the RW and NW plots at Rowel Branch were identifiable as headwater riverine wetlands according to the HGM system. According to the Soil Survey of Brunswick County, North Carolina (U.S. Soil Conservation Service 1986), the RW plot was located in two map units, the first named for the Foreston soil series (Aquic Paleudults) and the second named for Goldsboro series (Aquic Paleudults), while the NW plot was located in two map

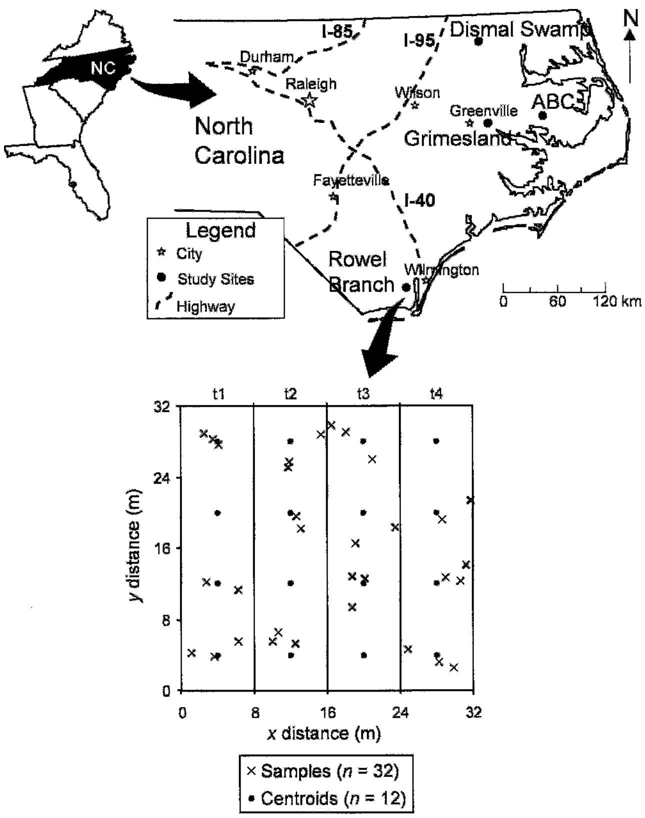

Figure 1. Map of the locations of the four paired wetland study sites in the Coastal Plain of North Carolina, USA and an example of the spatial sampling design showing the four transects (t1–t4), the placement of the centroids, and the locations of the sampling points (Modified from Bruland and Richardson 2005).

units, the first named for the Rains series (Typic Paleaquults) and the second for the Norfolk series (Typic Kandiudults). A short distance downstream, the floodplain area was located in a map unit named for the Muckalee series (Typic Fluvaquents). The site was restored from spring 2000 through summer 2001. The NW was located a few kilometers upstream of the RW.

The CW and NW plots at Grimesland were identified as mainstream riverine wetlands according to the HGM system. According to the Soil Survey of Pitt County (U.S. Soil Conservation Service 1974), the CW plot was located in a map unit named for the Lakeland soil series (Typic Quartzipsamments). However, based upon field observations, this plot most likely occurs within an unnamed inclusion that is wetter and more poorly-drained than the Lakeland series. The NW plot was located in the miscellaneous land type named swamp. The site includes *Taxodium distichum* [L.] Richard (cypress) swamp, bottomland hardwood forest, upland forest, and borrow-pit lakes. From fall 1999 to winter 2000, 3.1 hectares of wetland were created. The CW plot was located between Grindle Creek, a third-order tributary of the Tar River that flows through the site and one of the borrow pit lakes. The NW plot was located adjacent to the CW plot.

The RW and NW plots at the ABC site were classified as non-riverine mineral soil flats according to the HGM system. Based on the Soil Survey of Beaufort County (Natural Resources Conservation Service 1995), the RW and NW plots were located in a map unit named for the Leaf series (Typic Albaquults) but which, according to the map unit description, also includes soil in the Bayboro series (Umbric Paleaquults). Much of the inter-stream area was converted to agriculture in the 1960s. The agricultural area was restored from fall 2000 through winter 2001. The NW plot was located in part of the forested interstream area that was not converted to agriculture.

Both the RW and NW plots at the Dismal Swamp site were identifiable as non-riverine organic soil flats. Based on the Soil Survey of Gates County (Natural Resources Conservation Service 1996), each plot was located within a map unit that was named for the Scuppernong and Belhaven soil series (Terric Haplosaprists). Unlike the mineral soils of the other sites, the Scuppernong and Belhaven soils typically have a minimum of 40 cm of organic soil materials that contain between 30 and 90% organic matter. The site was originally a *Chamaecyparis thyoides* [L.] BSP. (Atlantic white cedar) swamp that had been cleared, ditched, and drained to facilitate silvicultural and agricultural activities. It was restored from summer 1996 to winter 1997.

Soil Sampling and Laboratory Analyses

At each site, flat areas in the CW/RW and NW zones were identified for which microtopography was generally within $\pm$ 0.5 m of the mean elevation. Plots 32 m $\times$ 32 m (0.1 ha) in size were established in the CW/RWs and in their paired NWs. This size was chosen because it was the largest plot that could fit onto all sites. To allow for geostatistical analysis of the data, a sampling design with four transects, separated by 8 m was established (Figure 1). At the two riverine sites (Rowel Branch and Grimesland), transects were oriented perpendicular to the direction of water flow, while at the non-riverine sites (ABC and Dismal Swamp), transects were oriented in a North-South direction. Each transect consisted of four centroids, around which samples were clustered at random directions and at random distances (all $<$ 4 m). This was advantageous because both uniform and random sampling designs fail to provide adequate sample points separated by short distances, which in turn, does not permit analysis of fine-scale spatial variability. In order to keep analysis of the soil samples manageable, one of the four centroids was randomly omitted from the sampling. Three cores were collected from two of the remaining centroids, while two cores were collected at the final centroid (Figure 1). Sixty four cores were collected per site (32 in the CW/RW and 32 in the NW) for a total of 256 cores.

