Linking microbial community structure to water quality function: investigating nitrogen cycling during early floodplain development

Basic Information

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1. Peralta, A.L., J.W. Matthews, E. Johnston, and A.D. Kent. In preparation. Abiotic correlates of microbial community structure and function vary within and among wetland ecosystems.
2. Peralta, Ariane. In preparation. Soil microbial community structure and function along environmental gradients: Implications for wetland nitrogen cycling. Ecology, Evolution and Conservation Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

PROJECT TITLE

Linking microbial community structure to water quality function: Investigating nitrogen cycling during early floodplain development

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PROBLEM AND RESARCH OBJECTIVES

Land use change and increased nutrient inputs in combination with wetland losses have exacerbated degradation of Illinois waterways and downstream waters. Floodplain restoration within agricultural landscapes along the Illinois waterways can potentially mitigate nutrient loading and improve water quality. Microorganisms are responsible for biogeochemical cycling of nutrients, but little is known about the relationship between microbial community composition and ecosystem processes within restored floodplain wetlands. Further, microbial processes and the populations responsible for microbially-mediated ecosystem functions are influenced by environmental factors that are likely to vary dramatically in response to changes during floodplain development. Understanding the response of microorganisms to local environmental soil factors is essential for restoration of wetland water quality functions.

Restoration of floodplain wetlands adjacent to major waterways could potentially reduce nutrient (nitrate) export and improve water quality to downstream coastal areas. Excess nitrates from agricultural runoff can be converted to gaseous forms through the processes of denitrification ($NO_3^- \rightarrow N_2$) and nitrification ($NH_4^+ \rightarrow NO_3^-$). Denitrification is an anaerobic process that occurs in saturated soils and sediments. In contrast, nitrification is an aerobic microbial process, which is responsible for nitrogen retention in soils. Both of these processes are sensitive to oxygen levels, and are responsive to fluctuations in water levels in the newly restored St. Joseph Wetland (Champaign County, IL) and Emiquon Preserve (Fulton County, IL). Changes in water saturation of soils will influence oxygen availability in the floodplain sediments, and will affect the balance between nitrification and denitrification activities.

We investigated the link between microbial community composition and activity, specifically related to nitrogen cycling, in response to changing water levels in early floodplain development. The objectives of this study were to (1) assess changes in nitrogen-specific microbial populations related to denitrification and nitrification along a moisture gradient and (2) quantify potential denitrification and nitrification rates along a moisture gradient. Comparison between nitrogen cycling functional groups provides the opportunity to test whether particular soil factors are associated with microbial community structure and function differently, depending on the environmental tolerance of the denitrifier or ammonia oxidizer communities. We predicted that changes along an upland to wetland gradient at different wetlands will result in variable shifts in microbial community composition and activity. Understanding how microorganisms vary in functional response can enhance management of water quality functions within restored wetlands.

METHODOLOGY

Field site

Restored floodplain wetlands at St. Joseph Wetland (IL-1; Champaign, IL) and Emiquon Preserve (IL-2; Fulton County, IL) were sampled in this study. The St. Joseph Wetland was converted from cropland to floodplain wetland in 2006. Depressional areas were excavated to enhance water retention of floodwaters originating from the Salt Fork River. The Emiquon Preserve contains approximately 3000 hectares of land bordering the Illinois River. Beginning in 2007, this site began a conversion from agriculture to its original state as a floodplain river ecosystem.

Sample collection

At each wetland site, four transects (20 m apart) were laid out perpendicular to the shoreline of the pond/lake. Along each transect, four plots were positioned along an upland to wetland gradient (A to D), according to distinct changes in plant community composition (Fig. 1). Topography and plant community composition were used as proxies for soil moisture changes within wetlands. Within a 4-m^2 area at each established plot, cover class of each plant species was assigned and recorded to survey herbaceous vegetation. No vegetation was present in the saturated plots (plot D at each transect) at Emiquon Preserve (Emiquon).

Wetlands were visited monthly from June to August 2009. Within a 1-m^2 quadrat at each plot, six soil cores (3.048 cm diameter) were collected to a 12 cm depth, combined, and homogenized. Samples were transported on ice, and stored at 4°C prior to processing in the laboratory. A subsample was collected for storage at -20 °C for microbial analysis.

