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DENITRIFICATION POTENTIAL OF DIFFERENT LANDUSE TYPES IN AN AGRICULTURAL WATERSHED, LOWER MISSISSIPPI VALLEY

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Denitrification in Lower Mississippi

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Abbreviations: AMOC –Anaerobically mineralizable organic carbon; DP – Denitrification Potential; LMV- Lower Mississippi alluvial valley

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ABSTRACT

Expansion of agricultural land and excessive nitrogen (N) fertilizer use in the Mississippi River watershed has resulted in a 3-fold increase in the nitrate load of the river since the early 1950's. One way to reduce this nitrate load is to restore wetlands at suitable locations between croplands and receiving waters to remove run-off nitrate through denitrification. This research investigated denitrification potential (DP) of different land uses and its controlling factors in an agricultural watershed in the lower Mississippi valley (LMV) to help identify sites with high DP for reducing run-off nitrate. Soil samples collected from seven land-use types of an agricultural watershed during spring, summer, fall and winter were incubated in the laboratory for DP determination. Low-elevation clay soils in wetlands exhibited 6.3 and 2.5 times greater DP compared to high-elevation silt loam and low-elevation clay soils in croplands, respectively. DP of vegetated-ditches was 1.3 and 4.2 times that of un-vegetated ditches and cultivated soils, respectively. Soil carbon and nitrogen availability, bulk density, and soil moisture significantly affected DP. These factors were significantly influenced in turn by landscape position and land-use type of the watershed. It is evident from these results that low-elevation, fine-textured soils under natural wetlands are the best locations for mediating nitrate loss from agricultural watersheds in the LMV. Landscape position and land-use types can be used as indices for the assessment/modeling of denitrification potential and identification of sites for restoration for nitrate removal in agricultural watersheds.

1. INTRODUCTION

The primary source of increased nitrate in surface waters is nitrogen (N) fertilizer applied to croplands (USEPA 1996). An increase in the nitrate concentration of water bodies is correlated with increased agricultural activity in river watersheds (Smith et al. 1987; Galloway et al. 2003). Nitrogen fertilizer use in the US increased by 300% from 1961 to 1999 and current usage consumes 13% of the inorganic N fertilizer used globally (Howarth et al. 2002). Thus, expansion of agricultural activities coupled with an increased use of synthetic N fertilizer in the US has resulted in excessive accumulations of reactive N in environments external to croplands (Galloway 2002; Howarth et al. 2002).

Extensive agricultural development and N fertilizer use over the past 200 years in the Mississippi River basin has increased nitrate loading into the river and the northern Gulf of Mexico (Turner and Rabalais 2003). Since the 1950's, N fertilizer use has increased 20-fold in the basin (Battaglin and Goolsby 1996), which has contributed to a 3-fold increase in the nitrate load of the Mississippi River (Turner and Rabalais 1994; Donner 2004). Agricultural run-off contributes about 74% of the current nitrate loading carried by the Mississippi River (Rabalais et al. 2002) and the increased nitrate loading is cited as one of the major causes of the extensive hypoxia in the northern Gulf of Mexico (Rabalais et al. 2002). A 30% reduction of the N load delivered by the Mississippi River has been recommended to reduce the hypoxia (EPA 2001; Mitsch et al. 2001).

The Lower Mississippi Alluvial Valley (LMV) has lost about 80% of its bottomland hardwood forests to other land uses primarily agriculture (Allen et al. 2001). The LMV was the largest floodplain ecosystem in the US covering about 23,300-km^2

area. Bottomland hardwood forests covered this floodplain and were flooded seasonally as a result of over-bank flooding by the Mississippi River. Due to growth in agriculture, the bottomland hardwoods were cleared, drained, ditched and cultivated for decades for row crop cultivation. This practice not only led to the loss of the NO_3 sinks in the form of greater denitrification rates of bottomland wetlands (Hunter and Faulkner 2001), but enhanced its potential of loading additional nitrate into surface waters including the Mississippi River through N fertilizer use, soil erosion (Mitsch et al. 2005; Rebich 2001; ARS 2001), mineralization of organic nitrogen, and direct drainage of the cultivated lands.

Measures and research recommended by Mitsch et al. (2001) and Mitsch and Day (2006) for reducing the NO_3 loading of the Gulf of Mexico from the Mississippi River basin include a) on-farm soil and N fertilizer management to enhance N use efficiency, b) alternative cropping and management systems for reducing N loss from croplands, and c) creation or restoration of wetlands and riparian ecosystems at suitable locations between croplands and water bodies to remove run-off nitrate before its outfall into the river. Like elsewhere in the basin, restoration of forested and riparian wetlands to reduce run-off nitrate through plant uptake and denitrification (Lowrance et al. 1984, Comin et al. 1997) in the LMV is recommended (Lindau et al. 1994). Moreover, re-connecting forested and riparian wetlands with the rivers for over-bank flooding is another measure suggested for nitrate removal from river water in the LMV (Mitsch and Day 2006; Lindau et al. 1994; Lowrance et al. 1997). Denitrification is one of the major biological processes for nitrate removal from soil and water. Soil organic carbon and NO_3 contents, moisture, temperature and texture affect the rate and extent of denitrification (Galloway et al.

2003). The status of these physicochemical soil properties are the result of interactions of topography, soil hydrology and soil management at basin and sub-watershed scales in the landscape (Florinsky et al. 2004; Lowrance et al. 1997; Peterjohn and Correll 1984). Therefore, it is important to discern the effects of topographic and landuse attributes on denitrification potential of agricultural watersheds.

