NOTE

Spatial Variability of Sediment Denitrification Across the Atchafalaya River Basin, Louisiana, USA

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Abstract Ecosystem-wide denitrification estimates generally depend on the degree of spatial variability in the system, but spatial variability is rarely assessed. To model nitrogen removal rates in the Atchafalaya River Basin we first identified trends in background and potential denitrification across this large floodplain. We conducted a laboratory study to quantify background and potential denitrification rates. Background and potential denitrification rates were significantly different. Background rates ranged from ranged from 0–1.35 $\mu gN\ g^{-1}\ d^{-1}$ and potential rates ranged from ranged from 26.72–710.47 $\mu gN\ g^{-1}\ d^{-1}$, illustrating the existence of denitrification hotspots across the landscape. Background rates were related to soil characteristics (carbon, nitrogen, nitrate), but potential rates appeared to be related to landscape position (spatial coordinates). Background denitrification showed a strong positive correlation with soil nitrate, and a negative correlation with soil nitrogen and soil carbon. Potential denitrification rates showed no significant correlations with any parameters tested. We observed a significant relationship between location and potential denitrification rates, with greater potential downstream than upstream, but not between location and background rates. This suggests that landscape scale studies should include additional qualifiers, such as habitat type and organic matter quality, for more reliable estimates of denitrification rates.

Keywords Acetylene block technique · Background denitrification · Nutrient removal · Potential denitrification · Wetlands

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Introduction

Floodplains are frequently identified as important sites for nutrient retention and removal (Brinson et al. 1984; Craft and Casey 2000; Gergel et al. 2005; Forshay and Stanley 2005; Noe and Hupp 2009). Denitrification in particular has the potential to remove significant amounts of nitrogen (N) from floodplains, as seasonal river inundation establishes anaerobic soil conditions, delivers nitrate, and preserves carbon (C). However, these conditions vary across both large and small scales, resulting in spatial heterogeneity of soils and spatial variability of biogeochemical reactions. Aspects of spatial variability have often been neglected when studying denitrification (Pina-Ochoa and Alvarez-Cobelas 2006).

Floodplains on the Coastal Plain of the Southeastern USA often provide the last opportunity for sediment storage and biogeochemical cycling before rivers discharge into estuaries (Hupp 2000). Sediment deposition within the bottomlands of the Coastal Plain generally results in a net elevation gain (Hupp 2000). Because sedimentation facilitates nutrient sequestration, suspended sediments are usually associated with adsorbed nutrients (Hupp 2000). Hence, an intact or restored floodplain can function as a long term sink for nutrients. Conversely, when elevation gain is such that the floodplain becomes isolated from

floodwaters, the potential for storage of river-borne nutrients is diminished. Soil conditions on a floodplain are largely influenced by the hydrologic conditions of the adjacent river. However, many rivers have seen significant hydrologic alteration due to the construction of dams, levees, and navigation channels.

The Atchafalaya River is the largest distributary of the Mississippi River. Attempts by the Mississippi to flow into the Atchafalaya were thwarted by the construction of the Old River Control Structure in 1963. Today, flow of Mississippi water down the Atchafalaya River is limited to approximately 30% of the total volume of the Mississippi. The Atchafalaya River flows 217 km from its origin at the confluence of the Red and Mississippi Rivers, to the Gulf of Mexico where it discharges into Atchafalaya Bay via the main stem of the river and the Wax Lake Outlet. Within the Atchafalaya River Basin (ARB), the reworking of sediments has resulted in variation in the conditions that are seen throughout the floodplain. Due to sedimentation, some areas of the floodplain are elevated such that they never have contact with river water. Other areas receive intermittent inundation, and some locations are permanently flooded. Wetlands on the floodplain of the Atchafalaya River function as water retention basins when river stage is high and as discharge areas when river stage is low (Xu 2010). Analysis of total suspended sediment samples collected across the Basin in the spring of 2008 (a high water year) suggests that only 21% of the floodwaters deposited sediments in the baldcypress region of the ARB (Scaroni, unpublished data). The retention of water on the floodplain has implications for downstream water quality; N transformations and removal generally occur where hydraulic residence time is increased (Cirmo and McDonnell 1997).