Cores were collected 9–15 July 2002 in 5-cm-diameter plastic sleeves from the upper 20 cm with a piston corer. Cores were stored on ice while transported to the laboratory. Upon arrival, cores were extruded and split in half vertically with a sharp knife. Half of the core was oven dried at 105°C for 24 hours to determine the moisture content. The other half of the core was kept at field moisture and passed through a 2-mm sieve. The field-moist sieved soil was analyzed for 2 M KCl extractable nitrate+nitrite–N (NO_3-N) and ammonium (NH_4-N) (Keeney and Nelson 1988) on a Bran and Lubbe TRAACS autoanalyzer (Bran + Lubbe, Inc., Delevan, WI, USA) and for deionized water extractable organic carbon (Kaiser and Zech 1996, Hunter and Faulkner 2001) with a Shimadzu TOC 5000 solution C analyzer (Shimadzu, Inc., Columbia, MD, USA).

The denitrification enzyme activity (DEA) (Tiedje 1982, Hunter and Faulkner 2001) was used as an index of denitrification potential. The DEA is useful for site comparisons because it offers a method by which the denitrification potential can be compared across different soil types (Groffman et al. 1992). As the DEA is an intensive laboratory procedure, a subset of eight randomly selected cores per plot was analyzed for a total of 64 cores. For the DEA, 20 g of sieved, field-moist soil were measured into 133 cm^3 mason jars. The lids of the jars were fitted with septa to allow for collection of gas samples with syringes. The soils were amended with solutions of glucose and potassium nitrate to ensure non-limiting substrate conditions, and chloramphenicol to inhibit protein synthesis. The slurries were made anaerobic by repeated flushing with N_2 gas. The jars were injected with 15 ml of H_2SO_4-scrubbed acetylene to inhibit N_2 production (Hyman and Arp 1987), vigorously shaken by hand, and then shaken at 100 rpm for 90 minutes on an orbital shaker. At 30, 60, and 90 minutes, 5-mL gas samples were collected from each jar with a syringe. Samples were stored prior to analysis by inserting the syringe needles into Butyl rubber stoppers (Whalen 2000). Nitrous oxide concentrations were determined with a Shimadzu (Columbia, MD, USA) GC-14A ^{63}Ni electron capture detector gas chromatograph (GC). Operating conditions and calibration of the GC were according to Whalen (2000). Nitrous oxide fluxes were calculated as the time-linear rate of concentration increase in the headspace of the mason jars. Nitrous oxide dissolved in sample water was corrected with the Bunsen equation (Hunter and Faulkner 2001), as were decreases in the volume of gas in the jars with repeated sampling. The DEA was calculated as the short-term rate of N_2O production in the jars and is indicative of the size of the denitrifying enzyme pool present in the soil.

Statistical and Geostatistical Analyses

As our spatial sampling design included sites that were located in close proximity to each other, the majority of the cores collected could not be considered independent. Therefore, while we calculated descriptive statistics (frequency distributions, means, and standard deviations) for each soil property measured at each plot, we did not compare these mean values with *t*-tests or analysis of variance because of the presence of spatial autocorrelation. The Shapiro-Wilk test (Shapiro and Wilk 1965) was used to determine if the frequency distributions departed significantly from normality (Statistica Version 5.5, Statsoft, Tulsa, OK). Cochran's test (Cochran 1947) was calculated (with Statistica) to test for homogeneity of the variances of the soil properties from the paired CW/RWs and NWs. Soil properties that had non-normal distributions were log-transformed to conform better to the normality assumption of the Cochran's test (McGuinness 2002). Variances were considered significantly different when $p < 0.05$.

As the sample plots at each site were chosen for their relatively homogeneous topography, isotropic semivariograms were expected to be used in conjunction with ordinary point kriging to model patterns of spatial variability across the plots. However, due to the relatively small sample sizes and erratic behavior of the data, the semivariograms had poor fits with the actual semivariance data for many plots. Thus, inverse distance weighting (IDW) (Isaaks and Srivastava 1989), another interpolation method similar to kriging, was selected for interpolation. Unlike kriging, where weights for each observation depend on the semivariogram parameters, the weights for IDW are inversely proportional to a power of the distance from the location being estimated (Isaaks and Srivastava 1989). Exponents between one and three are typically used with IDW, with two being the most common (Gotway et al. 1996). Tests with different IDW exponents indicated that two was optimal, as predicted values generated with this exponent showed the best fits with actual data in cross validation tests. GS+ software (Version 5, GammaDesign, Plainwell, MI) was used to generate the IDW maps and perform the cross validations.

Multiple step-wise regression (MSR) was used to determine the relationships among soil properties and the DEA. Multiple step-wise regression is a statistical tool used to predict the response of a dependent variable from a group of potential predictor variables. For the MSR, we used DEA as the dependent variable and moisture, NO_3-N, NH_4-N, and SOC as the independent variables. Previous studies have generally shown potential denitrification to be positively correlated with NO_3-3 and SOC (Robertson et al. 1988, Davidsson and Leonardson 1998, Ullah et al. 2005). The relationship of NH_4-N to DEA is less clear, as in some cases high NH_4-N may stimulate nitrification, while in other cases, it may indicate anaerobic conditions in which all NO_3-N has been denitrified. While volumetric moisture content has been shown to be related to denitrification in previous studies (Grundmann et al. 1988, Ball et al. 1997), it can change rapidly over short time intervals, and its correlation with DEA may be non-repeatable. The MSR equations were used to extrapolate DEAs for the other 24 soil cores at each plot. These extrapolated DEAs were used to produce maps of predicted DEA across the study plots with IDW.

RESULTS AND DISCUSSION

Plot Means, Frequency Distributions, and Variances

As there were considerable differences in bulk density across the plots and sites (Bruland and Richardson 2005), the soil chemical data were expressed on a volumetric rather than a mass basis. According to the Shapiro-Wilk test, moisture was normally-distributed in every plot. At Rowel Branch, Grimesland, and ABC, there was considerable overlap among the moisture frequency distributions for the CW/RWs and NWs, whereas at Dismal Swamp, there was much less overlap (Figure 2). Mean soil moisture was greater in the CW/RWs than in the paired NWs at all sites except for ABC (Table 1). The largest difference in moisture between a CW/RW and NW pair was at the Dismal Swamp site, where mean soil moisture in the RW was nearly triple that of the NW. The Cochran's test indicated that variances for moisture were significantly heterogeneous at each site (Table 2). At Rowel Branch and Dismal Swamp, variances were significantly higher in the RWs, whereas at Grimesland and ABC, variances were significantly higher in the NWs.