Agricultural watersheds in the LMV are not homogenous croplands, but are a mosaic of land uses including well, moderately and poorly drained soils under row crop cultivation, a network of drainage ditches and access roads, patches of bottomland hardwood forests and depressional wetlands. Based on the current land use, hydrology, and landscape position, these different land use types can either enhance or retard denitrification. Maintaining environmentally sound crop production in the LMV and reducing nitrate loading into aquatic ecosystems warrants investigation of landscape and environmental factors regulating denitrification potential in agricultural watersheds. Such research-based information is important for the assessment/modeling of denitrification potential at watershed scale and identification of sites for wetland restoration (White and Fennessy 2005) in the LMV. To our knowledge, there are no scientific studies available on this topic in the LMV. Our objectives were to 1) determine denitrification potential of different land use types of an agricultural watershed in the LMV, and 2) identify some of the environmental and landscape/land-use management factors regulating the denitrification potentials.

2. MATERIAL AND METHODS

2.1 Study Area

The study area is the 8.5 km^2 Beasley Lake watershed in Sunflower County, Mississippi (Figure 1) in which about 0.25 km^2 area is covered by the Beasley Lake. The watershed is part of the Yazoo delta region of Northwestern Mississippi formed by the alluvial deposits of the Mississippi River and its tributaries (Fisk 1951). Soils of the watershed range from coarse-textured silty-loam and loam deposits to fine-textured clay alluvium. Dominant soil series of the watershed are Sharkey clay (non-acidic montmorrilinitic, Vertic Haplaquept), Dowling (Very-fine, smectitic, nonacid, thermic Vertic Endoaquept), Alligator (Very-fine, smectitic, thermic Chromic Dystraquert), Dundee (Fine-silty, mixed, active, thermic Typic Endoaqualf), Dubbs silt loam (Fine-silty, mixed, active, thermic Typic Hapludalf), and Forestdale (Fine, smectitic, thermic Typic Endoaqualf), (NRCS 1959).

The elevation gradient between the highest and lowest points in the watershed is 5.5 meters. Current land uses consist of high (Ag-high) and low (Ag-low) elevation croplands, vegetated ditches (veg-ditches), un-vegetated ditches (unveg-ditches), natural forested wetland and depressional wetlands. Forested wetlands are dominated by bottomland hardwood tree species such as American elm (*Ulmus americana)*, Water oak (*Quercus nigra)*, Pin oak (*Quercus phellos*), Green ash (*Fraxinus pensylvanica)* Red maple (*Acer ruburum*), and Hackberry (*Celtis leavigata*). FW cover about 1.2 km^2 area in the watershed. Depressional wetlands (~0.1 km^2 area) are small depressions next to Beasley Lake, which remain ponded during winter and spring and are dominated by submerged and emergent wetland vegetation such as *Potamogeton spp.*, *Sagittaria spp., Scirpus spp. Typha spp.*, *Nymphaea spp. Andropogon* and *Panicum* species usually grow on the drier banks of the depressions. These depressions are the remnants of the swale and ridge topographic features of an ox-bow lake watershed where the swales developed into depressional wetlands. Ag-high (~5.3 km^2) landuse covers mainly well-drained soils while Ag-low (~ 1.5 km^2) covers poorly drained soils next to depressional wetlands and forested wetlands. A low elevation natural ditch next to the forested wetland was developed into a constructed wetland (~0.01 km^2) in spring of 2002 through excavation and installation of a water control structure to increase the aerial extent of the flooded soil. About 0.2 km^2 high and low-elevation croplands of the watershed drained into the constructed wetland. The heavy clay soil of the constructed wetland was similar to that of the nearby natural wetlands. The Sharky and Dowling clay soil of the constructed wetland supported *Potamogton spp., Sagittaria spp. Typha spp. Panicum spp.*, and *Andropogon spp*. The constructed wetland remained ponded in spring, fall and winter like that of depressional wetlands.

The major management activity in the watershed is crop cultivation. Cotton and corn are grown in the Ag.high croplands while soybean is grown in the Ag-low croplands with conventional tillage system. Recreational land uses include fishing and hunting in the Beasley Lake and in forested wetlands. Overhead irrigation is applied as needed for crop cultivation. Irrigation run-off and rain water are drained through the ditches into Beasley Lake. Due to cultivation and the shunting of agricultural runoff directly to the lake, sedimentation in the lake has increased to a degree where it now threatens its ecology (ARS 2001). Maintaining ditches under grass cover is one of the best management practices (BMP) implemented in the watershed by USDA to help reduce sedimentation in Beasley Lake (Rebich 2001). The dimension of the ditches ranges from 1 to 3 meters wide and 1-2 meter deep in the high-elevation areas and upto 5 meters wide and 4 meters deep in the low-elevation areas of the watershed. The banks of the veg.ditches are stabilized by planting and maintaining switch grass (*Panincum spp*.)

Seven land use types of the watershed were selected for this research (Ag-high, Ag-low, veg-ditches, unveg-ditches, forested wetlands, depressional wetlands and the constructed wetland). Eight sampling points were selected randomly in each land use type. Four soil cores (0-10 cm deep; 3 cm dia.) were collected from each sampling point of the seven land-use types of the watershed using a hand auger in March 2002, July 2002, October 2002 and January 2003. The four cores from each sampling point were composited and were transferred to the laboratory on ice and refrigerated at 4 ^{o}C under their original moisture content levels (field-moisture condition) for further analysis.