Soil chemical analysis and activity assays

For soil collected at each plot, on each sampling date at each wetland, gravimetric soil moisture was assessed from a 20-30 g subsample. In addition, a subsample of air-dried soil was ground into a fine powder and analyzed to quantify total organic matter content (total organic C and total N) using combustion methods (ECS 4010, COSTECH Analytical Instruments, Valencia, CA). Soil pH, available ammonium (NH_4^+-N) and nitrate (NO_3^--N), and soil texture were also determined for all samples. On fresh soil samples, denitrification potential of the soil microbial community was estimated using the acetylene inhibition method (Royer et al. 2004). Potential nitrification activity of the soil microbial community was assayed using a short-term incubation (Kandeler 1996).

Microbial community analyses

Since the majority of environmental microbes are not easily cultured using standard laboratory culture techniques (Torsvik et al. 2002), molecular microbial ecology methods were used to assess functional community composition in soil. Microbial populations of denitrifying bacteria and ammonia-oxidizing archaea were compared among samples using molecular analysis of terminal restriction length polymorphism (T-RFLP), where the genes involved in denitrificaiton and nitrification were targeted. Community analyses based on functional genes coding for nitrous oxide reductase (*nosZ*) and ammonia monooxygenase (*amoA*) were used to characterize populations of denitrifiers and ammonia oxidizers, respectively (Rosch et al. 2002, Rich et al. 2003, Francis et al. 2005, He et al. 2007). All of the microbial community analyses (total community and analyses based on functional genes) take advantage of DNA sequence variation in the target gene to differentiate microbial populations. We use these "DNA fingerprint"

approaches to create a census of microbial populations present in each sample. Microbial assemblages were compared among samples, and in relation to environmental gradients using multivariate analysis.

Statistical analysis

St. Joseph Wetland and Emiquon Preserve were analyzed separately since the environmental gradient was not identical at each site. Analysis of variance (ANOVA) was used to test for differences in microbial activity along the gradient over three months. Sampling month and plot along the gradient were considered fixed factors, plot within transect was a random factor, and month was used as a repeated measure. ANOVA was run in the MIXED procedure of SAS (PROC MIXED, SAS v9.2, SAS Institute).

Similarity matrices were generated for denitrifer and ammonia oxidizer communities by calculating the Bray-Curtis similarity coefficient for each pair of samples based on terminal restriction fragment (T-RF) relative fluorescence data generated from microbial analysis method T-RFLP. Nonmetric multidimensional scaling (NMDS) was used to visualize the relationship among microbial communities along the moisture gradient using the metaMDS function in the R Statistics Package (R development Core Team 2011). NMDS is based on the rank order relation of dissimilarities based on the Bray-Curtis similarity coefficient, where the largest distance between points on the NMDS plot denotes the most different microbial communities. The envfit function was used to fit microbial activity and soil factors significantly associated with microbial variation (based on PERMANOVA) on each NMDS plot.

Permutational (nonparametric) multivariate analysis of variance (PERMANOVA) was used to assess the effect of location and sampling date along the gradient (plot) within each wetland, and to assess the relationship among different environmental factors and potential microbial activity on microbial community variation within each wetland (Anderson 2001, McArdle and Anderson 2001).

PRINCIPAL FINDINGS AND SIGNIFICANCE

Soil characteristics along the upland to wetland gradient

The St. Joseph Wetland was characterized as a high fertility site, compared to lower soil fertility (lower SOM, inorganic N) at Emiquon Preserve (Tables 1 & 2). The soil pH gradient was relatively large at Emiquon, but narrow at St. Joseph. In addition, the St. Joseph site had more finely textured soils, while Emiquon was characterized as fine to coarsely textured along the gradient (Tables 1 & 2).

Microbial activity along the gradient

Higher soil fertility at the St. Joseph Wetland supported higher potential denitrification and nitrification rates (Tables 3 & 4) compared Emiquon. Potential denitrification rates generally increased from upland to wetland plots, while potential nitrification rates were highest in wet-dry transition plots at Emiquon and decreased over time (Tables 3 & 4).