Nitrate also was normally distributed in all plots except the RW and NW at Rowel Branch and the RW at Dismal Swamp. While there was overlap in the frequency distributions of NO_3–N from the RW and NW at Rowel Branch, there was much less overlap in the frequency distributions at the other three sites. Unlike moisture, mean NO_3–N concentrations were lower in CW/RWs than in their paired NWs. When compared to the CW/RWs, mean NO_3–N values in the NWs were nearly double for Rowel Branch, almost five times higher at Grimesland, four times higher at ABC, and six times higher at Dismal Swamp. The Dismal Swamp NW plot had the highest mean NO_3–N content. The Cochran's test revealed that variances of NO_3–N were significantly lower in the CW/RWs than in the NWs across all four sites.

There were numerous outliers and significant deviations from normality for NH_4–N at each of the CW/RWs and NWs. Mean NH_4–N concentrations were lower in CW/RWs than in NWs at all sites except for ABC. However, differences in NH_4–N between the CW/RWs and NWs were less pronounced for the riverine sites than for the non-riverine sites. There were no differences in the variances of NH_4–N at Rowel Branch, ABC, or Dismal Swamp, while at Grimesland, variances were significantly lower in the CW than in the NW. Lower mean values of NO_3–N and NH_4-N in the CW/RWs may be due to the removal of organic rich topsoil or the loss of organic matter due to increased decomposition during prior land uses. Low levels of inorganic nitrogen at these CW/RWs may hinder the development of microbial and vegetative communities.

According to the Shapiro-Wilk test, SOC was normally distributed in all plots except for the CW at Grimesland and both plots at ABC. There was also considerable overlap in the frequency distributions of SOC for all sites. For the riverine sites, mean SOC content was lower in the CW/RWs than in the NWs. Conversely, for the non-riverine sites, mean SOC content was greater in the CW/RWs. This pattern may be explained by the low bulk densities of the non-riverine NW plots (Bruland and Richardson 2005). While these sites had higher SOC concentrations than the NWs, the CW/RWs actually contained more SOC per unit soil volume. There were no significant differences in SOC variances for CW/RWs and NWs at Rowel Branch, Grimesland, or ABC, while at Dismal Swamp, the variance was significantly greater in the RW than in the NW. Overall, these results suggest that land-use

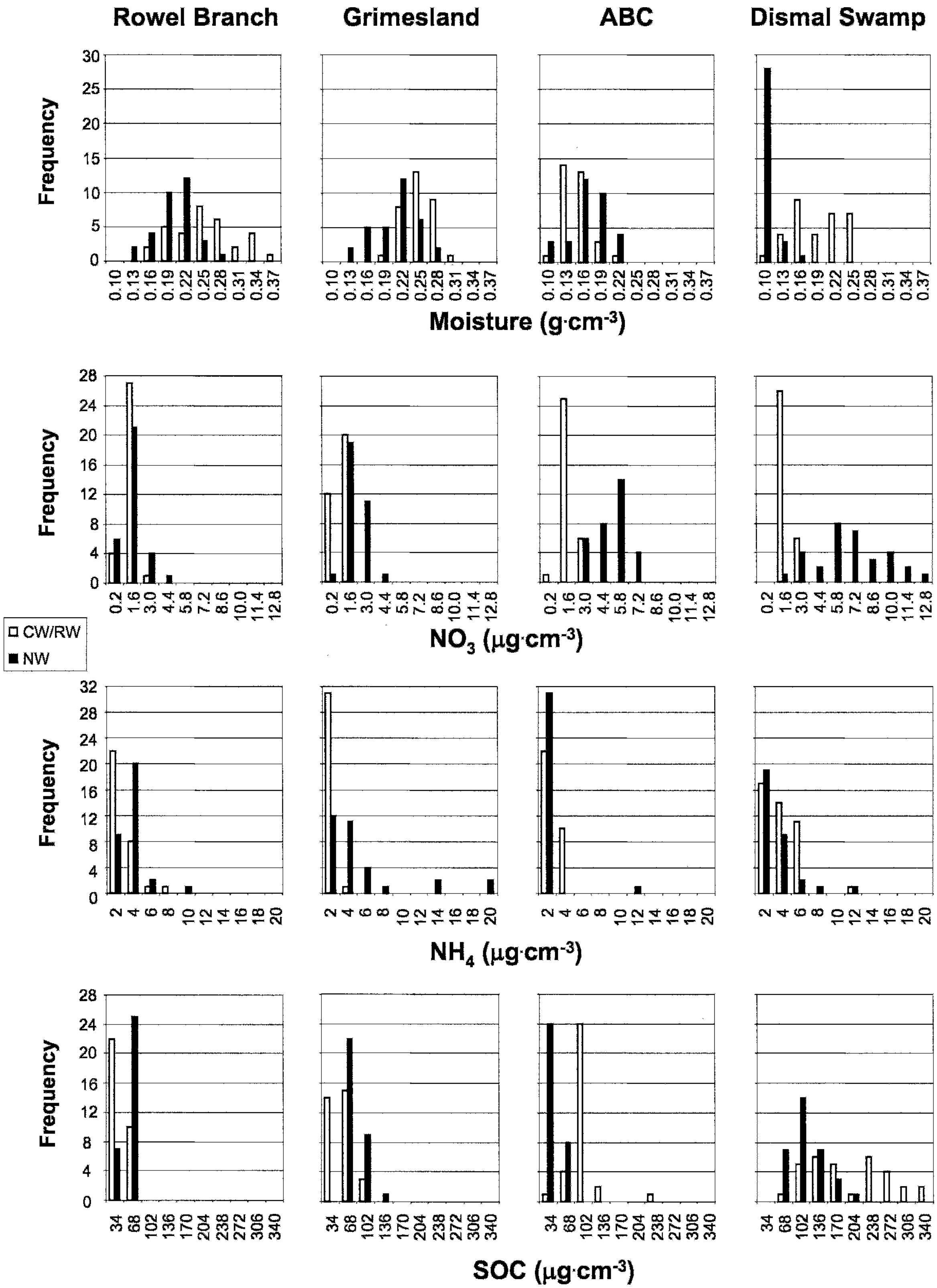

Figure 2. Frequency distributions of soil properties in the created/restored (CW/RW) and natural wetland (NW) plots at Rowel Branch, Grimesland, ABC, and Dismal Swamp.