2.2 Denitrification Potential (DP)

The inherent capacity of a soil to reduce and denitrify nitrate to N_2 gas under an unlimited supply of nitrate using organic carbon as an energy source under anaerobic conditions is called denitrification enzyme assay (Beauchamp and Bergstrom 1993; Groffman et al.1999). This assay measures the amount of denitrification enzymes available at the time of soil sampling. We modified this procedure by amending the soil slurries only with nitrate as we were interested in the denitrification potential (DP) of the different land use types under their existing soil carbon contents using the C_2H_2 block technique (Hill and Cardaci 2004). It is well established that adding carbon to nitrate amended slurries will increase denitrification rates (Hunter and Faulkner 2001; Groffman and Crawford 2003), however there is no practical way of increasing soil carbon at the landscape scale. Therefore, this approach is a more realistic evaluation of the existing ability of the different landscape units to remove nitrate from surface or ground water. For the purpose of this study DP is defined as the capacity of soil slurries to denitrify nitrate under anoxic conditions at room temperature (22 degress Celcius). Field moist soils were thoroughly homogenized by hand and brought to room temperature overnight before incubation. The next morning, six sub samples (moist equivalent of10 g dry soil) of the homogenized soil from each of the eight soil samples from each of the seven land use types were weighed into 6 replicate serum bottles (150 mL). Fifteen mL of 10 mg NO_3^- L^{-1} solution and 5 ml of de-ionized water were added to three of the six bottles to deliver 15 µg of NO_3^- g^{-1} dry soil, while 20 mL of de-ionized water was added to the remaining three bottles. The bottles were then capped airtight and purged with O_2-free N_2 gas for 20 minutes to induce anaerobic conditions. Ten percent of the serum bottle headspace was replaced with cleaned C_2H_2 gas to block the bacterial conversion of N_2O to N_2 gas. The bottles were then wrapped in Al foil and put on a reciprocating shaker for continuous shaking at room temperature (22 to 25 ^oC). Headspace gas samples were collected at 2, 4 and 6 hours with a syringe and stored in Beckton Dikinson Vacutainers®. The gas samples were analyzed on a Tremetrics 9001GC having a porapack Q column with ECD detector for N_2O concentration determination. The rate of N_2O production was calculated in ug N-N_2O g^{-1} h^{-1} using the 3 gas sample readings during the 6 hour incubation. Adjustments were made for soluble N_2O in the bottles using a Bunsen absorption coefficient of 0.54 at 25 ^oC.

2.3 Anaerobically Mineralizable Organic Carbon (AMOC)

Denitrification depends directly on the amount of mineralizable organic C available to the denitrifier population under anaerobic conditions (Singh et al. 1988; Gale et al. 1992). Since denitrification is an anaerobic process, the amount of mineralizable organic C available under anaerobic conditions would help explain any trend in the DP among different land use types. Field moist soil (equivalents of 5 g oven-dried soil) from each soil sample were weighed into 150 mL duplicate serum bottles. Twenty ml of 50 mg NO_3^- L^{-1} solution was added into each bottle, which delivered 200 µg NO_3^- g^{-1} soil. The bottles were capped airtight and purged with oxygen-free N_2 gas for 20 minutes to induce anaerobic conditions. After purging, the bottles were wrapped in aluminum foil and were shaken for 15 minutes on a reciprocating shaker. After shaking, the bottles were stored at room temperature (22-25 ^oC). The headspaces of the bottles were sampled with a syringe at 1, 24, 48 and 72 hours of incubation and stored in 5-mL Beckton Dikinson Vacutainers®. The gas samples were analyzed on a Tremetric 9001 GC fitted with a methanizer and an FID detector for CO_2 concentration determination (Ullah et al. 2005). The gas production over the length of incubation remained linear in all the landuse types. The amount of CO_2 produced was calculated as µg C-CO_2 g^{-1} h^{-1}. Corrections were made for soluble CO_2 in the incubation bottles by using the Bunsen Adsorption coefficient of 0.752 at 25 ^oC.

2.4 Total soil carbon and nitrogen

Total soil carbon (C) and nitrogen (N) were determined using a Thermo Finnigan CNS Analyzer. Soil samples were oven dried, pulverized and thoroughly homogenized. A sub-sample of about 35 mg was weighed into a tin capsule for automated analysis to determine concentrations of organic C and total N. These values and bulk density measurements were used to calculate Mt of C and N ha^{-1}.

2.5 Soil nitrate, bulk density, porosity, water-filled pore space and texture

Field-moist soil equivalents of 5 g oven-dried soil were weighed into 250 mL duplicate bottles. Fifty mL of 2M KCL solution was added to each bottle. The bottles were put on a reciprocating shaker for continuous shaking for 1 hour. After shaking, the bottles were centrifuged at 3000 rpm for 5 min and were then filtered into 20 mL scintillation vials through a No. 42 Whatman filter paper. The samples were stored in a freezer until analysis for nitrate on a Lachat automated flow injection analyzer. Average values for each soil sample were determined and reported in mg N Kg^{-1} oven-dried soil. At each land use type eight intact soil cores (2.5 cm dia. x 10 cm long) were taken and transferred to the lab for the determination of soil moisture and bulk density. Soil porosity was determined using the equation of 1- (bulk density/particle density). Percent water-filled pore space (WFPS) was determined for each landuse type for all season according to Ullah et al. (2005). Soil particle size distribution was determined by the filtration method according to Sheldrick and Wang (1993).