Denitrifier structure-function relationship along the gradient within each wetland

Location along the gradient significantly associated with distinct denitrifier community composition along the environmental gradient (PERMANOVA: St. Joseph: $R^2 = 0.3627$,

$P = 0.0010$; Emiquon: $R^2 = 0.4043$, $P = 0.0010$) (Fig. 1). At St. Joseph Wetland, sampling date significantly related to denitrifier community variation (PERMANOVA, $R^2 = 0.0928$, $P = 0.0040$) but not at Emiquon. Based on length of the denitrification vector on the NMDS plots, the relationship between composition and function was stronger at St. Joseph Wetland compared to Emiquon (Fig. 1).

The relationship between local soil factors and denitrifier community composition varied between restored wetlands. At the St. Joseph site, distinct denitrifier communities were observed in upland compared to wetland plots, but there was no measured soil factor that significantly associated with community variation (Fig. 1A). At the Emiquon site, soil pH and percent clay significantly related to denitrifier community variation along the gradient (PERMANOVA, pH: $R^2 = 0.2159$, $P = 0.0010$, clay: $R^2 = 0.0439$, $P = 0.016$) (Fig. 1B).

In summary, denitrifier community structure and function was decoupled at Emiquon Preserve, where potential denitrification rates were observed to change but composition stayed constant over time. Soil pH was significantly associated with denitrifier community composition at Emiquon, where pH ranged the most widely.

Ammonia oxidizer structure-function relationship along the gradient within each wetland

Location along the gradient significantly linked to differences in ammonia oxidizer community composition (St. Joseph: $R^2 = 0.2975$, $P = 0.0010$; Emiquon – $R^2 = 0.5314$, $P = 0.0010$) (Fig. 2). Sampling date did not influence ammonia oxidizer variation (data not shown). Based on the nitrification vector associated with the NMDS plots, potential nitrification rate at the St. Joseph site was observed to increase toward wetland plots (Fig. 2A), while potential nitrification rate increased toward plots at the wet-dry transition zones at the Emiquon site (Fig. 2B).

At St. Joseph, soil pH significantly related to differences in ammonia oxidizer community composition along the gradient (PERMANOVA, $R^2 = 0.0722$, $P = 0.0110$) (Fig. 2A). Soil pH, total organic carbon (TOC), ammonium-N and percent clay significantly associated with ammonia oxidizer community variation along the gradient at the Emiquon site (PERMANOVA, pH: $R^2 = 0.955$, $P = 0.0010$, TOC: $R^2 = 0.0209$, $P = 0.0440$; ammonium: $R^2 = 0.0347$, $P = 0.0110$, clay: $R^2 = 0.0365$, $P = 0.0100$) (Fig. 2B).

In summary, ammonia oxidizer community structure and function were decoupled, where function changed over time but composition did not. Soil pH, texture and organic matter concentration, factors that do not temporally change over short timescales, tended to associate with ammonia oxidizer community change among wetland sites.

NOTABLE ACHIEVEMENTS

- Microbes involved in nitrogen cycling variably respond to environmental conditions within restored wetland ecosystems. Understanding how soil factors influence these microorganisms will allow enhanced restoration of specific water quality functions.
- Restoring wetland water quality functions can be complicated by site-specific environmental factors.
- To predict microbial functional response to restoration activities, it is imperative that microbial functional groups related to nutrient cycling processes are specifically investigated.

- Microbial functional groups respond to the environment differently, and the assumption that microorganisms are extremely diverse and therefore functionally redundant can misguide management of nutrient cycling functions within wetlands.

STUDENT SUPPORTED WITH FUNDING

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Degree sought: Ph.D. (expected August 2011)

PRESENTATIONS

Peralta. A.L., J.W. Matthews, E. Johnston, S. Ludmer, and A.D. Kent (2011). Linking microbial community structure to water quality function in restored floodplain wetlands. Midwest-Great Lakes Society for Ecological Restoration Chapter Annual Meeting. Springfield, IL, USA. April 1-3, 2011. Oral presentation.