Table 1. Plot means (± 1 standard deviation) for the soil properties of the four paired plots sampled in this study.

Site	Plot	Moisture (g cm^{-3})	NO_3-N (μg cm^{-3})	NH_4-N (μg cm^{-3})	SOC (μg cm^{-3})
Rowel Branch	RW†	0.24 ± 0.05‡	0.49 ± 0.52	1.69 ± 1.31	31.5 ± 8.50
Rowel Branch	NW	0.19 ± 0.03	0.86 ± 0.78	2.75 ± 1.53	42.1 ± 8.94
Grimesland	CW	0.24 ± 0.03	0.30 ± 0.22	0.73 ± 0.53	41.0 ± 15.3
Grimesland	NW	0.20 ± 0.04	1.40 ± 0.76	4.21 ± 4.83	62.0 ± 16.5
ABC	RW	0.14 ± 0.02	1.06 ± 0.61	1.65 ± 0.93	85.1 ± 29.7
ABC	NW	0.15 ± 0.03	4.50 ± 1.17	0.98 ± 1.85	29.8 ± 10.1
Dismal Swamp	RW	0.17 ± 0.04	1.08 ± 0.67	1.99 ± 1.68	181 ± 79.8
Dismal Swamp	NW	0.06 ± 0.03	6.23 ± 2.78	2.22 ± 2.41	91.5 ± 36.3

† CW = created wetland; RW = restored wetland; NW = natural wetland.
‡ n = 32 for each plot.

and creation/restoration activities decreased the variability in certain soil properties, such as NO_3-N, while they increased the variability in others soil properties, such as SOC.

Median DEAs were lower in three of the four CW/RWs than in their NW pairs (Figure 3). Only at the Dismal Swamp site was the median DEA greater in the RW than in the NW. This was probably related to agricultural activity in the Dismal Swamp RW that caused increases in soil nitrate concentrations due to fertilization and soil compaction due to operation of agricultural machinery. Denitrification enzyme activities tended to be lower in riverine than in non-riverine sites and especially low in riverine CW/RW sites. This result was similar to that reported in a study of denitrification in RWs and NWs in Louisiana (Hunter and Faulkner 2001).

As individual measurements of DEA have been shown to be highly correlated with field rates of ambient denitrification (Schipper et al. 1993), as well as annual soil denitrification rates (Ambus 1993), these results indicate that the CW/RWs sampled in this study did not possess microbial communities that have the same capacity to perform denitrification as their NW pairs. Such inferences about microbial communities are justified from the results of the DEA assay, as the limiting factors of denitrification (anaerobic conditions, NO_3-N, and C) are present in excess, microbial growth is inhibited (by the addition of chloramphenicol), and the N_2O gas produced is assumed to be a function

Table 2. Comparison of the variances of the paired plots for soil moisture, nitrate+nitrite (NO_3-N), ammonium (NH_4-N), and soluble organic carbon (SOC) with the Cochran's test.

Site and Soil Property	Relationship of the variances	Cochran's C	*p*-value†
Rowel Branch Moisture	RW > NW	0.72	**0.01**
Rowel Branch NO_3-N ‡	RW < NW	0.69	**0.03**
Rowel Branch NH_4-N ‡	RW = NW	0.56	0.48
Rowel Branch SOC	RW = NW	0.53	0.78
Grimesland Moisture	CW < NW	0.68	**0.04**
Grimesland NO_3-N	CW < NW	0.92	< **0.01**
Grimesland NH_4-N ‡	CW < NW	0.88	< **0.01**
Grimesland SOC †	CW = NW	0.64	0.11
ABC Moisture	RW < NW	0.68	**0.04**
ABC NO_3-N	RW < NW	0.79	< **0.01**
ABC NH_4-N ‡	RW = NW	0.59	0.32
ABC SOC ‡	RW = NW	0.53	0.75
Dismal Swamp Moisture	RW > NW	0.69	**0.03**
Dismal Swamp NO_3-N ‡	RW < NW	0.70	**0.02**
Dismal Swamp NH_4-N ‡	RW = NW	0.64	0.12
Dismal Swamp SOC	RW > NW	0.83	< **0.01**

† Variances were considered significantly different when $p < 0.05$ and are shown in bold.
‡ These soil properties were log-transformed to better conform to the assumption of normality required by the Cochran's test.

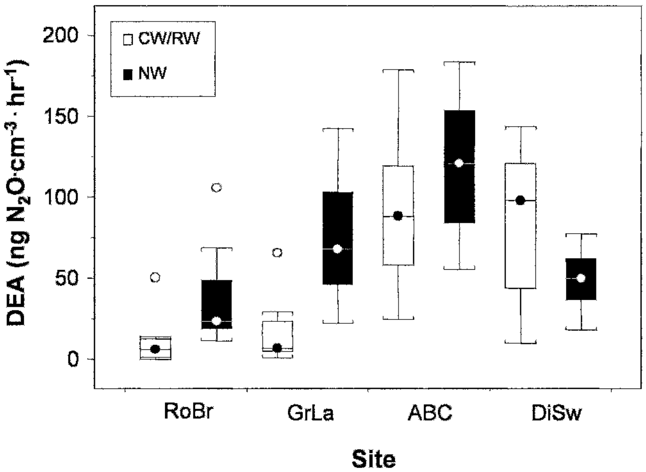

Figure 3. Box and whisker plots of the median (circle with horizontal line), upper and lower quartiles (box), and outliers (circles) for the denitrification enzyme activities of the created/restored (CW/RW) and natural wetland (NW) samples collected for each plot. Error bars represent 1.5 times the interquartile range.

only of the level of enzyme present in the sample (Robertson et al. 1999). It is important to make the caveat that the DEAs reported in this study were only measured once during the summer of 2002 due to the intensive nature of the sampling and subsequent laboratory analysis. As the DEA provides a snap-shot of the soils at the time of sampling (Luo et al. 1996), the patterns observed in July 2002 may not be consistent throughout the year or from one year to another. However, as the low NO_3–N and SOC levels in the CW/RWs are not likely to undergo rapid increases in the near future, these trends will probably persist for several years. Moreover, DEAs measured elsewhere in NWs were consistently greater than CW/RWs across four different seasons (Hunter and Faulkner 2001).