2.6 Statistical Analysis

Differences in DP among the landscape units within each season were analyzed by a two-way analysis of variance using the General Linear Model in SAS (SAS Institute 1998). Landscape was treated as main effect, nitrate amendment was treated as a sub-plot effect and season was treated as repeated measures variable in the ANOVA model. Post ANOVA tests were conducted with Fisher's protected LSD at 5% significance level. Linear regression of the yearly averaged DP on total soil C, N, and bulk density of the 7 land use types was done using SAS. Significant differences in physio-chemical properties of soils of the 7 land use types were determined using one-way ANOVA. Pearson correlation coefficients among DP, AMOC and soil moisture were calculated for each season. The data were analyzed for normality and homogeneity of variance of the residuals using the proc univariate procedure in SAS and Shapiro-wilk test of normality of the residual at $p > 0.05$.

3. RESULTS

3.1 Landscape position and Landuse type effects on Denitrification Potential

The natural and constructed wetlands had significantly greater ($p < 0.05$) DP than the Ag-high and Ag-low sites in all seasons (Figures 2). Forested wetlands showed the highest DP while Ag-high showed the lowest during the four seasons when additional NO_3 was added during the assay compared to their unamended soils (Figures 2 and 3). The DP of forested wetlands was 4.5 to 11 times greater than the Ag-high during the year. Similarly, the forested wetlands showed 2.4 to 4.3 times greater DP than the Ag-low sites in all the seasons. Except in summer, Ag-high and Ag-low had similar DP values ($p > 0.05$). On average, forested wetlands exhibited 3.0 and 2.7 times greater DP than those of veg-ditches and unveg-ditches, respectively (Table 1).

Depressional and constructed wetlands showed similar DPs, except in summer when depressional wetlands had 1.5 times greater DP than the constructed wetland. On average both depressional and constructed wetlands had 3.7 to 7.4 and 1.2 to 3.7 times greater ($p < 0.05$) DP than that of the Ag-high and Ag-low respectively during the four seasons. Depressional and constructed wetlands also showed 1.2 and 1.6 times greater DP than veg.ditches and unveg-ditches, respectively, but were less than the DP of forested wetlands (Table 1).

When the DP of individual land use types were averaged for the four seasons (Table 1), the DP of veg-ditches was 4.1 times of the Ag-high ($p <0.05$) and 1.7 times of Ag-low, although statistically not significant ($p > 0.05$). Moreover, veg-ditches had 1.3 times greater DP than unveg-ditches. On the whole forested wetlands exhibited significantly greater DP (p <0.05) than the rest of the land use types. Similarly, depressional and constructed wetlands had higher DP than unveg-ditches, Ag-high and Ag-low sites (Table 1), but had lower DP than the forested wetlands. The DP of veg-ditches was more variable and thus was not significantly different than Ag-low, unveg-ditches, constructed and depressional wetlands ($p > 0.05$).

3.2 Environmental variables and Denitrification Potential

Among the environmental variables measured, nitrate amendment, season, organic carbon availability, bulk density and %WFSP contents significantly influenced DP of all the land use types. When amended with additional nitrate, forested and depressional wetland soils showed 55% and 67% greater DP compared to the DP of soil without nitrate additions (Figures 2 and 3). Constructed wetland and unveg-ditches showed an increase in DP with NO_3 enrichment during fall and winter only while veg-ditches responded to nitrate addition in summer and fall. Ag-high and Ag-low soil did not respond in terms of increased DP to nitrate amendment during the four seasons (Table 1), which shows that their DPs were limited by factors other than nitrate, probably by the availability of organic C and lack of anaerobosis.

Season significantly affected the DPs of all the land uses except Ag-high and unveg-ditches (Table 1). When the DPs of all the land use types were averaged together and tested for a seasonal effect, the winter DP was found the lowest than the rest of the seasons. Lower winter soil temperatures are speculated to have limited the denitrifier activity. Similar seasonal effect on denitrification potential of forested wetland soils in the LMV was observed by Hunter and Faulkner (2001).

Significant differences in a number of soil properties among the different land use types were observed (Table 2). Wetlands had 1.8 times greater total soil organic C than the cultivated soils. Higher soil organic C in wetlands contributed to improved soil structure resulting in lower bulk densities and high soil porosities in wetlands than the Ag-high and Ag-low sites. Anaerobic incubation of soils from the 7 land use types showed that the amount of anaerobically mineralizable organic carbon (AMOC) in wetlands soil was 1.4 times those of the cultivated soils (Table 3). Similarly, veg-ditch soil had a relatively lower soil bulk density, higher porosity (Table 2) and 1.3 time greater AMOC values compared to those of the cultivated soils. Unveg-ditches had similar soil bulk density, porosity and AMOC values to those of the cultivated soils. High AMOC values observed in the wetland and veg-ditch soils supported greater DP compared to those in the cultivated soils. AMOC showed a significant correlation with DP of nitrate amended soils during the four seasons ($p<0.05$). AMOC values of the spring, summer, fall and winter significantly correlated with DP ($p <0.05$) with r value of 0.51, 0.57, 0.75 and 0.81, respectively (Table 3). Simple linear regression identified significant influence of total soil N, C and bulk density on DP of the nitrate amended soils with r^2 values of 0.80 0.78, and 0.80 respectively. Soil moisture content also correlated significantly with DP in each of the four seasons (Table 4). The Pearson's correlation coefficients of spring, summer, fall and winter soil moisture with DP were 0.46, 0.44, 0.69 and 0.57 respectively at $p <0.05$.