Peralta. A.L., J.W. Matthews, and A.D. Kent (2010). Linking microbial community structure to water quality function: Investigating nitrogen cycling influenced by hydrology in wetland ecosystems. Illinois Water Conference 2010, Champaign, IL, USA. Oct. 3-5, 2010. Poster presentation.

Peralta. A.L., J.W. Matthews, and A.D. Kent (2010). Linking microbial community structure to water quality function: Investigating nitrogen cycling influenced by hydrology in wetland ecosystems. The 13th International Symposium on Microbial Ecology, Seattle, WA, USA. August 22-27, 2010. Poster presentation.

PUBLICATIONS

Dissertation in preparation

Peralta, Ariane. In preparation. Soil microbial community structure and function along environmental gradients: Implications for wetland nitrogen cycling. Ecology, Evolution and Conservation Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

Publication in preparation

Peralta, A.L., J.W. Matthews, E. Johnston, and A.D. Kent. In preparation. Abiotic correlates of microbial community structure and function vary within and among wetland ecosystems.

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FIGURES AND TABLES

Figure 1. Experimental design established at wetland sites. Four plots along an upland to wetland gradient were established within each of four replicate transects (TR 20 m apart).

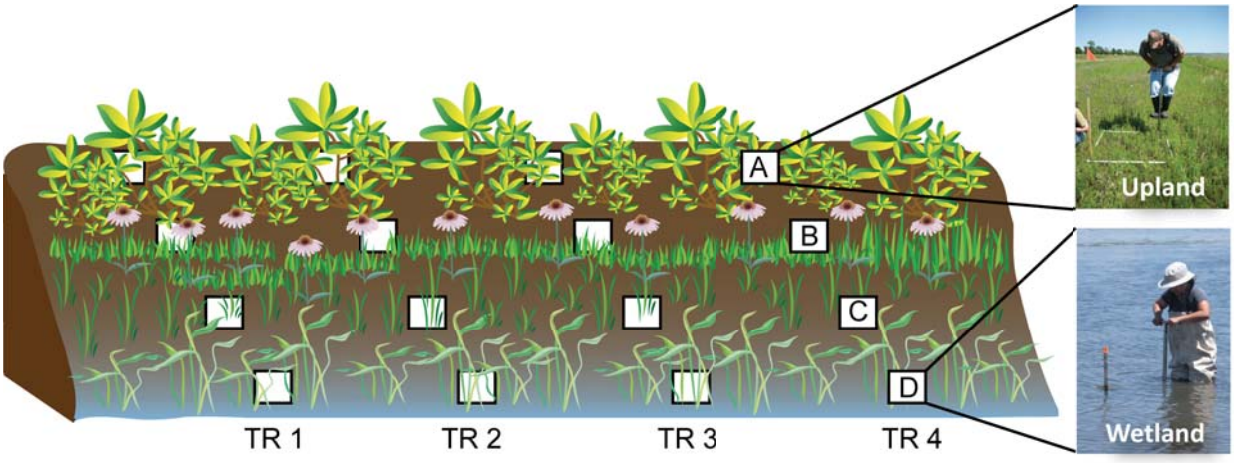

Figure 2. Non-metric multidimensional scaling plot of denitrifier community composition. Symbols are colored white to black and differ in shape to represent samples along an upland to wetland gradient at St. Joseph Wetland (IL-1) and Emiquon Preserve (IL-2). Each point represents the community composition of the denitrifiers based on T-RFLP relative fluorescence at different wetland sites. Arrows on plots relate denitrifier communities with soil factors and potential denitrification. The length and direction of the arrow corresponds to the relative correlation between environmental factor and ordination.