Spatial Distributions of Soil Properties and DEA in CW/RW versus NWs

As shown in the interpolated maps (Figure 4), NO_3–N in the CW/RWs had much less spatial variability than NO_3–N in the NWs. Concentrations of NO_3–N in the riverine CW/RWs were homogeneous, ranging only from 0 to 0.8 $\mu g\ cm^{-3}$. In contrast, NO_3–N in the NWs was much more variable, ranging from 0–3.2 $\mu g\ cm^{-3}$. While numerous NO_3–N hotspots were observed in each of the riverine NWs, only two NO_3–N hotspots were observed in the Rowel Branch RW, and none were observed in the Grimesland CW. Concentrations of NO_3–N in the non-riverine RWs were also homogeneous, ranging only from 0 to 3.0 $\mu g\ cm^{-3}$ compared to NO_3–N in the non-riverine NWs that ranged from 0–12 $\mu g\ cm^{-3}$. As with the riverine wetlands, NO_3–N hotspots were observed in each of the non-riverine NWs, while no NO_3–N hotspots were observed in the RWs. A similar pattern was observed with NH_4–N across the riverine and non-riverine wetlands with homogeneous distributions in CW/RWs and heterogeneous distributions in NWs (Figure 5).

The nearly uniform distribution of NO_3–N and NH_4–N in the CW/RW plots may have been caused by a number of factors. First, the ABC and Dismal Swamp sites were both under agricultural production for at least 10 years prior to restoration and subjected to a variety of practices that homogenize soil properties, such as tillage, liming, and fertilization. Previous studies have shown that land-use activities such as agriculture, grazing, and surface mining tend to homogenize soil properties (Whisenant et al. 1995, Marriott et al. 1997, Boerner et al. 1998, Paz-Gonzalez et al. 2000). Second, the earth moving, mixing, and grading that occurred during creation/restoration may have homogenized NO_3–N and NH_4–N concentrations by mixing patches in the horizontal direction and profiles in the vertical direction. Third, homogeneous inorganic nitrogen concentrations in the CW/RWs may be a result of the uniform relationship between processes producing NO_3–N and NH_4–N, such as mineralization of organic matter, and processes consuming NO_3–N and NH_4–N, such as denitrification, plant assimilation, and microbial immobilization.

The homogeneous distribution of NO_3–N across the CW/RWs suggested that CW/RW soils only experienced a limited range of soil chemical conditions and associated biogeochemical transformations. This also relates back to the observation that the microtopographic variability in the CW/RWs was more subdued than that of the NWs, which would affect both the hydrology and soil chemical processes in these plots. In contrast, the highly variable distribution of NO_3–N in the NWs indicated that NW soils experienced wider ranges in NO_3–N concentrations and that certain areas of the plot supported higher rates of NO_3–N production and consumption than others. This trend persisted across all four HGM subclasses and across CW/RWs with different land-use histories and supported the hypothesis that prior land-use and mitigation activities tend to homogenize wetland soils.

The uniform soil chemical conditions in the CW/RWs may also favor the growth and expansion of annual plants that can effectively exploit large homogeneous patches of nutrients (Gross et al.