4. DISCUSSION

Significant differences in the DPs of nitrate ameneded soils of different land use types (Figures 2) are attributed to differences in the position and management of different land use types in the watershed. Low-lying heavy clay soil (Sharkey, Dowling and Alligator soil series) under forested and depressional wetlands showed 6.3-fold greater DPs than the Ag-high silt-loam soils. Groffman and Tiedje (1989) reported similar observation of significant influence of soil texture and drainage (surrogates for landscape position) class in forest soils on denitrification potentials. Mohn et al. (2000) also found low denitrification rates in drained mounds compared to wetland soils. Our data shows that landscape position is a significant regulator of DP in soils as poorly-drained, fine-textured soils in natural wetlands supported higher denitrifier activity than the coarse textured Ag-high soils. It is worthy to note that Ag-low soil had an average 2.5 times higher DP than that of Ag-high soils, which implies that topographic position led to the explicit differences in DP of these sites.

As expected, forested, depressional and constructed wetlands exhibited 3.0, 2.0 and 2.1 times greater DP than the pedogenically similar (Table 2) Ag-low soils, respectively. Drainage and cultivation of the Ag-low soils over the years led to lower available organic C substrate (Table 2) and moisture contents (Table 4), which resulted in lower denitrifier activity compared to similar soils under wetlands in the watershed. In another study in the same watershed, we observed significantly greater denitrification rates in forested wetlands than the rates of an adjacent cultivated site under variable soil moisture contents (Ullah et al. 2005), demonstrating that cultivation diminished the capacity of these soils to denitrify nitrate at rates similar to forested wetlands. The natural vegetation cover and the resulting soil litter production in forested and depressional wetlands provide higher organic carbon substrate for supporting greater denitrifier activity than similar textured Ag-low soils. This observation implies that pedogenically similar soils at the same elevation in a watershed under row crops cultivation sustain lower denitrification potential than under wetlands.

The higher denitrifier activity in the veg-ditches is attributed to its maintenance as a grassed waterway, which resulted in maintaining high soil moisture regime and producing greater AMOC contents compared to croplands and unveg-ditches. This finding supports the current practices of maintaining veg-ditches as a BMP for erosion control and water quality improvement as recommended by the USDA (ARS 2001) in the region.

The DPs observed in spring, summer and fall of all the land use types were significantly greater ($p < 0.05$) than their DPs of winter except Ag.high and unveg-ditch soils (Table 2), respectively. This observation suggests that the lower average winter soil temperatures (ranging from 6 to 9 ^{o}C) and higher % WFPS substantially reduced soil denitrifier activity (Table 4). Lower winter temperatures and higher WFPS percentage compared to the summer values suppressed microbial activity (Magg et al. 1997) and reduced the supply of mineralizable organic C to denitrifiers (Table 3) (Mohn et al. 2000; Mogge et al. 1998; Klein and Logtestijn 1996). Even though %WFPS of soils in winter were relatively lower than the fall %WFPS, lower winter temperature led to significantly lower AMOC production than the fall values of all the land use types (Table 3). This indicates that lower soil temperature exerts significant controls over microbial activity. Lower denitrifier activity may pose greater risk of nitrate loss from agricultural watersheds in winter.

Unlike the Ag-high and Ag-low soils, nitrate availability was found limiting DPs in forested and depressional wetlands, because nitrate additions to these soils led to increases in DPs during the four seasons compared to DPs of Ag-high and Ag-low sites (Figures 2 and 3). When averaged over the four seasons, forested and depressional wetlands showed 3 and 4-fold increase in DP under additional nitrate compared to the unamended soils. Higher denitrification rates under additional nitrate can happen, if the process is not limited by the availability of organic C (Weier et al. 1993). No response of Ag-high and Ag-low soils to nitrate amendment shows that DP in these land uses was limited by the availability of organic C (Table 1). Constructed wetland responded with increased DP to nitrate additions in fall and winter only. The increase in DP of the constructed wetland in response to nitrate additions after 6-9 months of its construction demonstrate that wetland restoration on abandoned marginal lands in low-elevation areas of the watershed can enhance denitrifier activity within and/or after the first growing season. Veg-ditches also responded with increased DPs to nitrate additions. These results depicts that wetlands and veg-ditches can denitrify additional nitrate coming from cultivated soils or other external sources. This finding is in agreement with the findings of similar studies conducted regarding nitrate removal potential of riparian wetlands and vegetated buffer-strips in agricultural watersheds (Lindau et al. 1994; Lowrance et al. 1995; Groffman and Crawford 2003; Groffman et al. 2002; Ingrid-Brettar et al. 2002; Clement et al. 2002; DeLaune, et al. 2005).

As expected, higher total soil carbon, mineralizable organic C, wetter soil conditions, greater soil porosity and fine-clay texture of natural and constructed wetlands (Table 2) supported greater denitrifier activity than those observed in the Ag-high and the pedogenically similar Ag-low and unveg-ditch soils. This result is in agreement with the findings of Hill and Cardaci (2002), Davidsson and Stahl (2000), and Groffman and

Tiedje (1989) who reported significant control of denitrification potential by available organic carbon, soil moisture contents and soil texture classes. At the biogeochemical scale, soil moisture content, soil texture, available C and nitrate are the major regulators of denitrifier activity in soils (Patrick and Reddy, 1976; Myrold et al.1998; Weitz et al. 2001) and the same factors were found significantly affecting DPs in the Beasley watershed. The status of these biogeochemical scale regulators of DP is a result of the interaction of soil oxygen diffusion rate/dynamics, landscape position, plant community structure, physical disruption (landuse), ecosystem type, microbial biomass, and organic matter production characteristics of a given landscape unit (Myrold et al.1998; Florinsky, et al. 2004). Florinsky et al. (2004) reported significant control of denitrifier activity in soils by landscape position of a site and its subsequent influence on soil moisture and organic carbon availability. The authors concluded that higher denitrifying activity in low-elevation areas was mostly affected by the redistribution and accumulation of soil moisture and available organic carbon due to their additional gains from high-elevation areas along the slope (Florinskey, et al. 2004). In the Beasley watershed, lower elevation land uses under wetlands maintained higher soil moisture contents, greater soil organic carbon pools, and lower bulk densities that resulted in higher DP in wetland soils than either the high elevation or low elevation soils under cultivation. These findings show that landscape position and landuse type exerts significant controls on denitrifying activity in a watershed. Any effort to model and predict denitrification potential at watersheds scale such as the LMV should include topographic and land use aspects of a watershed as key controllers of DP besides the biogeochemical scale variables in the modeling process. Such an approach can help compute/predict relatively more realistic