The formation of a large area of hypoxia (dissolved oxygen levels <0.2 mg L^{-1}) in the Gulf of Mexico is attributed to N loading from the Mississippi and Atchafalaya Rivers (Rabalais et al. 2002). Recent studies indicate that 14% of the total nitrogen (TN) that enters the ARB does not discharge into the Gulf of Mexico; this is equivalent to 50,544 Mg tons TN annually sequestered or removed within the ARB (Xu 2006). Sedimentation and plant uptake are the major processes contributing to nitrogen retention, while the denitrification pathway is considered a permanent removal of N from the system. Nutrient retention studies tend to calculate a mass balance of N outputs from N inputs; quantifying the contribution of each of these three processes to total N retention and removal are not common, and are generally restricted to lakes (Saunders and Kalff 2001). However, Saunders and Kalff (2001) have shown that certain wetlands retain twice as much N as lakes at a given N load. Because the ARB contains a variety of habitats, including lakes, wetland areas, and bottomland hardwood forests, it is likely that there is considerable variability in N retention and removal rates across the Basin. Our goal was to examine spatial variability as well as look for trends in denitrification across the ARB.

Study Area

The ARB is located in south-central Louisiana, and covers approximately 3,400 km^2. The floodplain consists of three major habitat types: bottomland hardwood forests, baldcypress swamps, and lakes. These tend to follow a north to south gradient, with the northern ARB dominated by bottomland hardwoods, and the southern ARB predominately lakes. The ARB is bounded to the east and west by protection levees to prevent flooding of adjacent agricultural areas and towns; however, these levees reduce the size of the floodplain for the Atchafalaya River. The river itself has a high sediment load, and transports around 84×10^6 metric tons of sediment annually (Allison et al. 2000). While much of this sediment discharges into Atchafalaya Bay, contributing to delta formation (Roberts 1998), a portion is deposited on the floodplain within the ARB. This deposition leads to natural habitat succession, and is transitioning the ARB from a lake-dominated ecosystem to a predominately bottomland hardwood forest ecosystem. As sedimentation continues, there will be a further reduction in the total area of the ARB that has contact with floodwaters. In addition to alteration for flood control, the natural hydrology of the system has been further disrupted for pipeline and highway construction, navigation, and timber removal. However, despite this history of human intervention, the Atchafalaya Basin still contains large areas of uninhabited wilderness that provide extensive natural resources for fish and wildlife habitat.

Methods

Sample Collection

Bulk sediment samples were collected from 10 sites throughout the ARB during May 2007 (Fig. 1). Sites were haphazardly selected based on accessibility and proximity to public land, and were separated by at least 3 km but not more than 63 km. Samples were sealed in 4-L wide-mouth heavy duty polyethylene (HDPE) bottles, put on ice during transport to the laboratory, and then stored at 2°C. Subsamples were analyzed for N and C using a Leco C-N Analyzer (Leco Corp., St. Joseph, MI, USA). Extractable nitrate and ammonium were analyzed colorimetrically (Method 365.4, USEPA 1983). Particle size was determined
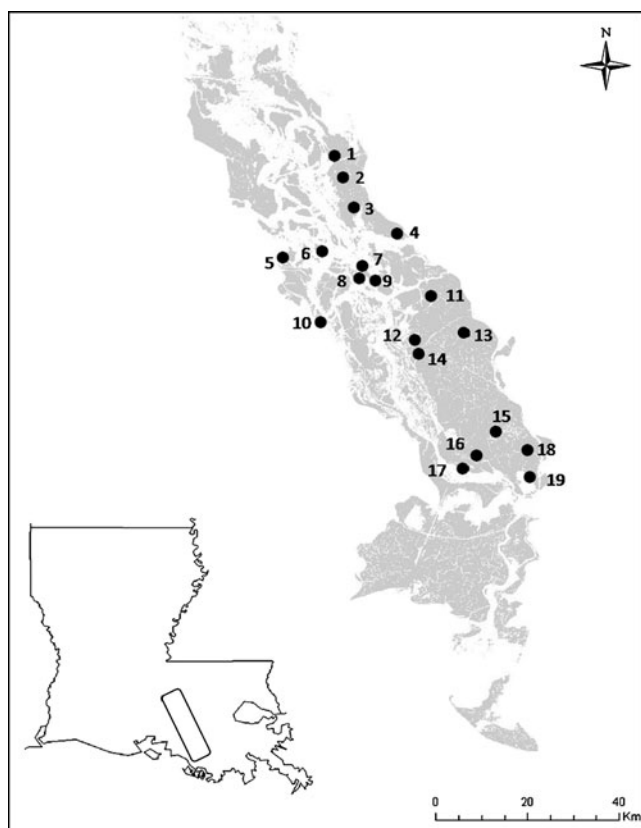
Fig. 1 Location of sampling sites in the Atchafalaya River Basin