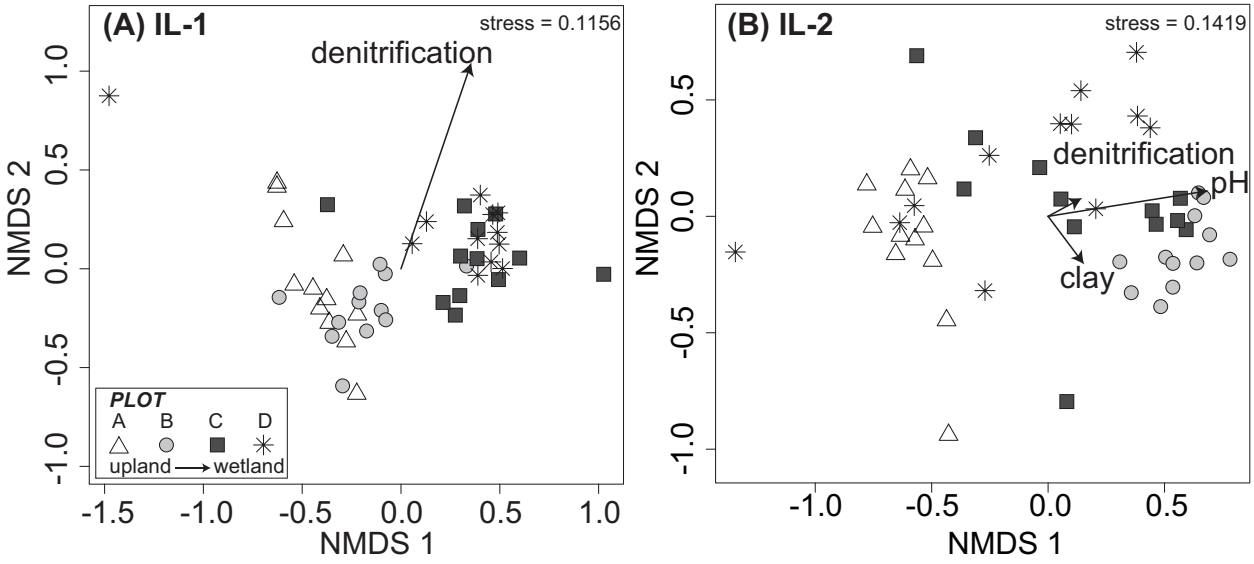

Figure 3. Non-metric multidimensional scaling plot of ammonia oxidizer community composition. Symbols are colored white to black and differ in shape to represent samples along an upland to wetland gradient at St. Joseph Wetland (IL-1) and Emiquon Preserve (IL-2). Each point represents the community composition of the ammonia oxidizers based on T-RFLP relative fluorescence at different wetland sites. Arrows on plots relate ammonia oxidizer communities with soil factors and potential nitrification. The length and direction of the arrow corresponds to the relative correlation between environmental factor and ordination.