DPs at watershed scale and identify sites for restoration in large river basins (White and Fennessy, 2005). Application of digital terrain models for predicting microbial activities of watersheds based on inputs of both biogeochemical and landscape scale variables into the model have been found feasible in Canada (Florinsky et al. 2004).

The results of this research demonstrate that denitrification potential in soils is regulated by more than one biogeochemical scale factor and is significantly influenced by both the landscape position and land use types in a watershed. Marshal (1999), Groffman et al. (1999), and Florinsky et al (2004) also reported that denitrification cannot be predicted on the basis of one factor, rather a combination of factors are involved in the regulation of the denitrification process. Therefore, it is suggested to consider landscape scale variables (landscape position and land use type) in addition to biogeochemical scale variables in assessing/modeling denitrification potential of soils and identification of sites for wetland restoration for water quality improvement in the LMV. Our findings suggest that fine-textured low-elevation sites in agricultural watersheds are the best candidates for wetland restoration for nitrate removal in the LMV. Such sites can accumulate higher soil organic carbon and retain higher soil moisture for sustaining persistent and enhanced denitrification rates than coarse-textured Ag-high soils in agricultural watersheds.

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FIGURES LEGEND

Figure 1. Location map of the Beasley Lake watershed, Mississippi, USA.

Figure 2. Denitrification potential of different land use types amended with nitrate in spring, summer, fall and winter with standard error of the means (same letters on top of each bar show no significant differences in DPs among the land uses within each season at $p = 0.05$).

Figure 3. Denitrification potential of different unamended land use types in spring, summer, fall and winter with standard error of the means.

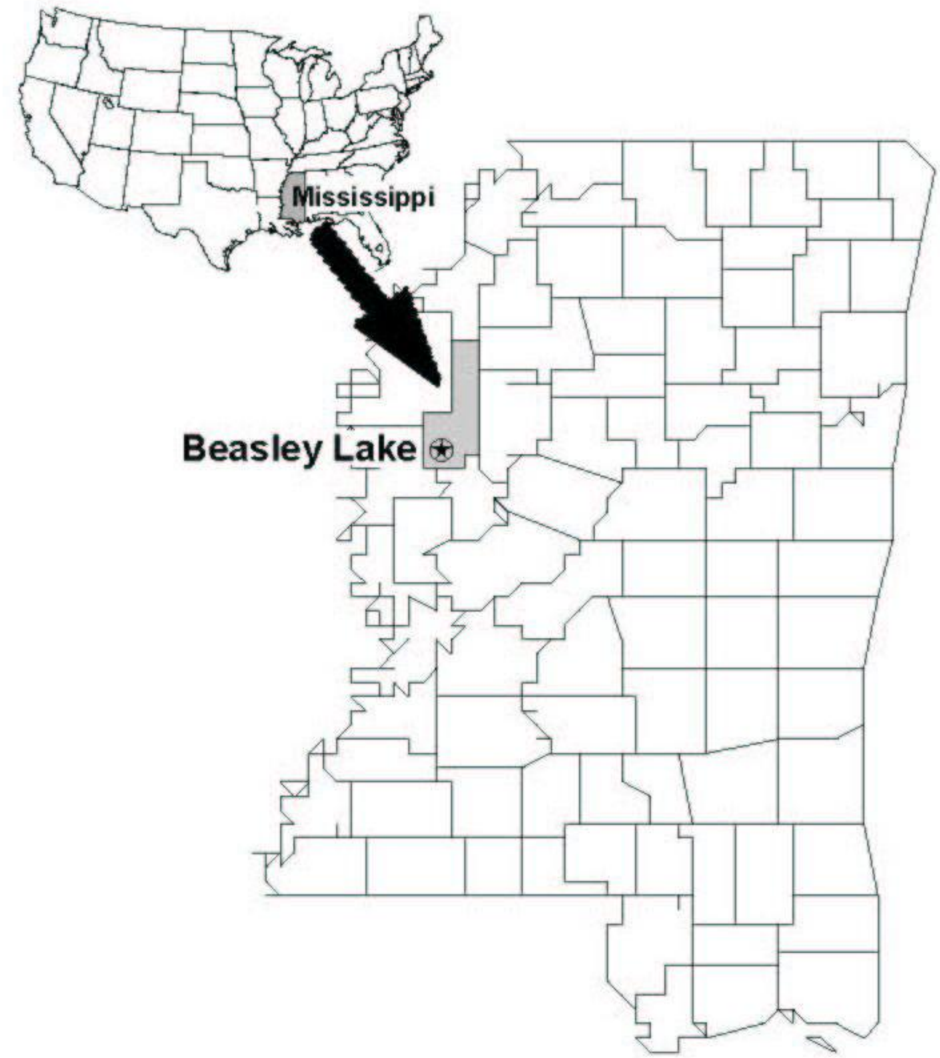

Figure 1. Location map of the Beasley Lake watershed, Mississippi, USA.