by the Hydrometer Method (Gee and Bauder 1986). A conductivity meter was used to analyze samples for conductivity and salinity. A pH meter was used to determine the pH of samples. Samples were weighed before and after they were dried in an oven to determine moisture content. To increase spatial variability in our study, we included sediment samples that were collected during the prior growing season (August 2006) from nine additional sites (Fig. 1). Sites were separated by at least 5 km but not more than 74 km. These samples had been frozen and stored in the laboratory since collection. Subsamples were analyzed for C and N shortly after they were collected in 2006. While the long storage time of the previously collected samples is a concern, Stenberg et al. (1998) saw less of an effect on microbial biomass and activity in soils that had been frozen, as opposed to refrigerated. Another study investigating the response of denitrifying bacteria to freeze/thaw cycles did not observe a decrease in microbial biomass as a result of freezing (Sharma et al. 2006). Sharma et al. (2006) observed a burst of N_2O emissions immediately following thawing, so before we began the experiment we let the sediment samples equilibrate after thawing. Denitrification experiments were then carried out at room temperature.

We used the indirect acetylene block technique (Groffman 1994) to estimate denitrification rates. This technique is subject to criticism as it systematically underestimates denitrification rates (Groffman et al. 2006). Acetylene blocks the reduction of nitrous oxide to N_2 gas; however, incomplete inhibition can occur over longer time periods. Acetylene also inhibits nitrification, which can mask the importance of denitrification in systems with low external nitrate, where denitrification is primarily supported by internal nitrification. However, over short terms this is generally less of a problem (Ryden and Dawson 1982).

Background Denitrification

Duplicate microcosms were established for each site ($n=19$) by adding approximately 10 g of wet sediment to 40-ml glass vials capped with rubber septum tops. Following sediment addition, 10-ml of deionized water (no nitrate added) was added to all vials ($n=38$), which were subsequently agitated with a vortex mixer. Sealed vials were purged with ultra high pure N_2 gas for 1 min to remove O_2. A 2-ml gas tight syringe was used to remove 4 mL of gaseous headspace from all vials, which was then replaced by 4-ml purified acetylene (15% v/v C_2H_2/air). Vials were again agitated by the vortex mixer, and left undisturbed for 24 h in the dark. Gas samples were collected, at time 0 and at 24 h, with a syringe and injected into a Shimadzu GC-14A gas chromatograph (Shimadzu Scientific Instruments, Inc., Columbia, MD, USA) fitted with a 1-ml sampling loop, Porpak Q 1.8 m ss column, electron capture detector (ECD) and calibrated with certified N_2O gas standards (Scott Specialty Gases, Inc., Plumsteadville, PA, USA). Ultra high pure N_2 was the carrier gas and the instrument operated at temperatures of 40, 100, and 290 °C for the oven, injector, and ECD detector, respectively (Lindau et al. 1998).

Potential Denitrification

We replicated the methodology used for the background denitrification experiment to determine denitrification potential. However, the addition of 10-ml of deionized water was replaced by addition of 10-ml of 100 $mg L^{-1}$ NO_3-N solution. This high nitrate concentration was used to ensure that nitrate was not limiting denitrification.

Statistical Methods

Background denitrification and potential denitrification rates were analyzed using PROC MIXED in SAS to characterize denitrification within and across habitats (SAS Institute 2006). Correlations between N_2O emissions and soil components (N, C, NO_3^-, and NH_4^+) were tested

using the PROC CORR function in SAS (SAS Institute 2006). The PROC REG function in SAS was used to test for a relationship between denitrification rates and site coordinates (recorded using the Universal Transverse Mercator projection, zone reference 15R), expressed as UTM east and UTM north (SAS Institute 2006).

Results

Total N in the soil samples ranged from 0.1–1.4%. Total C ranged from 1.12–22.6%. Soil nitrate ranged from a minimum value of 0.07 $mgkg^{-1}$ to a maximum value of 98.3 $mgkg^{-1}$. Soil ammonium values ranged from 1.1 mg kg^{-1} to 487.4 $mgkg^{-1}$ (Table 1).