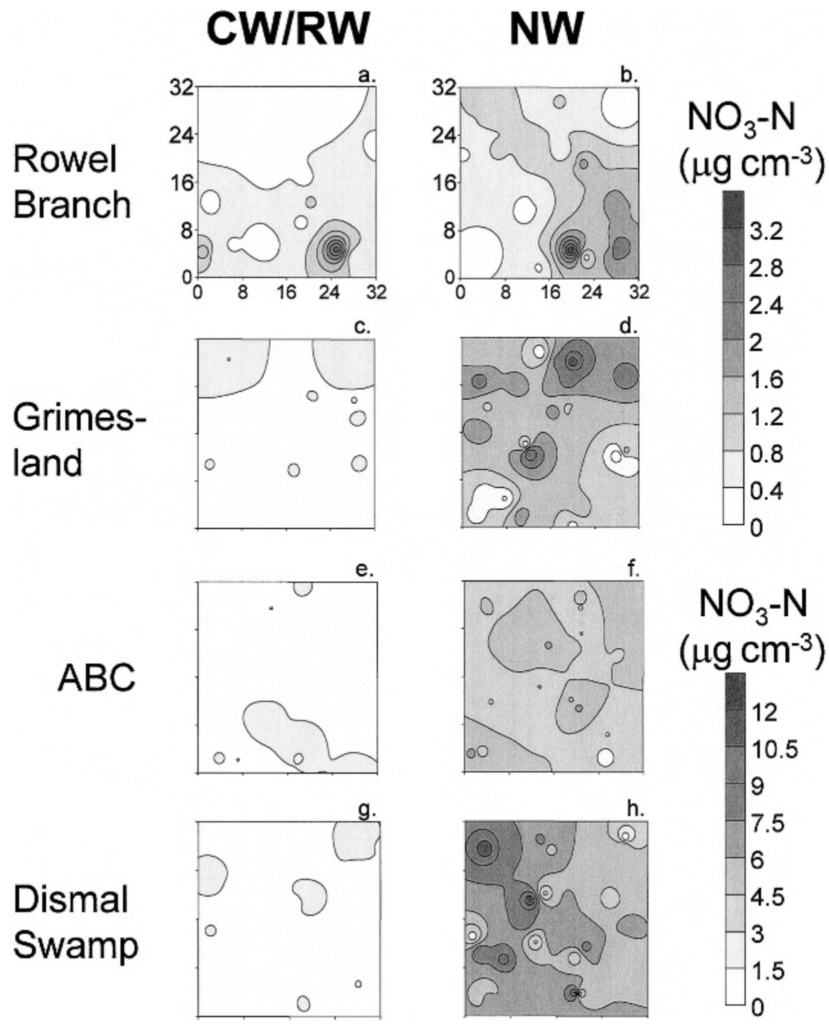

Figure 4. Spatial distribution of nitrate (NO_3-N) at the Rowel Branch restored (a) and natural wetland (b), the Grimesland created (c) and natural wetland (d), the ABC restored (e) and natural wetland (f), and the Dismal Swamp restored (g) and natural wetland (h). The upper scale corresponds to the riverine sites (Rowel Branch and Grimesland) and the lower scale applies to the non-riverine sites (ABC and Dismal Swamp).

1995) at the expense of the development of desired wetland plant species or diversity. As soil micro-heterogeneity has been associated with species richness (Huston 1994, Vivian-Smith 1997), and species richness has been correlated to denitrification potential (Chabrerie et al. 2001), CW/RWs with uniform soil conditions and low species richness may also have lower denitrification potentials.

While NO_3–N and NH_4–N in CW/RWs had less spatial variability than NO_3–N and NH_4–N in paired NWs, there were no consistent differences across the HGM subclasses for other soil properties such as SOC (Figure 6) and moisture (maps not shown). For example, at Rowel Branch, SOC content was much more homogeneous in the RW (Figure 6a) than in the NW (Figure 6b). However, SOC content in the RW plots for Dismal Swamp and ABC (Figures 6e and 6g) showed comparable if not greater spatial structure than their NW pairs (Figures 6f and 6h). Although the presence of spatial structure for SOC and moisture in CW/RWs was unexpected, it was plausible. There are a variety of factors that may have contributed to this heterogeneity, including (1) the action of physical processes such as sedimentation and erosion have begun to influence the distribution of soil properties across the plots and (2) the action of biological processes such as root growth, litterfall, and bioturbation have affected soil structure and chemistry in localized areas. Furthermore, it is possible that prior land-use and creation/restoration activities did not decrease spatial variability in moisture and SOC but that they may have increased it. The lack of spatial structure in both the CW/RWs and NWs may also be due to variability at scales smaller or larger than our plots or outlier values that weakened the ability

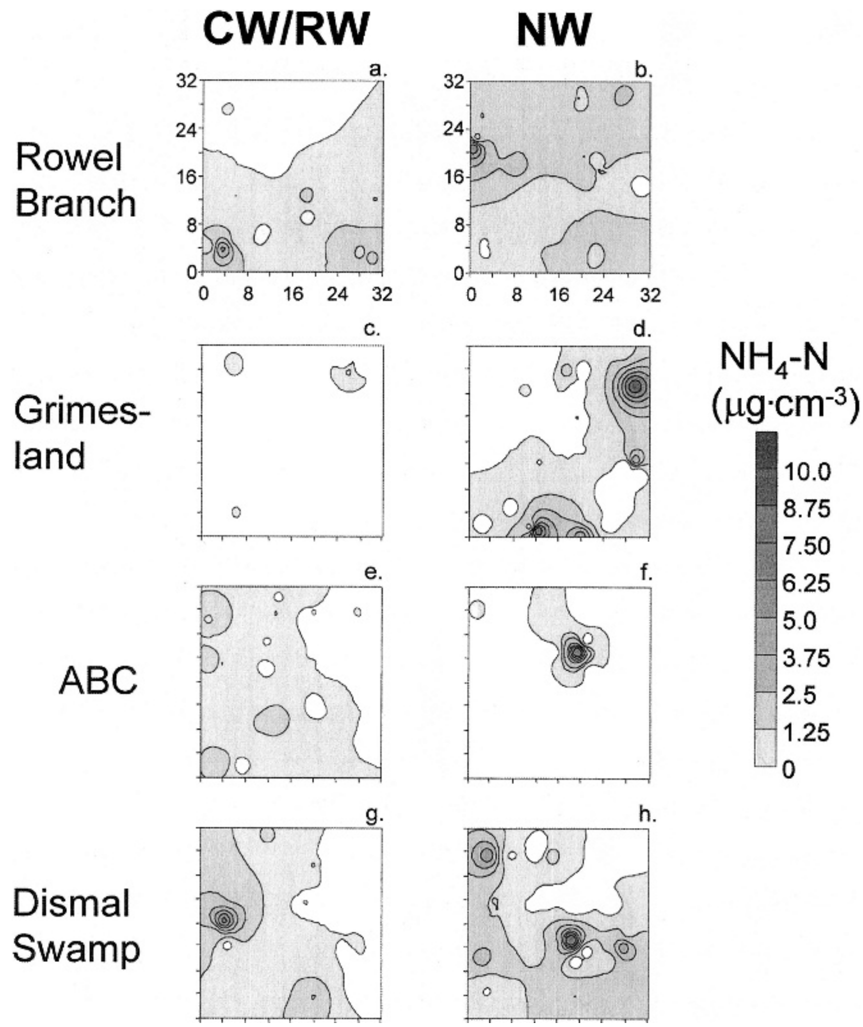

Figure 5. Spatial distribution of ammonium (NH_4-N) at the Rowel Branch restored (a) and natural wetland (b), the Grimesland created (c) and natural wetland (d), the ABC restored (e) and natural wetland (f), and the Dismal Swamp restored (g) and natural wetland (h).

of the geostatistical analyses to detect spatial patterns.

At six of the eight plots, NO_3–N was included in a statistically-significant MSR model that predicted DEA (α=0.1, Table 3). At four plots, NO_3–N alone explained greater than 58 % of the variance in the DEAs. Other studies have also shown NO_3–N to be the most important predictor variable of DEA (Groffman and Tiedje 1989, Cooper 1990). None of the measured soil properties were significantly related to DEA at the Dismal Swamp NW. Soluble organic carbon was included as significant predictor of DEA for three of the plots. This was similar to the study of denitrification in RWs and NWs from Louisiana that reported only weak correlations between SOC and DEA (Hunter and Faulkner 2001). Moisture was a significant predictor for two plots. Ammonium was selected as a predictor of DEA at Dismal Swamp but the overall MSR model was not statistically significant.