Table 2. Mean Physio-chemical properties of soils of the7 land use types in Beasley watershed. Means (S.E of the means) followed by the same lower-case letter indicate no significant difference ($p > 0.05$) among the different land use types.

Soil parameters	Forested wetland	Dep.wetland	Constructed wetland	Veg-ditches	Unveg-ditches	Ag-low	Ag-high
Bulk Density (g cm^{-3})	0.90 (0.03) a	0.96 (0.01) a	1.20 (0.2) b	1.17 (0.03) b	1.24 (0.4) b	1.24 (0.9) bc	1.31 (0.4) c
Porosity cm^3 cm^{-3}	0.66 (0.98) a	0.64 (0.99)a	0.55 (0.91)b	0.56 (0.98) b	0.53 (0.98) b	0.53(0.96) bc	0.51(0.98) c
Clay (%)	51 (0.5) a	47 (1.2) a	45 (1.2) a	51 (2.5) a	45 (5.4) a	48 (2.1) a	23 (0.001) b
Silt (%)	47 (0.7) a	49 (3) a	45 (1.6) a	44 (3.5) a	33 (2.8) b	46 (1.3) a	65 (0.8) c
pH	5.4 (0.05) a	5.3 (0.03) a	5.5 (0.07) a	5.6 (0.07) a	5.6 (0.04) a	6.1 (0.05) b	6.3 (0.03) b
Total C 0-10 cm(Mt ha^{-1})	297 (30) a	167 (18) b	218 (9) b	189 (11) b	156 (44) bc	168 (13) bc	85 (26) c
Total N 0-10 cm (Mt ha^{-1})	26 (2) a	17 (1.4) b	23 (0.9) b	24 (2.2) b	13 (2.6) c	11 (1.0) c	5 (3.5) d
NO_3 (mg kg $soil^{-1}$)	5.7 (1.7) a	6.5 (2.5) a	6.1 (2.8) a	7.6 (3.2) a	7.4 (1.9) a	8.0 (1.6) a	7.7 (3.2) a

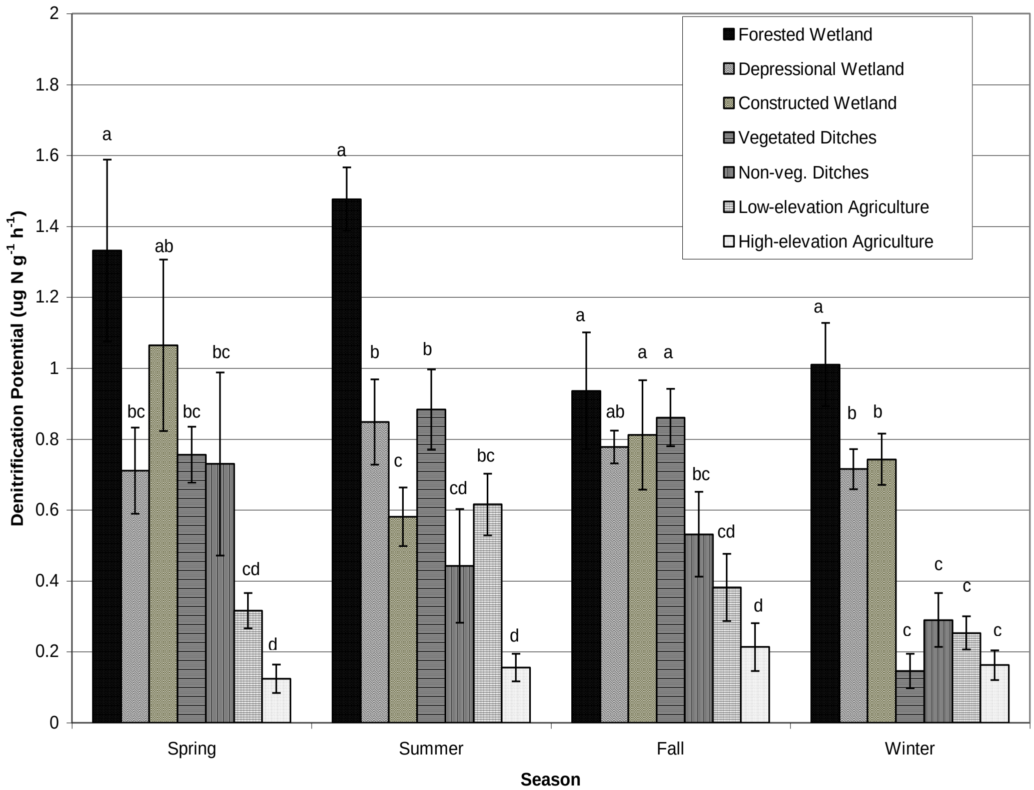

Figure 2. Denitrification potential of different land use types amended with nitrate in spring, summer, fall and winter with standard error of the means (same letters on top of each bar show no significant differences in DPs among the land uses within each season at p =0.05).