Background denitrification rates and potential denitrification rates differed among sites ($p<0.0001$ and $p=0.0005$, respectively), and also differed from each other ($p<0.0001$). Background denitrification rates showed a positive correlation ($r=0.74$, $p<0.0001$) with soil nitrate, and negative correlations with soil C ($r=-0.38$, $p=0.0183$) and soil N ($r=-0.32$, $p=0.0475$). Potential denitrification rate showed no correlations with any of the soil parameters tested. Soil N showed positive correlations with soil C ($p<0.0001$) and soil ammonium ($p=0.0035$). Average denitrification rates for each site, and ranges, are shown in Table 2.

Simple linear regression between spatial coordinates of our sites and corresponding denitrification rates did not detect a relationship for background denitrification rates ($R^2=0.10$, $p=0.31$), suggesting that patterns of spatial variability in background denitrification rates across the ARB are lacking. However, a significant relationship existed between spatial coordinates and denitrification potential ($R^2=0.32$, $p=0.004$). In general the highest rates were found in the southeast, downstream area of the ARB (Fig. 2). Soil nitrate did not show a spatial relationship across the ARB ($R^2=0.19$, $p=0.23$), but soil C was related to location ($R^2=0.32$, $p=0.004$). Soil C levels were highest in the north-central (upstream) and southeast (downstream) areas of the ARB (Fig. 3).

Discussion

Riparian soils flood more often than their upland counterparts, which can lead to anaerobic conditions in the soil and subsequent buildup of organic matter (Schipper et al. 1993). Through decomposition, this supply of C becomes available to fuel denitrifying bacteria; hence a positive correlation is often seen between soil organic C and denitrification potential (Gale et al. 1993; D'Angelo and Reddy 1999). While soil C and potential denitrification were not correlated, we observed a negative correlation between soil C and background denitrification. Plant communities differ across the ARB, thus the bioavailability of C will differ as well. Habitat types, hydrologic conditions, or quality of

Table 1 Sediment characteristics of samples collected from the Atchafalaya River Basin, LA

Site	Total C (%)	Total N (%)	NO_3-N $mgkg^{-1}$	NH_4-N $mgkg^{-1}$	Flooded	pH	EC (mS/cm)	Salinity (ppt)	Moisture (%)	Sand (%)	Silt (%)	Clay (%)
1	14.5	0.8	0.2	89.8	Yes	5.5	0.6	0.3	83.4	22.6	11.2	66.2
2	22.6	1.4	0.6	91.0	Yes	5.4	0.6	0.3	90.0	30.2	16.8	53
3	9.1	0.7	1.1	77.1	Yes	6.0	0.2	0.1	58.6	13.3	14.7	72
4	2.7	0.2	0.4	137.5	Yes	6.3	0.6	0.3	66.6	5.7	43.9	50.4
5	1.2	0.1	3.7	6.9	No	5.6	0.0	0	18.8	65.3	23.7	11
6	1.7	0.1	6.2	8.7	Yes	6.4	0.2	0.1	35.0	2.5	48.4	49.1
7	1.6	0.1	2.1	11.0	Yes	5.6	0.3	0.2	42.0	1.8	28.5	69.7
8	3.1	0.2	40.6	1.3	No	7.0	0.4	0.2	32.7	9.5	58.9	31.6
9	13.3	0.9	1.7	27.4	Yes	5.9	0.4	0.2	78.3	17.9	15.2	66.9
10	2.4	0.2	12.6	1.1	No	6.0	0.3	0.1	37.7	2.8	32.4	64.8
11	1.9	0.2	66.0	9.9	No	7.0	0.4	0.2	24.0	58.2	31.5	10.3
12	1.1	0.1	32.8	1.1	No	6.6	0.2	0.1	17.4	78.5	14.2	7.3
13	2.3	0.2	34.8	2.3	No	7.1	0.4	0.2	27.3	15.4	66.6	18
14	7.8	0.8	0.1	487.4	No	4.4	1.9	1	71.6	10.5	15.5	74
15	9.1	0.8	4.9	105.0	No	4.7	1.4	0.7	70.7	12	28.5	59.5
16	3.2	0.3	98.3	27.1	No	5.6	0.8	0.4	48.8	4.1	34	61.9
17	1.4	0.2	1.2	2.7	Yes	6.7	0.8	0.4	48.3	16.3	60.4	23.3
18	2.7	0.3	0.1	24.3	Yes	6.9	1.8	0.9	71.3	3	41.5	55.5
19	3.3	0.3	1.0	35.2	Yes	7.0	1.9	1	71.4	3.4	31.6	65