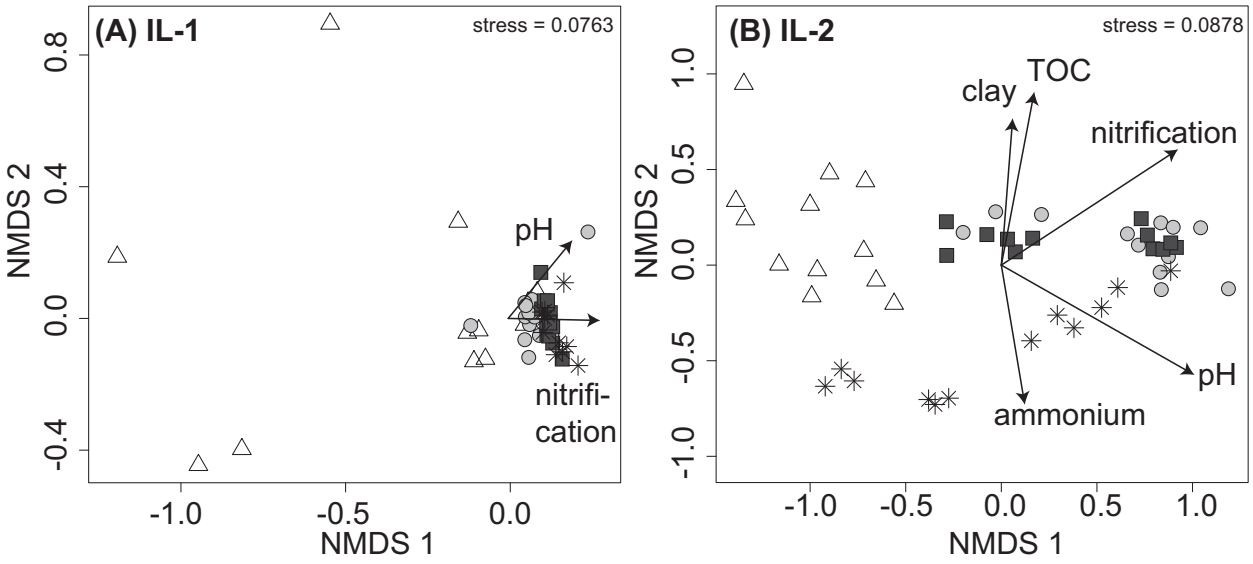

Table 1. Summary of soil factors (mean ± SD) along the upland to wetland gradient (plots A – D) at St. Joseph Wetland. Soil texture (%sand, %silt, %clay), pH and soil organic matter (total organic C – TOC, total nitrogen – TN, and C:N ratio – CN) were averaged along the gradient over all sampling months (A). Soil moisture and inorganic N (ammonium-N, nitrate-N) were averaged along the upland to wetland gradient for each sampling month (B).

(A)

soil factor	A			B			C			D		
sand	50	±	10	45	±	11	31	±	5	21	±	6
silt	29	±	6	33	±	8	42	±	4	47	±	4
clay	21	±	4	22	±	4	27	±	1	33	±	2
pH	7.68	±	0.79	8.14	±	0.45	8.10	±	0.34	8.05	±	0.31
TOC	14.59	±	2.49	19.18	±	1.99	27.07	±	1.99	32.38	±	2.86
TN	1.16	±	0.20	1.42	±	0.21	1.86	±	0.16	2.22	±	0.37
CN	12.73	±	2.21	13.67	±	1.18	14.60	±	0.56	14.77	±	1.40

(B)

June												
soil factor	A			B			C			D		
moisture	17.84	±	4.08	26.75	±	2.65	42.06	±	2.75	44.48	±	6.08
ammonium	0.838	±	0.025	1.088	±	0.325	2.425	±	0.807	2.700	±	0.634
nitrate	1.650	±	0.311	2.000	±	0.204	3.025	±	0.301	2.500	±	0.381
July												
soil factor	A			B			C			D		
moisture	17.00	±	2.46	24.08	±	3.19	37.25	±	5.90	40.17	±	7.44
ammonium	0.350	±	0.108	0.325	±	0.087	0.475	±	0.206	0.438	±	0.197
nitrate	3.963	±	0.634	3.550	±	0.917	5.675	±	1.796	4.975	±	1.019
August												
soil factor	A			B			C			D		
moisture	10.89	±	2.03	19.44	±	4.61	34.28	±	4.27	35.98	±	2.83
ammonium	0.500	±	0.183	0.400	±	0.147	0.575	±	0.132	0.788	±	0.284
nitrate	1.925	±	0.272	2.200	±	0.358	3.350	±	0.402	4.238	±	0.686

(UNITS: sand, silt, clay: %; TOC: mg C kg^{-1} soil; TN: mg N kg^{-1} soil; CN: wt/wt; moisture: %; ammonium: µg NH_4^+-N g^{-1} soil; nitrate: µg NO_3^--N g^{-1} soil)

Table 2. Summary of soil factors (mean ± SD) along the upland to wetland gradient (plots A – D) at Emiquon Preserve. Soil texture (%sand, %silt, %clay), pH and soil organic matter (total organic C – TOC, total nitrogen – TN, and C:N ratio – CN) were averaged along the gradient over all sampling months (A). Soil moisture and inorganic N (ammonium-N, nitrate-N) were averaged along the upland to wetland gradient for each sampling month (B).