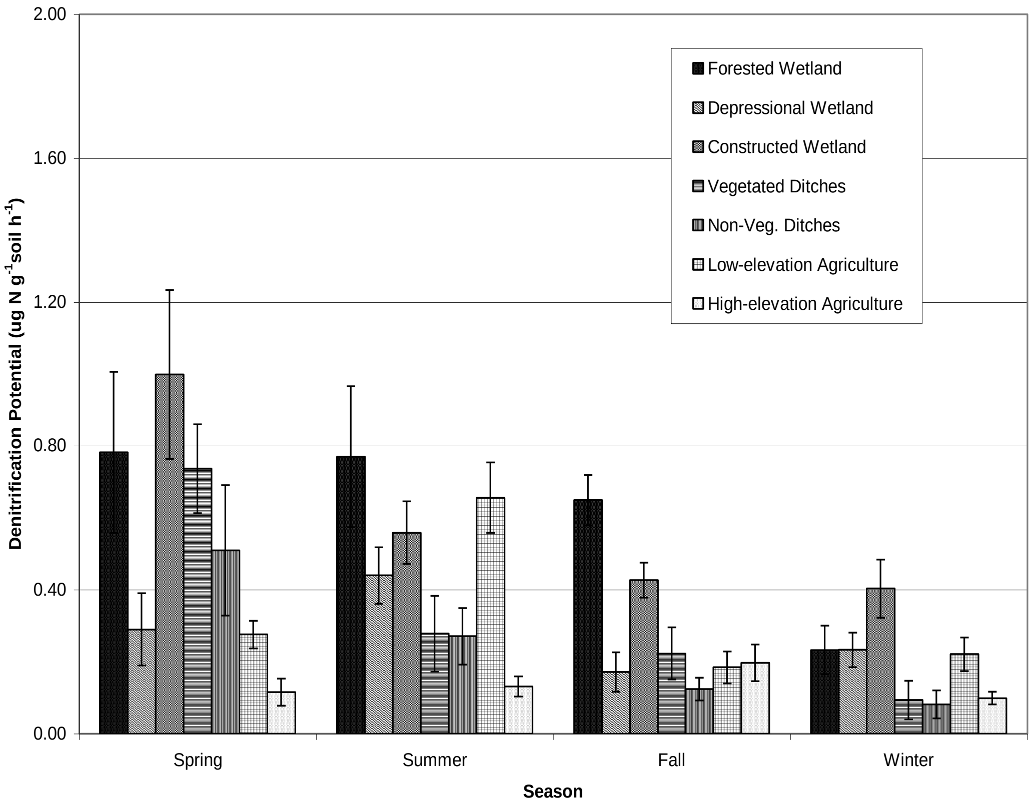

Figure 3. Denitrification potential of different land use types (no nitrate amended soils) in spring, summer, fall and winter with standard error of the means.

Table 1. Yearly average denitrification potential (DP) of the 7 landuse types with post-ANOVA comparisons between 7 land use types. Same lower-case letters following mean values shows no significant difference among the different land use types at $p = 0.05$. Effects of NO_3 amendment of soil slurries and season on denitrification potential of the 7 land use types are also shown using pooled variance two-sample t-test and ANOVA, respectively at $p < 0.05$.

Land use types	DP of NO_3 amended soils ($ug\ N\ g^{-1}\ h^{-1}$)	NO_3 addition effects on DP	Seasonal effects on DP
Forested wetland	1.18 (0.13) a	*	*
Depressional wetland	0.77 (0.03) b	*	*
Constructed wetland	0.82 (0.10)b	*	*
Veg-ditches	0.66 (0.17) bc	*	*
Unveg-ditches	0.50 (0.09) c	*	ns
Low-elevation Agric.	0.40 (0.08) cd	ns	*
High-elevation Agric.	0.16 (0.02) d	ns	ns

*: significant effect, ns: non-significant effect

Table 3. Mean anaerobically mineralizable organic carbon content of the 7 land use types with standard error of the means and its Pearson's correlation coefficients with DP

Season	----------------------------------AMOC (µg C-CO_2 produced g^{-1} soil h^{-1})---------------------							
	Forested wetland	Dep. wetland	Constructed wetland	Veg-ditches	Unveg-ditches	Ag-low	Ag-high	Pearson's correlation with DP (n=57)
Spring	0.78 (0.10)	0.63 (0.07)	0.82 (0.11)	0.77 (0.07)	0.14 (0.03)	0.46 (0.00)	0.35 (0.07)	0.51*
Summer	1.26 (0.13)	1.29 (0.03)	1.21 (0.05)	0.91 (0.05)	0.85 (0.04)	0.88 (0.03)	0.89 (0.04)	0.57*
Fall	1.03 (0.02)	1.04 (0.03)	0.99 (0.04)	0.98 (0.05)	0.89 (0.03)	0.91 (0.02)	0.66(0.01)	0.75*
Winter	0.86 (0.04)	0.70 (0.04)	0.70 (0.04)	0.52 (0.03)	0.62 (0.03)	0.59 (0.06)	0.72 (0.02)	0.81*

* Significant difference at $p < 0.05$

Table 4. Seasonal water-filled pore space percentages with standard error of the mean and its correlation co-efficient with denitrification potential of the 7 land use types. Last row of the table shows seasonal average soil temperatures of Beasley watershed.

Land use Types	Water-filled pore space (%)			
	Spring	Summer	Fall	Winter
Forested wetland	62 (1.0)	48 (0.9)	59 (1.0)	53(1.0)
Depressional wetland	63 (1.0)	28 (1.0)	52 (1.0)	44 (1.0)
Constructed wetland	51(1.0)	29 (1.0)	55 (1.0)	48 (1.0)
Veg-ditches	47 (1.0)	61 (1.0)	68 (.9)	50 (1.0)
Unveg-ditches	49 (1.0)	60 (1.0)	58 (1.0)	51 (1.0)
Low-elevation croplands	56 (1.0)	34 (1.0)	56 (1.0)	45 (1.0)
High-elevation croplands	34 (1.0)	28 (1.0)	45 (1.0)	32 (1.0)
Correlation of soil moisture with DP	0.46*	0.44*	0.69*	0.57*
Soil Temp. ^{o}C	22	28	21	9

*Significant at $p < 0.05$, and n= 56