Table 2 Background and potential denitrification rates ($\mu gNg^{-1} d^{-1}$)

Site	Mean Background	Std Dev	Min	Max	Mean Potential	Std Dev	Min	Max
1	0.51	0.15	0.40	0.62	68.55	65.58	22.17	114.92
2	0.40	0.05	0.36	0.43	26.72	13.65	17.06	36.37
3	1.04	0.16	0.92	1.15	159.54	12.23	150.89	168.19
4	0.08	0.07	0.03	0.13	332.24	354.32	81.69	582.78
5	0.45	0.09	0.38	0.52	72.08	53.46	34.27	109.88
6	1.03	0.06	0.99	1.07	369.00	40.50	340.36	397.63
7	0.74	0.02	0.73	0.75	271.53	6.99	266.59	276.47
8	0.65	0.02	0.64	0.67	250.10	25.29	232.22	267.98
9	0.31	0.00	0.31	0.31	90.52	15.97	79.23	101.81
10	0.00	0.00	0.00	0.00	336.08	216.03	183.32	488.83
11	0.11	0.05	0.08	0.14	256.11	28.95	235.64	276.58
12	1.35	0.02	1.33	1.37	250.97	4.39	247.86	254.07
13	1.35	0.02	1.34	1.36	257.68	1.00	256.97	258.39
14	0.00	0.00	0.00	0.00	710.47	22.68	694.43	726.50
15	0.00	0.00	0.00	0.00	309.55	70.11	259.97	359.12
16	0.18	0.24	0.01	0.35	133.91	30.69	112.21	155.61
17	0.35	0.36	0.09	0.60	249.15	6.96	244.23	254.07
18	0.04	0.03	0.02	0.06	44.83	7.79	39.32	50.34
19	0.45	0.01	0.44	0.45	128.46	0.54	128.08	128.84
Mean	0.48	0.45	0.00	1.37	227.23	172.08	17.06	726.50

organic matter may be stronger predictors of denitrification potential than C. Schipper et al. (1993) indicated that in anaerobic and organic C-rich environments, denitrifying bacteria will have a competitive advantage for organic C and nutrients. However, when comparing soils across habitat types other variables could be more important than organic C concentrations.

Pina-Ochoa and Alvarez-Cobelas (2006) found that annual in-situ denitrification rates were highest in rivers and lakes, followed by coastal systems, and estuaries. These findings highlight the concept that environmental variability at scales of habitat and ecosystem level are often confounded and may explain inconsistencies between controlling variables (such as C) and denitrification rate.
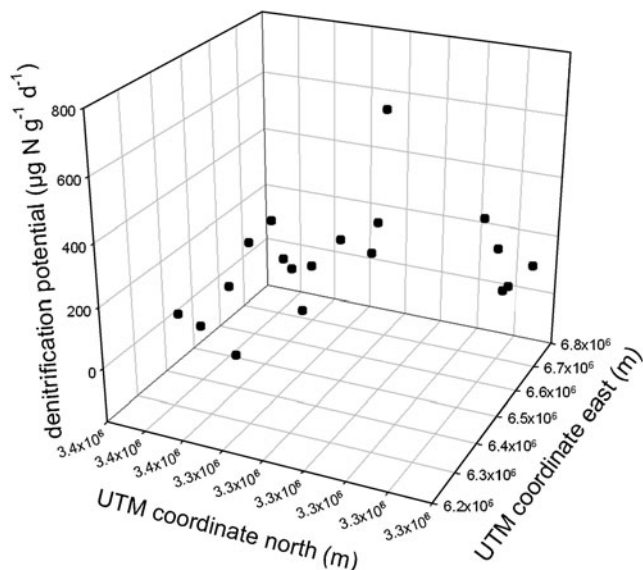