Interpolated maps of the predicted DEA generally showed lower and more uniform distributions in the riverine CW/RWs than in the riverine NWs (Figure 7). In some cases, the predicted DEAs of the CW/RWs were zero, suggesting that denitrifier populations were absent. Thus, riverine CW/RWs may be much less effective at denitrification than the NWs they were designed to replace, and this may contribute to the decreased removal of nitrate by wetlands at the landscape scale. Variability of predicted DEAs in the non-riverine plots were more comparable in RWs and NWs. In fact, at the Dismal Swamp site, variability of DEA was greater in the RW than in the NW, as the IDW mapping predicted six DEA hotspots in the RW compared to one hotspot in the NW. This suggested that RWs located

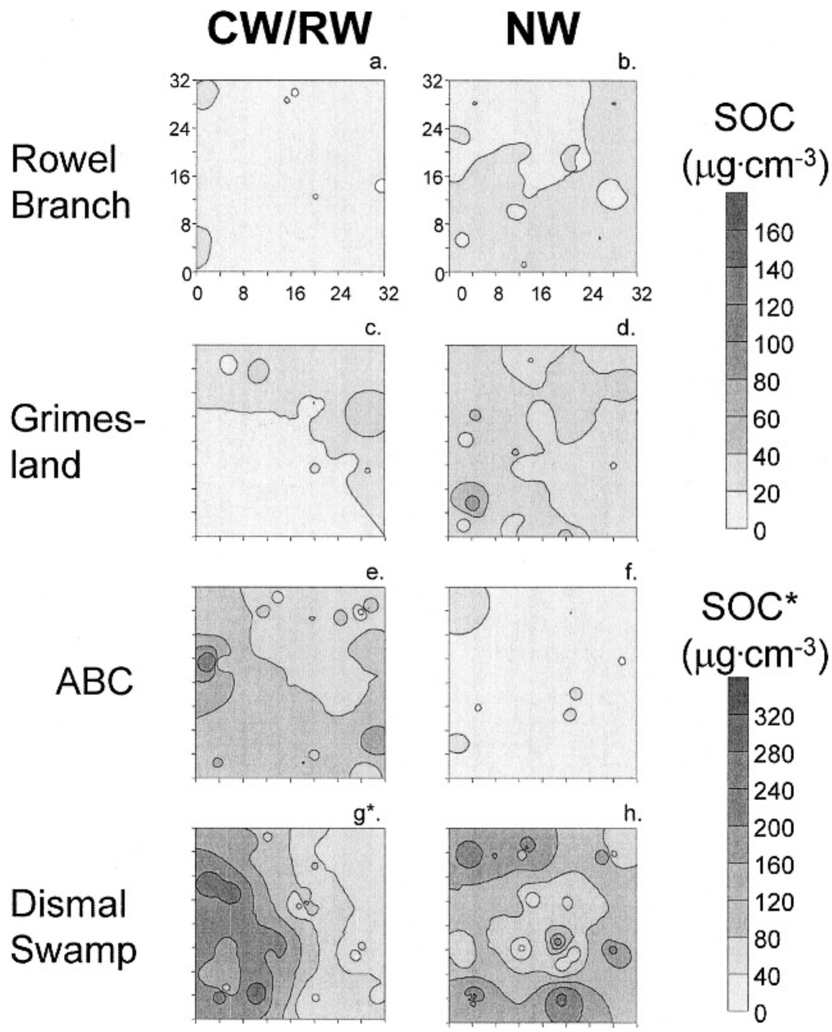

Figure 6. Spatial distribution of soluble organic carbon (SOC) at the Rowel Branch restored (a) and natural wetland (b), the Grimesland created (c) and natural wetland (d), the ABC restored (e) and natural wetland (f), and the Dismal Swamp restored (g) and natural wetland (h). The upper scale corresponds to plots a, b, c, d, e, f, and h, and the lower scale applies to plot g.

on former agricultural land may be effective at denitrification in their first few years of development. As with this study, another study of denitrification in a riparian area in New Zealand reported similar patterns in the spatial distributions of nitrate and DEA (Schipper et al. 1993).

Thus, for NO_3-N across all HGM subclasses and for DEA in riverine wetlands, the results from this

Table 3. Results of stepwise multiple regression analysis for denitrification enzyme activity (DEA) at the created/restored (CW/RW) and natural wetland (NW) plots across all four sites.

Site and Plot	DEA Predictor(s)	Regression Coefficient(s)	Cumulative r^2	p-value
Rowel Branch RW	NO_3-N	16.6	0.69	0.02
Rowel Branch NW	NO_3-N	56.6	0.74	0.01
Grimesland CW	SOC	1.09	0.63	0.02
Grimesland NW	NO_3-N, SOC, Moisture	44.3, 1.7, −954	0.79	0.08
ABC RW	NO_3-N	57.2	0.59	0.10
ABC NW	NO_3-N	31.3	0.97	< 0.01
Dismal Swamp RW	SOC, NH_4-N	0.49, −37.4	0.55	0.13
Dismal Swamp NW	NO_3-N, SOC, Moisture	−4.0, −1.3, 1699	0.95	0.01