(A)

soil factor	A			B			C			D		
sand	26	±	6	33	±	5	33	±	3	45	±	8
silt	50	±	4	42	±	4	43	±	2	35	±	5
clay	25	±	2	25	±	1	25	±	2	20	±	3
pH	5.72	±	0.31	7.56	±	0.22	7.38	±	0.49	7.17	±	0.18
TOC	14.13	±	1.10	14.84	±	3.08	14.24	±	3.14	7.17	±	1.38
TN	1.27	±	0.09	1.15	±	0.07	1.30	±	0.21	0.73	±	0.12
CN	11.17	±	0.64	12.88	±	2.30	10.86	±	0.82	9.87	±	0.69

(B)

June												
soil factor	A			B			C			D		
moisture	23.95	±	2.68	24.63	±	2.12	30.81	±	4.03	30.02	±	3.60
ammonium	3.675	±	3.960	3.138	±	0.687	5.788	±	0.757	10.313	±	4.920
nitrate	1.338	±	0.609	2.025	±	1.317	1.438	±	0.184	1.300	±	0.082
July												
soil factor	A			B			C			D		
moisture	30.58	±	6.79	26.10	±	3.14	33.61	±	4.70	30.44	±	2.26
ammonium	1.575	±	0.444	2.963	±	1.998	6.950	±	3.992	17.438	±	6.427
nitrate	3.300	±	1.340	2.988	±	0.899	2.663	±	1.430	2.763	±	1.274
August												
soil factor	A			B			C			D		
moisture	20.21	±	3.73	24.01	±	2.62	30.92	±	2.45	26.01	±	0.97
ammonium	0.700	±	0.261	3.588	±	1.920	4.625	±	1.963	11.763	±	2.464
nitrate	2.113	±	0.206	2.238	±	0.206	2.175	±	0.545	1.850	±	0.238

(UNITS: sand, silt, clay: %; TOC: mg C kg^{-1} soil; TN: mg N kg^{-1} soil; CN: wt/wt; moisture: %; ammonium: µg NH_4^+-N g^{-1} soil; nitrate: µg NO_3^--N g^{-1} soil)

Table 3. Summary of potential denitrification and nitrification rates (mean ± SD) along the upland to wetland gradient (plots A – D). Microbial activity was averaged across transects along the gradient for each sampling month at St. Joseph Wetland (A) and Emiquon Preserve (B).

(A) St. Joseph

June		A			B			C			D	
nitrification	2.461	±	1.041	4.358	±	1.448	6.793	±	1.450	8.172	±	1.655
denitrification	897	±	126	1539	±	431	3455	±	1202	3286	±	1473
July		A			B			C			D	
nitrification	2.553	±	1.440	3.835	±	1.393	5.399	±	0.994	6.301	±	0.940
denitrification	114	±	56	109	±	43	91	±	50	50	±	23
August		A			B			C			D	
nitrification	2.562	±	1.161	4.060	±	1.547	6.481	±	0.733	6.972	±	0.914
denitrification	33	±	11	76	±	50	195	±	84	249	±	180

(B) Emiquon

June		A			B			C			D	
nitrification	0.119	±	0.055	3.749	±	1.211	2.852	±	1.205	0.596	±	0.683
denitrification	486	±	326	660	±	412	650	±	438	179	±	150
July		A			B			C			D	
nitrification	0.048	±	0.062	3.758	±	0.396	1.944	±	1.502	0.809	±	0.596
denitrification	103	±	19	174	±	47	535	±	188	402	±	147
August		A			B			C			D	
nitrification	0.033	±	0.026	3.163	±	0.619	1.471	±	1.042	0.588	±	0.657
denitrification	22	±	7	38	±	19	35	±	12	18	±	7

Table 4. Summary of analysis of variance (ANOVA) results. ANOVA of the main effects (plot along gradient and month) and the interactions of main effects were carried out on potential denitrification and nitrification rates at St. Joseph Wetland (A) and Emiquon Preserve (B). Effects were considered significantly different at $P < 0.05$.

(A) St. Joseph

denitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	10	<0.0001
month	2, 33	259.89	<0.0001
month*plot	6, 33	8.37	<0.0001

nitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	14.52	<0.0001
month	2, 33	5.93	0.0063
month*plot	6, 33	1.5	0.2084

(B) Emiquon

denitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	6.28	0.0017
month	2, 33	18.02	<0.0001
month*plot	6, 33	2.55	0.0384

nitrification			
effect	df	*F*-value	*P*-value
plot	3, 33	24.04	<0.0001
month	2, 33	4.66	0.0165
month*plot	6, 33	2.03	0.0892