Fig. 2 Denitrification potential ($\mu gNg^{-1} d^{-1}$) vs. spatial coordinates (UTM) showing a general trend of increasing denitrification rates along a northwest to southeast trajectory
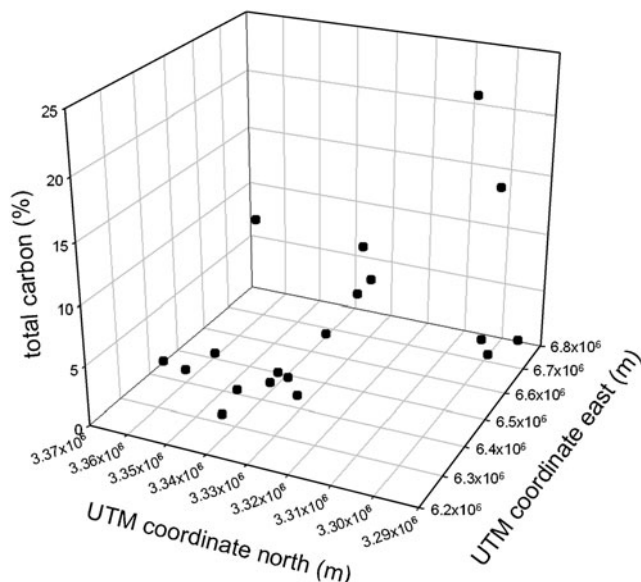

Fig. 3 Total carbon vs. spatial coordinates (UTM) showing an increase in total carbon along a northwest to southeast trajectory

Pina-Ochoa and Alvarez-Cobelas (2006) corroborated our observation of a correlation between background denitrification and soil nitrate; they also found that nitrate concentration was the best explanatory variable for differences in denitrification rates across ecosystem types. In our experiment samples that received nitrate additions were spiked with non-limiting quantities (100 mg L^{-1} NO$_3$$^-$-N), and because all other limiting factors besides C were controlled for, we expected to see C as the factor most limiting denitrification rates. The lack of a statistical correlation between C and potential denitrification might be attributed to the failure to fractionate organic matter and determine what percent of soil organic C was labile, and thus bioavailable to denitrifiers.

In our study, a number of possible reasons exist for total C in the system failing to correlate with denitrification potential. Because plant communities differ across the ARB, the quality of organic matter probably differs as well. The recalcitrant nature of baldcypress needles could explain why some sites with adequate soil C content did not show a significant relationship between soil C and denitrification potential. We sampled from the top 10-cm of the soil profile, which is the area where denitrifying organisms are most prevalent. In habitats experiencing rapid sedimentation, labile organic C could be buried at deeper depths in the soil profile. Dodla et al. (2008) estimated labile organic C during a study of potential denitrification rates across forest swamp, freshwater marsh, and saline marsh in Southeast Louisiana and found that labile organic C had a positive correlation with potential denitrification rates.

The lack of a trend in spatial variability for background denitrification rates and soil nitrate in the ARB could also be due to the fact that plant communities and water levels do not strictly conform to a distinct north-south or east-west gradient. A category that encompasses a number of these variables influencing denitrification, such as habitat type, may better explain differences in biogeochemical cycling. However, trends in spatial variability for potential denitrification rates and soil C observed in this study suggest that C may be more limiting of denitrification potential at the landscape scale.

Adding to the difficulty in distinguishing the contributions of denitrification to ecosystem-scale N removal is the fact that biogeochemical cycling can vary over small scales and short time periods (Seitzinger et al. 2006). "Hot spots" and "hot moments" result when key elements in the denitrification pathway are brought together in a particular place, or after an episodic water release re-activates accumulated reactants (McClain et al. 2003). Within the ARB specifically, reactants accumulate in the soil during dry periods when the river is not in contact with the floodplain. Litterfall primes the system with C from decaying organic matter, and N is introduced from nitrification during aerobic periods. This sets up a system where anaerobic conditions limit the denitrification reaction. When water transports additional nutrients across the floodplain, newly induced anaerobic conditions enhance reaction rates (McClain et al. 2003), leading to a hot moment for denitrification. Because some of our samples were collected dry (Table 1), we might have measured a temporal hot moment at some sites. This could have affected our analysis, which indicated that denitrification potential is related to landscape position. Future experiments will measure denitrification rates from these dry sites with and without flooding in the laboratory.

Brief 24-hour studies such as this one cannot elucidate denitrification dynamics over time. Actual peak denitrification rates for soil types that take more than one day to reach maximum emissions might have been missed. Pulses of nitrate that enter the ARB seasonally or following storms will not be considered; these could lead to spikes in denitrification rates. Fluctuating water levels can accelerate denitrification rates (Bowden 1987), so sites should be sampled before and after floods to better understand patterns in denitrification. Additional studies are needed to determine the role of organic matter quality and habitat classification in predicting potential denitrification rates in the ARB.

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