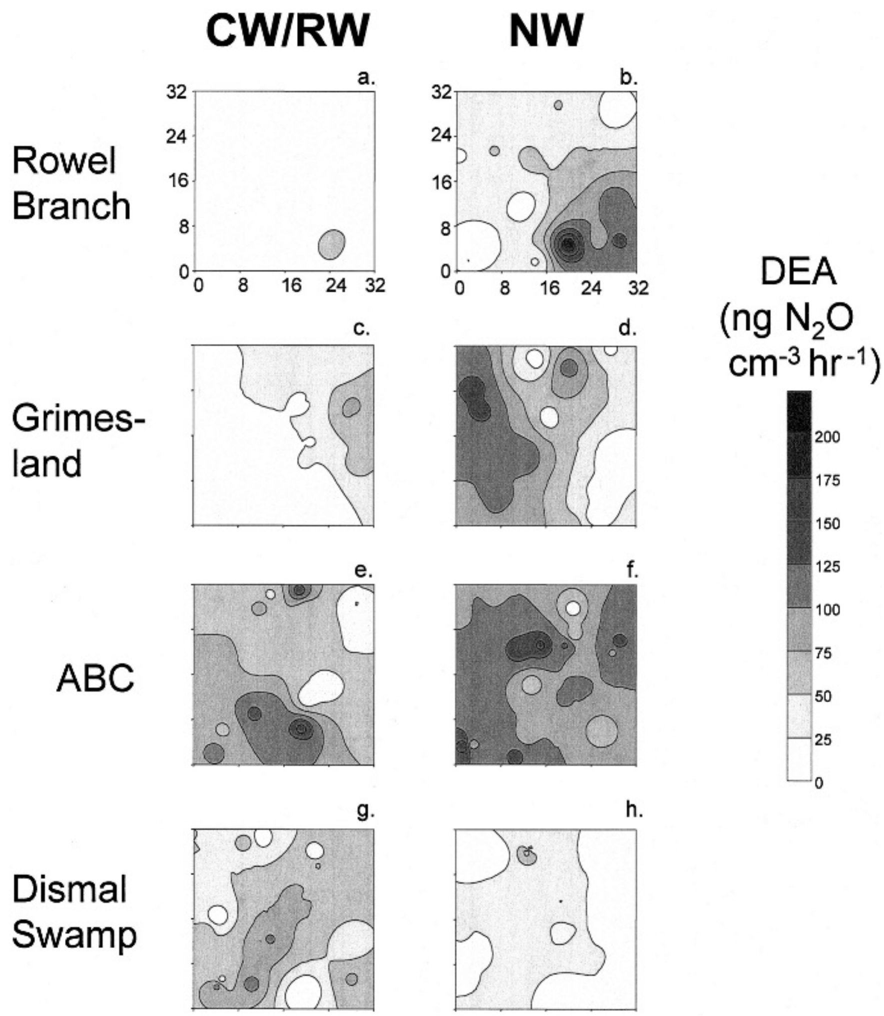

Figure 7. Spatial distribution of predicted denitrification enzyme activities (DEA) from the Rowel Branch restored (a) and natural wetland (b), the Grimesland created (c) and natural wetland (d), the ABC restored (e) and natural wetland (f), and the Dismal Swamp restored (g) and the natural wetland (h).

study confirmed the hypothesis that soil properties of CW/RWs were more homogeneous than those of their NW pairs. However, for other soil properties, such as moisture, SOC, and DEA in non-riverine wetlands, soil properties in CW/RWs showed equal if not greater heterogeneity than their NW pairs. This may be related to the fact that SOC variability may be a result of recalcitrant organic C that was not as affected by recent land-use activities as other soil properties. Nitrate and DEA variability, on the other hand, may reflect labile C pools and nutrient flow patterns that have only recently developed in CW/RWs. Thus, it appeared that prior land-use and mitigation activities had the potential to decrease, increase, or have no effect on the variability of soil properties and processes in created and restored wetlands.

Importantly, due to the inherent variability of soil properties observed in the NWs, attempts to characterize denitrification potential in these areas by taking a single soil sample or even a few random samples are inappropriate. For example, if in this study, only three cores from each plot were collected in random locations, the rich spatial structure that existed in the denitrification potential and related soil properties of these sites would not have been captured. If one or more of the three cores had been located in hotspots, denitrification would have been overestimated, while if none of the three cores had been located in hotspots, denitrification would have been underestimated. As noted in other recent studies of wetland soils (Johnston et al. 2001, Bruland and Richardson 2004), results from this study indicate that sampling schemes in wetlands should utilize clustered or stratified random sampling designs that capture fine, intermediate, and coarse scale spatial structure. Despite the additional sampling and labwork required for spatially explicit research, it is not only worthwhile but also necessary in order to further our understanding of denitrification dynamics. This type of research can provide insights on how to reproduce patterns of natural variation in restored and created wetlands.

SUMMARY AND CONCLUSIONS

The frequency distributions, Cochran's tests, and interpolated maps generally supported the hypothesis that variability denitrification-related of soil properties was lower in CW/RWs than in NWs. Variances of NO_3-N were significantly more homogeneous in CW/RWs than in NWs across all sites. Furthermore, the homogeneous spatial distributions of NO_3-N and NH_4-N in each of the CW/RW plots indicated that this phenomena was consistent across a range of HGM subclasses and land-use histories. The homogeneous distribution of NO_3–N and NH_4-N across the CW/RW plots suggested that CW/RW soils only experienced a limited range of soil chemical conditions and associated biogeochemical transformations. Such conditions are not conducive to the development of the full range of biogeochemical cycling that occurs in NWs. In contrast, the highly variable distribution of NO_3–N and NH_4–N in the NW plots indicated that the NWs experienced wider ranges in biogeochemical conditions and transformations. Unlike NO_3–N and NH_4–N, spatial distributions of SOC and moisture were not consistently more homogeneous in CW/RWs than in NWs, suggesting that prior land use and mitigation activities might increase or have no effect on spatial variability at certain sites or for certain properties.

At six of the eight plots, NO_3–N was a significant predictor of DEA, and at four plots NO_3–N alone explained greater than 58% of the variance in the DEA. Distributions of predicted DEA were homogeneous in riverine CW/RWs, while in non-riverine CW/RWs, they were more heterogeneous and comparable to non-riverine NWs. Thus, denitrification potential and related soil properties in CW/RWs and NWs appeared to be influenced by a complex interplay of factors including prior land-use (agriculture, upland), type of mitigation (restoration versus creation), and hydrogeomorphic setting (riverine versus nonriverine). Results from this study provide critical insights on how to mimic the complexities of natural wetlands in created and restored wetlands and will serve as the foundation for future spatially explicit comparisons of soil properties and processes in created and restored versus natural wetlands.

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